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Volume Editors

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Contents

XI **Foreword**

1 **Helminth-Induced Immunoregulation of an Allergic Response to Food**
C. Nagler-Anderson, Charlestown, Mass.

1 Abstract
1 Microbes and Allergy
3 Enteric Helminth Infection Acts as a Th2-Polarizing Mucosal Adjuvant
5 Enteric Helminth Infection Induces Th2 Responses without Atopy
8 How Does Helminth Infection Protect against Allergy: Immunoregulatory T or B Cells
11 Conclusions
11 References

14 **The Mutual Influence of Nematode Infection and Allergy**
D. Negrão-Corrêa, *M.M. Teixeira*, Belo Horizonte

14 Abstract
14 The Worldwide Relevance of Nematode Infections
15 Nematode Infections and the Hygiene Hypothesis
17 The Model of *S. venezuelensis* Infection in Rodents
18 *S. venezuelensis* Infection Induces Eosinophilic Airway Inflammation and Airway Hyperreactivity
20 Airway Hyperreactivity Is Shut Off by *S. venezuelensis* Infection but Prevents Parasite Infection
22 Role of a Th2 Immune Response for *S. venezuelensis* Elimination from the Intestine
25 Concluding Remarks

26 Acknowledgements
27 References

29 Human Schistosomiasis Decreases Immune Responses to Allergens and Clinical Manifestations of Asthma
M.I. Araujo, *E.M. de Carvalho*, Salvador

29 Abstract
29 Immunological Response and Clinical Forms of Schistosomiasis
31 Down-Modulation of Type 1 Immune Response in *S. mansoni* Infection
35 Immune Response in Atopy
36 Influence of Parasite Infection on the Development of Atopy and Asthma
42 References

45 Proteases in Helminth- and Allergen-Induced Inflammatory Responses
S. Donnelly, *J.P. Dalton*, Sydney; *A. Loukas*, Brisbane

45 Abstract
48 Proteases Are Major Allergens Derived from Various Organisms
49 How Do Proteases Act as Allergens?
49 Disruption of the Epithelial Barrier
50 Modulation of Dendritic Cell (DC) Function
50 Modulation of B and T Cell Function
50 Activation of Mast Cells Via Protease Activated Receptors
51 Alteration of the Protease/Anti-Protease Balance
52 Proteases Provoke Allergenic Responses to Non-Peptidolytic Molecules
53 Do Parasites Exacerbate or Protect against Allergy?
54 Helminth Parasites Secrete Proteases
54 Can Parasite Proteases Sensitise Individuals to Environmental Proteases *Via* Cross-Reactive IgE?
57 Parasite Peptidolytic Activity May Sensitise Individuals to Allergens
57 Degranulation of Mast Cells and Basophils
57 Modulation of T Cell Responses
58 Recruitment and Activation of Alternatively Activated Macrophages
58 Are Helminth Proteases Allergens?
61 Conclusion
62 Acknowledgements
62 References

65 Mechanisms Underlying Helminth-Induced Th2 Polarization: Default, Negative or Positive Pathways?
D. Jankovic, *S. Steinfelder*, *M.C. Kullberg*, *A. Sher*, Bethesda, Md.

65 Abstract
66 The Immune Response during Infection with *Schistosoma mansoni*
67 Role of IL-4 in *S. mansoni*-Induced Th2 Polarization
69 *S. mansoni*-Induced Th2 Polarization Requires DC: Evidence against a Default Pathway
71 *S. mansoni* Th2 Polarization As A Negative Pathway: Down-Regulation of DC Functions

73 *S. mansoni* Th2 Polarization As A Positive Pathway: Selective Activation of DC
74 How Do SEA-Conditioned DC Promote IL-4 Secretion in CD4+ Cells?
77 Conclusions
77 Acknowledgements
78 References

82 **Regulation of Dendritic Cell Function by Pathogen-Derived Molecules Plays a Key Role in Dictating the Outcome of the Adaptive Immune Response**
E.J. Pearce, *C.M. Kane*, *J. Sun*, Philadelphia, Pa.

82 Abstract
83 Dendritic Cells and Their Role in Th Cell Activation
84 Th Response Polarization
85 The Interaction of SEA with DCs
86 Eggs and SEA Induce Different Responses – An Unexpected Finding
87 SEA Inhibits TLR-Initiated DC Maturation
88 TLR-Ligand-Activated DCs Provide Negative Instruction for Th2 Response Polarization
89 Acknowledgements
89 References

91 **Glycans Modulate Immune Responses in Helminth Infections and Allergy**
I. van Die, Amsterdam; *R.D. Cummings*, Oklahoma City, Okla.

91 Abstract
93 Glycan Ags in Schistosomes
95 Schistosome Glycan Ags Generate High Levels of Anti-Glycan Abs in Infection
97 Helminth- and Allergen-Derived Glycan Ags Induce Th2 Responses
98 Immunogenic Glycan Ags Shared between Helminths and Allergens Are IgE Epitopes
100 Glycan Ags Regulate Immune Responses via Interaction with Host Lectins
101 C-Type Lectins on Antigen-Presenting Cells Recognize Glycan Ags
102 Recognition of Schistosome- or Allergen-Derived Glycan Ags by Antigen-Presenting Cells
102 Schistosome Egg Glycan Ags Interact with DC-SIGN
103 L-SIGN Binds Schistosome Egg Glycan Ags
103 Interaction of Schistosome Glycan Ags with hMGL
104 The Mannose Receptor Recognizes Ags from Schistosome Eggs and House Dust Mite Der p 1
104 Host Protection Mechanisms May Include the Binding of Glycosylated Ags by Soluble Lectins in Schistosomiasis and Allergy
105 Interaction of Helminth Glycan Ags and Allergens with Collectins
105 Galectin-3 Recognizes LDN Glycan Ags within Schistosome SEA
106 Does IL-10 Production Induced by Parasite Glycans Contribute to Protection Against Allergy?
108 Concluding Remarks
109 Acknowledgements
109 References

113 Role of the Natural Killer T Lymphocytes in Th2 Responses during Allergic Asthma and Helminth Parasitic Diseases
F. Trottein, *T. Mallevaey*, *C. Faveeuw*, *M. Capron*, Lille; *M. Leite-de-Moraes*, Paris

113 Abstract
114 Classification and General Features of NK T Cells
115 NK T Cells in Autoimmune, Neoplastic and Infectious Diseases
117 NK T Cells in Th2 Inflammatory Response during Allergic Asthma
118 Mechanisms of NK T Cell Activation and Mode of NK T Cell Actions during Allergic Asthma
119 NK T Cells during Helminth Infection
122 Mechanisms by which NK T Cells Become Activated during Infection
122 NK T Cells in Regulatory Mechanisms during Th2-Related Inflammation
124 Conclusions
124 Acknowledgements
125 References

128 The Mast Cell and Gut Nematodes: Damage and Defence
J.L. Pennock, *R.K. Grencis*, Manchester

128 Abstract
129 Regulation of Mucosal Mast Cells
131 Adhesion and Homing of Mast Cells to the Gut
132 Effector Mechanisms
136 Current Ideas
137 Acknowledgements
138 References

141 Basophils, Basophilia and Helminth Infections
E. Mitre, *T.B. Nutman*, Bethesda, Md.

141 Abstract
142 Basophil Biology
142 Lineage
142 Growth and Development
143 Trafficking
144 Basophil Function
144 Basophil Activation
144 IgE-Mediated Activation
145 Non-IgE Activation
146 Degranulation
146 Activation Markers
146 Effector Molecules
147 Histamine
147 Other Effector Molecules
148 Immunomodulatory Capabilities
148 Cytokine Release
148 Immunomodulatory Properties of Histamine
149 Basophils and the Immune Response to Helminth Infections

150 Basophilia
151 Basophil Activation in Helminth Infections
151 Helminth Antigens as 'Super Allergens'
152 Potential Roles of Basophils in Helminth Infections
154 References

157 Innate, Adaptive and Regulatory Responses in Schistosomiasis: Relationship to Allergy
F.C. Hartgers, *H.H. Smits*, Leiden; *D. van der Kleij*, Rijswijk; *M. Yazdanbakhsh*, Leiden

157 Abstract
158 Innate Immune Responses in Schistosomiasis
160 Adaptive Immune Responses in Schistosomiasis
162 Downregulation of the Immune Response during Chronic Schistosome Infection
162 Modulation of Innate Responses
165 Modulation of Adaptive Responses
165 Modulation via Regulatory T Cells
167 Relationship between Schistosomiasis and Allergy
167 Prevention of Atopy
168 Role of Regulatory T Cells
169 IgG4 Antibodies and IL-13 Decoy Receptor
171 The Window of Immune Suppression
172 Concluding Remarks
172 Acknowledgements
172 References

176 Regulatory T Cells Induced by Parasites and the Modulation of Allergic Responses
M.S. Wilson, *R.M. Maizels*, Edinburgh

176 Abstract
177 The Cellular Basis of Allergic Diseases
179 Regulatory T Cell (Treg) Biology
181 Regulatory T Cells in Allergic Diseases
183 Helminth-Mediated Immune Regulation and Treg Cells
184 Helminth Infection and Allergic Disease
186 Model Systems for Infection and Allergy
189 Alternative Models
190 Molecular Mechanisms at the Parasite-Allergy Interface
191 Infectious Tolerance and the Specificity of Treg Cells
192 Conclusions
193 References

196 Author Index

197 Subject Index

Foreword

One of the key words of immunology at the beginning of the 21st century is 'regulation'. Twenty-five years later the Th1/Th2 paradigm, the concept of regulatory cell populations, is now in the heart of our understanding of immune response.

Helminths and allergic conditions are recognized as the main Th2 cell inducers. The negative association of allergic manifestations and helminth infections has been debated for over 30 years. It is, however, only in the recent past, that modulation of allergy by helminth infections has been clearly substantiated and shown to be consistent with the activity of regulatory cell populations, which control effector mechanisms of both Th1 and Th2 types.

Although remarkable progress has been made in identifying the molecular events required for Th2 differentiation, a number of questions which are addressed in this volume point to essential challenges.

Several contributions illustrate the critical importance of characterization of helminth molecules with Th2 or regulatory inducing activities and their modes of action in dendritic cells.

The large emphasis given to glycan epitopes highlights the profound immunomodulatory properties of glycan antigens and their role in inducing two key regulatory cytokines IL-10 and TGF-β. It is striking that the specificity of helminth infection does not influence the profile of the regulatory response: Schistosomes, *Onchocerca, Wuchereria* or gut nematodes for instance, induce similar patterns of cytokine production, the regulation appearing more related to the chronicity of infections than to the pathogen itself.

Although the identification of regulatory cell populations has progressed, we are left with a global notion of heterogeneity and a rather unclear respective role of the various incriminated populations: regulatory T and B cells, natural killer T cells, mast cells and basophils, etc. The concept that primary and secondary regulatory populations may account for their heterogeneity is very stimulating, and the role of Fox p 3 as a master control gene is very attractive.

Whereas most of the contributions discuss the down-modulation of allergy by helminths, there is also some evidence that allergy or predisposition to atopic diseases may protect against helminth infections. It is, on the other hand, of particular interest that removal or inhibition of regulatory T cells leads to the effective clearance of infection and restoration of antigen specific activity.

In practical terms, one may expect that allergen-specific immunotherapy, which generates populations of allergen-specific regulatory T cells, producing IL-10 and TGF-β, can significantly reduce allergic manifestations. Conversely, successful immunization against helminth infections and the development of efficient vaccines will certainly rely on a subtle balance between the induction of appropriate effector mechanisms and the expression of regulatory responses.

In this context, the various contributions to this volume dedicated to Parasites and Allergy reveal a new dimension of host-parasite interactions and of the importance of anti-inflammatory responses in chronic helminthiasis. They also provide a novel insight on the possible modes of down-modulation of unwarranted immune responses. They finally pave the way to new directions of research for the successful immunization against helminths and the prevention of inflammatory responses in allergic and autoimmune diseases.

André and Monique Capron, Lille

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Helminth-Induced Immunoregulation of an Allergic Response to Food

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Abstract

Work from our laboratory has shown that an enteric helminth infection can act as an adjuvant to prime for a Th2-biased response to a typically tolerogenic form of dietary antigen. Helminth infection did not, however, prime for an allergic response. Using a model in which systemic anaphylactic symptoms and antigen specific IgE are induced in C3H/HeJ mice by repeated intragastric administration of peanut antigen with the mucosal adjuvant cholera toxin we showed that an enteric helminth infection protects against the development of food allergy. Helminth-dependent protection against allergy was abrogated when the helminth-infected, allergen-sensitized mice were treated with neutralizing antibodies to IL-10. Recent work from our laboratory and others has implicated helminth induced immunoregulatory cells in protection against allergy. We will discuss the characteristics of the immunoregulatory cell populations that have been described and the mechanism(s) by which they may function in the suppression of allergy.

Microbes and Allergy

The prevalence of allergic disease and asthma has increased dramatically during the last 30–40 years. Initial attempts to correlate this increase with exposure to environmental pollutants or other stimuli were largely inconclusive. This was perhaps demonstrated most strikingly when asthma rates were compared in individuals with similar genetic heritage but different environmental exposures in the former East and West Germany. Although it was expected that asthma rates would be higher among children living in the East, where air pollution levels were very high, asthma incidence was actually much higher in the West [1]. By contrast, there is a compelling body of evidence demonstrating an inverse

correlation between the incidence of allergy and exposure to microbial infection. Earlier studies centered on specific pathogens. A study of Japanese children documented an inverse correlation between exposure to *M. tuberculosi*s (as assayed by delayed-type hypersensitivity responses to tuberculin) and a markedly increased prevalence of asthma [2]. In Italian military recruits, respiratory allergy was less frequent in individuals with antibody responses indicative of heavy exposure to orofecal and foodborne microbes like hepatitis A [3]. Another study showed that, in Africa, vaccination with *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) correlated with a reduced prevalence of allergic disease [4]. Cumulative microbial exposure in early childhood has been the focus of more recent work. A decreased incidence of (or protection against?) allergy has been associated with exposure to older children either in large families [5, 6] or by early attendance at daycare [7], animals (residence on a farm with livestock [8] or household pets [9]) or an anthroposophic lifestyle that includes limited vaccination and antibiotic use and a microbe-rich diet [10]. Noting that susceptibility to allergy inversely correlated with family size, Strachan [5] suggested that the increasing incidence of asthma and allergy might be attributable to reduced exposure to childhood Th1 polarizing infections brought about by vaccination and improvements in sanitation. In the absence of imprinting for Th1-biased memory cells early in life, the inherent Th2 bias of immune responsiveness at mucosal surfaces would proceed unchecked, leading to allergic hyperreactivity in genetically susceptible individuals. Originally formulated as the 'hygiene' hypothesis, recent studies have specifically implicated childhood exposure to endotoxin in determining susceptibility to asthma and allergic disease [11, 12].

However, two other observations argue against a Th1/Th2 counter-regulation model of susceptibility to allergy. Allergic responses and helminth infection are uniquely characterized by their induction of Th2-polarized immune responses and IgE. In the developing world, where chronic helminthiasis is still largely endemic, there is actually a *lower* incidence of allergy [13]. Moreover, an increased prevalence of autoimmune diseases like type 1 diabetes, multiple sclerosis and Crohn's disease (all characterized by Th1-biased inflammatory responses) parallels the increasing incidence of allergy and asthma [14]. In an attempt to reconcile these observations with Strachan's original hypothesis, Wills-Karp et al. [15] proposed that the primary consequence of encounter with all types of microbial stimuli is the induction of immunoregulatory mediators critical for the prevention of the immune hyperreactivity which characterizes both allergy and autoimmune disease. Earlier work had reported tantalizing links between the suppression of allergic hyperreactivity by helminth infection, and its reversal by treatment with anti-helminthics [16]. More recently, Yazdanbakhsh and colleagues have evaluated various immunological parameters to explain the

reduced skin test reactivity to a ubiquitous allergen (housedust mites) observed in helminth-infected children in Gabon [17]. Only high-level production of *Schistosome* antigen-specific IL-10 significantly correlated with reduced skin test reactivity. Other work has confirmed a reduced risk of atopy in helminth-infected children despite high levels of parasite-induced total IgE [18]. In a murine model, we have found that an enteric helminth infection can act as an adjuvant to prime for a Th2-biased response to a typically tolerogenic form of dietary antigen [19, 20]. However, helminth infection protects against the anaphylactic symptoms and the antigen-specific IgE induced in a model of food allergy. Helminth-dependent protection against allergy was abrogated when the helminth-infected, allergen-sensitized mice were treated with neutralizing antibodies to IL-10 [21]. In this chapter, we will begin by reviewing work from our laboratory on helminth-induced Th2 responses and protection against allergy. We will then examine work from several different laboratories indicating that this protection is mediated via the parasites' ability to induce immunoregulatory T and B cells. Finally, we will propose a model for the mechanism(s) by which helminth infection can protect against the induction of an allergic response.

Enteric Helminth Infection Acts as a Th2-Polarizing Mucosal Adjuvant

We discovered that an ongoing enteric helminth infection could act as an adjuvant for the response to a dietary antigen in experiments aimed initially at examining whether non-responsiveness to orally administered antigen could still be induced in a chronically activated mucosal microenvironment. In noninfected mice, intragastric administration of a model dietary antigen, prior to peripheral immunization with antigen in adjuvant, typically induces systemic non-responsiveness to subsequent antigen challenge (reviewed in [22]). We chose *Heligmosomoides polygyrus* as our microbial 'activating agent' because it is a natural murine helminthic parasite with a strictly enteric life cycle. Between 24 and 72 h after ingestion (or experimental inoculation) parasitic third-stage larvae enter the wall of the small intestine and migrate into the muscularis externa beneath the mucosa. Adults emerge into the lumen 8–9 days after the initial inoculation and establish a chronic infection in many strains of mice [23]. Like other helminths, this parasite induces a polarized Th2 cytokine response, particularly in the mesenteric lymph nodes that drain the gut-associated lymphoid tissue, as well as peripheral blood eosinophilia and elevated levels of polyclonal serum IgG1 and IgE [23]. We examined the response to intragastrically administered ovalbumin (OVA) at 8 days postinfection with

H. polygyrus, at the peak of the mucosal immune response. Both infected and non-infected mice were immunized with OVA in the footpads 2 weeks prior to harvesting the draining popliteal lymph nodes (PLN) for restimulation in vitro with varying doses of OVA or plate-bound anti-CD3 [23]. We found that, while non-responsiveness for a Th1 type (IFN-γ) response to antigen rechallenge in vitro was maintained, helminth-infected mice were primed for a Th2 response to this typically tolerogenic form of dietary antigen [23]. An examination of serum OVA-specific IgG1 and IgG2a levels provided a direct ex vivo measurement of the ability of this enteric infection to alter the response to dietary antigen and showed that nonresponsiveness for a Th2-dependent isotype of IgG (IgG1) could not be induced in the helminth-infected mice [23].

These initial experiments showed that an enteric helminth infection can elicit a Th2-biased immune response to a normally tolerogenic form of orally administered antigen. While the polarized, parasite-induced, Th2 cytokine response provides the priming microenvironment for T cell differentiation, other stimuli are needed to tilt the response towards immunity rather than tolerance. Experimentally, immune responses towards otherwise non-immunogenic antigens are achieved through the co-administration of adjuvants, such as complete Freund's adjuvant (a mixture of killed mycobacteria in oil), which have been thought to mimic the inflammation induced by microbial antigens/products. The effects of adjuvants on the immune response are 3-fold: they influence antigen presentation, clonal expansion and T cell differentiation (reviewed in [24]). We therefore examined whether an ongoing enteric infection could act as an adjuvant to alter the response to another antigen. We showed that, in addition to creating a Th2-polarized cytokine environment that influences T cell differentiation, this enteric helminth infection upregulates the expression of costimulatory molecules required for T cell activation. CD86 (B7.2) and, to a lesser extent CD80 (B7.1), expression was increased on professional APC populations in the mucosa (Peyer's patch and mesenteric lymph node), but not the periphery (spleen). We made use of an adoptive transfer model in which TCR transgenic OVA-specific T cells were labeled with the vital dye carboxyfluorescein diacetate succinimidyl ester (CFSE). CFSE divides equally into daughter cells at each cell division. Flow cytometric analysis can be used to measure the loss of CFSE fluorescence intensity in gated TCR transgenic T cells to provide a proliferative history of these antigen-specific T cells. We found that, in noninfected mice, OVA feeding induced a reduction in the proliferative capacity of OVA-specific TCR transgenic T cells in the draining lymph nodes upon challenge with antigen in incomplete Freund's adjuvant. In response to subsequent antigen challenge noninfected mice exhibited a profound reduction in proliferative capacity that resulted ultimately in functional nonresponsiveness due to the failure to expand antigen-specific clones and/or cell death. By contrast, when oral

antigen was presented in the context of enteric helminth infection, the infection acted as an adjuvant. The proliferative capacity of the adoptively transferred transgenic T cells was restored and, in the Th2-biased cytokine environment accompanying this infection, OVA feeding primed for an OVA-specific Th2 type (IL-4) response (fig. 1) [20]. Our results also showed that, like other adjuvants, helminth infection enhances the survival and antigen-specific expansion of TCR transgenic T cells.

Enteric Helminth Infection Induces Th2 Responses without Atopy

If helminth infection primes for a Th2 response to dietary antigen, can it also induce an allergic response to food? Using a model in which intragastric administration of peanut (PN) antigen, in the presence of the mucosal adjuvant cholera toxin (CT), results in the production of antigen-specific IgE and systemic symptoms of anaphylaxis in TLR-4 mutant C3H/HeJ mice, we compared the response to antigen administered to helminth-infected mice to that seen after sensitization with PN plus CT alone. Like helminth infection, CT upregulates costimulatory molecule expression on mucosal APC and acts as a Th2-polarizing mucosal adjuvant [25]. Helminth infection did not, however, prime for a PN-specific IgE response. Moreover, as shown in figure 2a, both the PN-specific IgE response and anaphylactic symptoms were greatly reduced in helminth-infected mice. The reduction in PN-specific IgE was associated with a marked reduction in the ability of splenic T cells harvested from helminth-infected mice to secrete IL-13 in response to PN challenge in vitro (fig. 2b). Yet the helminth-specific polarized Th2 response, characterized by the production of Th2 cytokines (IL-4 and IL-13) and elevated polyclonal serum IgG1 and IgE remained intact. This suggested that the parasite's ability to downregulate the allergic response occurred at the level of allergen presentation by APCs, presumably dendritic cells. Helminthic parasites stimulate the production of immunoregulatory mediators that are likely to play a role in maintaining chronicity of infection without a marked induction of pathology. In particular, elevated levels of IL-10 have been associated with protection against allergic disease in helminth-infected African children [17]. We therefore examined whether, in our model, treating the sensitized, infected mice with neutralizing antibodies to IL-10 altered helminth-mediated protection against allergy. We found that, in mice sensitized with PN plus CT, treatment with anti-IL-10 abrogated the ability of helminth infection to protect against allergic symptoms and downregulate PN-specific IgE (fig. 2c). Subsequent work from our laboratory indicates that CD4+ T cells are responsible for helminth-mediated

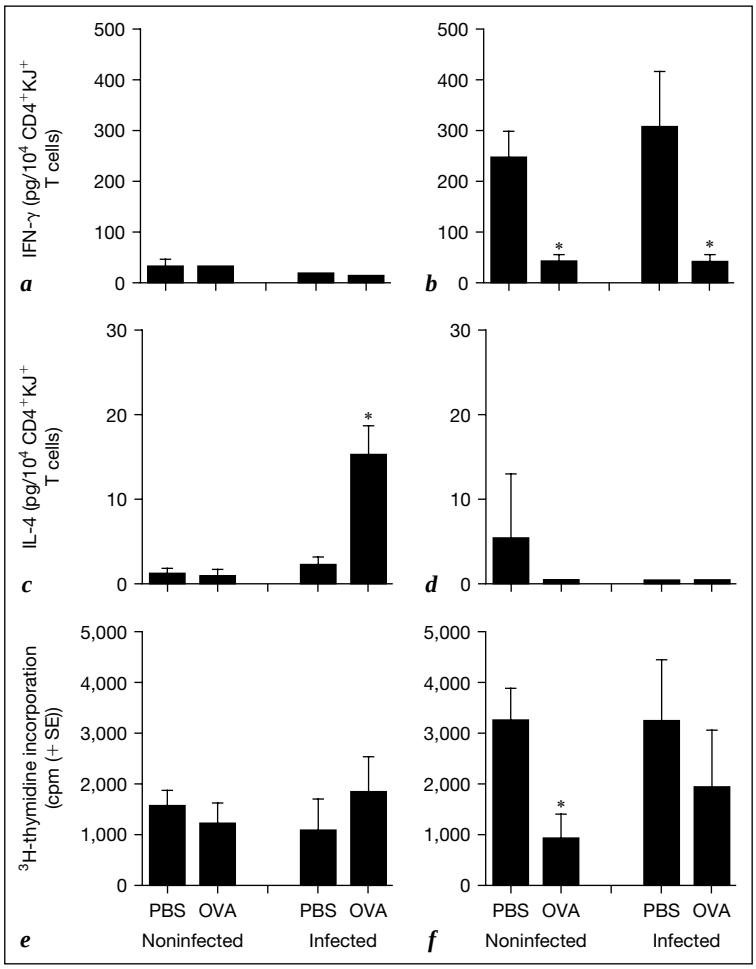

Fig. 1. OVA feeding primes for an antigen-specific Th2 response in helminth-infected mice. Mice were adoptively transferred with OVA-specific TCR transgenic T cells (KJ1-26) from DO.11.10 mice prior to OVA feeding to both infected and noninfected mice. PLN cells from each mouse were collected at both 3 (***a, c, e***) and 10 (***b, d, f***) days after immunization and cultured with OVA (100 μg/ml). IFN-γ (***a, b***) and IL-4 (***c, d***) secretion into the culture supernatants 72 h after the initiation of the culture was determined by ELISA. The data is expressed as the mean pg/ml of IFN-γ or IL-4 produced per 104 input CD4+ KJ1-26+ cells ± SEM. The antigen-specific proliferative response to restimulation with OVA in vitro was determined by ^{3}H-thymidine incorporation (***e, f***) and is expressed as the mean cpm ± SEM for 3 mice in each group. *$p < 0.05$ by Student's t test. By day 10 OVA feeding induced systemic non-responsiveness to in vitro challenge for a Th1-type (IFN-γ) response in both helminth-infected and noninfected mice. By contrast, OVA feeding primed for an antigen specific Th2 (IL-4) response (day 3). Reprinted from Shi et al. [20] with permission, copyright 2000, The American Association of Immunologists.

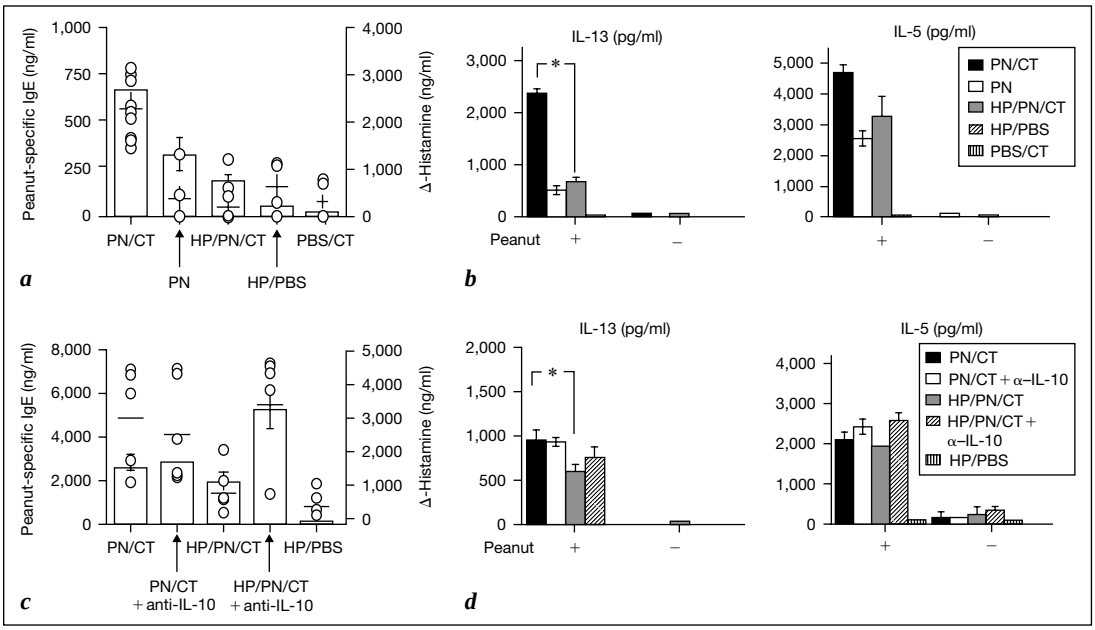

Fig. 2. Helminth infection inhibits IgE mediated anaphylaxis. ***a*** Helminth (Hp) infected mice sensitized with PN plus cholera toxin (CT) produced significantly less PN specific IgE than mice that received PN plus CT alone. Bar graphs depict mean PN-specific IgE (± SEM) per group. A change in plasma histamine levels at antigen challenge correlated with the onset of anaphylactic symptoms. Plasma histamine levels were determined using an enzyme immunoassay kit. Each circle represents one mouse. ***b*** Spleen cells from individual mice were restimulated in the presence or absence of PN (200 μg/ml) in vitro for 72 h. Culture supernatants were analyzed by ELISA. PN + CT sensitized, helminth infected made significantly less PN specific IL-13 than noninfected mice. ***c*** PN + CT-sensitized helminth-infected mice treated with neutralizing antibody to IL-10 exhibited marked anaphylactic symptoms at challenge and made high levels of PN-specific IgE. ***d*** The PN-specific IL-13 response was partially restored in anti-IL-10-treated helminth-infected mice. Adapted from Bashir et al. [21], with permission, 2002, The American Association of Immunologists.

protection against allergy in this model. Mice were infected with helminth five days before the start of sensitization with PN plus CT. CD4+ T cells were harvested from the spleen and MLN of either infected or noninfected mice 2 days after sensitization with PN plus CT and transferred to naïve recipients. The noninfected recipients were then sensitized according to our standard protocol (3 doses of PN/CT) prior to sacrifice 28 days after the first sensitization. Transfer of CD4+ T cells from helminth-infected (but not noninfected) mice protected against the development of PN-specific IgE and allergic symptoms,

suggesting a role for immunoregulatory T cells in helminth-mediated protection against allergy in PN/CT sensitized mice [Bashir and Nagler-Anderson, unpubl. obs.].

How Does Helminth Infection Protect against Allergy: Immunoregulatory T or B Cells

Immunoregulatory cells have recently experienced a dramatic resurgence [26]. This has been fueled, in part, by the characterization of a subset of CD4+CD25+ regulatory T cells (Tregs). These 'natural' Tregs are largely thymus derived and play a central role in regulating self-reactive T cells and preventing autoimmune disease. The association of this subset with the transcription factor Foxp3 defined the CD4+CD25+ T cell subset as a distinct lineage and identified Foxp3 as the master control gene for the development and function of these cells [27–29]. Patients with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX) lack the human Foxp3 homolog and exhibit severe autoimmune disease as well as inflammatory bowel disease and allergy. Whether or not these natural Tregs can also be induced in the periphery, particularly in response to microbial infection, has been a matter of some controversy, exacerbated by the lack of reliable cell-surface marker. CD4+CD25+ T regs protect against intestinal inflammation in murine models of inflammatory bowel disease induced by the transfer of CD45RBhi T cells to immunodeficient recipients [30–32]. However, an IL-10-secreting, T regulatory 1 (Tr1) subset reactive against luminal bacteria has also been implicated in the control of intestinal inflammation [33–35]. Whether the IL-10-secreting Tregs identified in the gut-associated lymphoid tissue (GALT) in these models are necessarily derived from the CD4+CD25+ Foxp3 lineage is still unknown. It is also unclear if the CD25+ Tregs that arise in the intestines of immunodeficient mice also occur in immunocompetent individuals. However, it is clear that immunoregulatory T cells play an important role in preventing uncontrolled inflammatory responses in the intestinal mucosa and in maintaining tolerance to the commensal bacterial flora. Indeed, a more global role for Tregs in the control of anti-microbial reactivity has been proposed [36]. Sacks and colleagues have shown that naturally occurring CD4+CD25+ Tregs aid the survival of the protozoan parasite *Leishmania major* by promoting the early establishment of infection in Rag−/− mice [37]. IL-10 produced by the CD4+CD25+ Tregs is required for persistence of *L. major* infection in these immunodeficient hosts. Pathogen persistence leads to the maintenance of parasite reservoirs required for the development of an effective memory response. Natural Tregs therefore act to dampen the response to a level sufficient to

contain and control the infection (and minimize pathology) while still allowing the long-term persistence required for immunological memory. The immunosuppression induced by chronic helminth infection may involve a similar host-parasite compromise.

As mentioned above, we have preliminary data suggesting that regulatory CD4+ T cells can transfer helminth-mediated protection against allergy. Recent work from other laboratories has begun to characterize regulatory T and B cell populations in mice infected with a different helminthic parasite, *Schistosoma mansoni*. Hesse et al. [38] transferred wild type or IL-10−/− CD4+ T cells to RAG−/− or RAG−/−IL-10−/− mice in an attempt to identify both T cell and non-T cell sources of IL-10. They found that most of the T cell IL-10 was produced by the CD4+CD25+ natural Treg population; however both CD4+CD25− T cells and non-T cell populations also produced IL-10. Although both IL-10-secreting innate effectors and CD4+ T cells appear to cooperate to minimize host pathology, the presence of the IL-10-secreting natural Tregs was particularly important for host survival [38]. Subsequent work from McKee and Pearce identified two populations of immunoregulatory CD4+ T cells isolated from *S. mansoni*-infected wild-type mice [39]. CD4+CD25+Foxp3+ T cells secreted IL-10 and suppressed the proliferation of other CD4+ T cells, characteristics typical of natural Tregs. CD4+CD25− T cells also made IL-10, as well as the Th2 cytokines IL-5, IL-6 and IL-13 and lacked Foxp3 and suppressive activity. Both of these IL-10-secreting populations work together to suppress IL-12 production by DC and drive Th2 polarization in Schistosome-infected mice. Although activated T cells typically express CD25, in chronically infected mice Th2 cells were clearly identifiable by both their secretion of cytokines and their lack of expression of CD25. Neither of these studies have, however, examined a role for either natural or induced Tregs in protection against allergy. While data from our laboratory supports a role for T cell IL-10 in protection against allergy, other cellular sources of IL-10 may also be important. Indeed, recent work from Mangan et al. [40] has implicated IL-10-producing B cells in protection against the systemic anaphylaxis induced by i.p. sensitization with Pen-V. They reported that, in this model, protection against anaphylaxis is independent of CD4+CD25+ Treg. As in the two prior studies, IL-10-producing CD4+ T cells were detectable in Schistosome-infected mice; however, depletion of CD4+ T cells did not influence helminth-mediated protection against anaphylaxis. Although B cell depletion abrogated the ability of Schistosome infection to confer protection against allergy, B cell transfer failed to confer protection and instead exacerbated the anaphylactic response. The authors suggest that, like T cells, multiple subpopulations of B cells exist, only some of which exhibit immunoregulatory function [40].

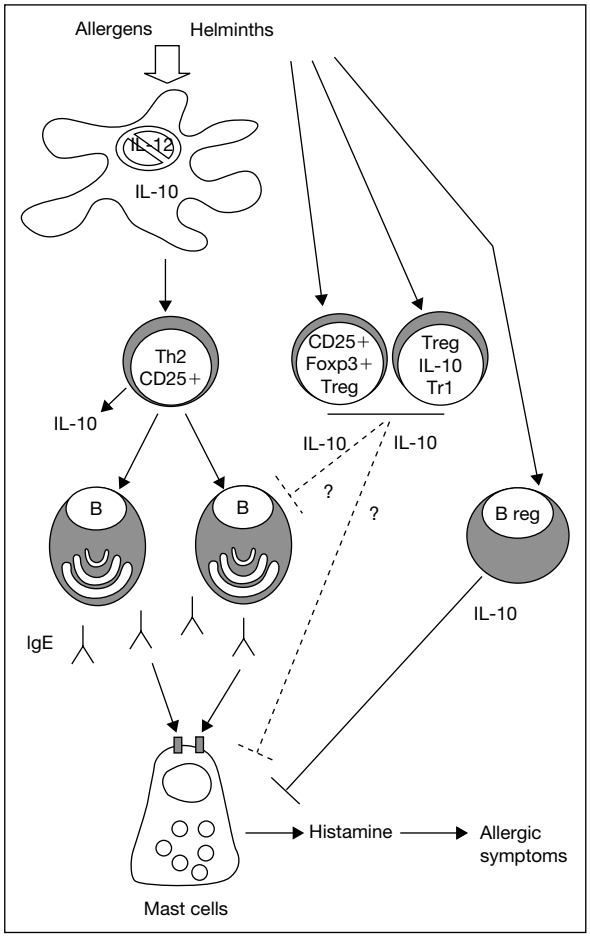

Fig. 3. A model for helminth mediated protection against allergic disease. Both allergens and helminths uniquely induce a polarized Th2 cytokine response and the production of IgE. To ensure their own survival and chronicity while minimizing pathology to the host, helminths also induce a variety of immunoregulatory cell populations. These include natural CD4+CD25+ Tregs, induced Tr1-like CD25− Tregs and regulatory B cells, all of which appear to act, at least in part, through the production of IL-10. In addition to being an important mediator for immunoregulatory cell function, IL-10 plays a critical role in driving the Th2 cytokine response. Other immunoregulatory mediators may also be involved. Helminth-induced immunoregulatory cells have been reported to protect against IgE-mediated anaphylaxis by inhibiting both the production of allergen-specific IgE and the symptoms induced by IgE-mediated receptor cross-linking on allergic effector cells such as mast cells.

Conclusions

Taken together, the available data suggests that both allergens and helminths induce a polarized Th2 cytokine response and the production of IgE. As illustrated in figure 3, helminth infection also induces immunoregulatory cell populations that contribute to the chronicity of infection while minimizing host pathology. These immunoregulatory cells also mediate protection against allergy. A better understanding of the cell types and mediators responsible for this protection may suggest mechanisms for the therapeutic use of helminth-derived products for protection against allergic disease.

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The Mutual Influence of Nematode Infection and Allergy

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Abstract

Several studies have now shown that the prevalence of helminth infections is negatively correlated with the prevalence and/or severity of allergic diseases. Here, we describe studies in rodents infected with *Strongyloides venezuelensis* examining the mutual influence of nematode infection and allergy. *S. venezuelensis* has a lung cycle, much akin to the human hookworm and Strongyloidiasis, and induces airway eosinophilia, local IgE and mucus production, and airway hyperreactivity. Both the Th2 and functional responses are relevant for the ability of rodents to deal with *S. venezuelensis* infection. Nevertheless, the parasite elicits the release of cytokines, such as IL-10, which are capable of regulating immune and functional manifestations. In infected animals, allergic inflammation prevents parasite migration and establishment. Nevertheless, the parasite is capable of regulating the allergic response, preventing part of the tissue damage and functional changes induced by allergy. Understanding the mechanisms by which helminths regulate inflammation may potentially lead to the development of strategies aimed at controlling unwanted inflammation in allergic and autoimmune diseases.

The Worldwide Relevance of Nematode Infections

Parasitic infections by gastrointestinal nematodes have always been highly prevalent in the human population and likely have been a very important element in the development of the immune system. In 1947, Stoll outlined the worldwide prevalence of helminth infections and estimated that 31% of North-Americans and 36% of Europeans were infected by one or more parasites. Recent studies estimate that at least a quarter of the world's population harbor one or more specie of gastrointestinal nematode. However, most infected

people now live in developing countries and the prevalence of the disease in the developed world is significantly lower [1].

The most prevalent nematode species infecting humans is *Ascaris lumbricoides*, with 1,472 million people infected, followed by 1,298 million individuals infected with hookworms and 1,049 million with *Trichuris trichiura* [1, 2]. Another important human nematode infection is *Strongyloides stercoralis* that afflicts 30–100 million people in 70 countries, mainly in the tropical and subtropical regions of the world [3].

Nematode Infections and the Hygiene Hypothesis

The global prevalence of allergic diseases such as asthma has increased markedly over the past few decades, especially in the most developed areas of the world [4, 5]. The overall increased frequency of asthma has been followed by an alarming increase in fatal and severe cases of the disease, especially in children. Although asthma has a genetic predisposition component, the fast increase of asthma incidence indicates that environmental factors would be responsible for the epidemic behavior of the disease. Low educational and social levels, changes in certain types of air pollution (particulates, for example) and indoor exposure to allergens have been associated with asthma development. Interestingly, asthma prevalence has risen especially in the developed world, where the overall air quality and socioeconomic level of the population has improved in recent years [4, 5]. This is in remarkable contrast with the prevalence of chronic infectious diseases worldwide, i.e. whereas nematode infections are greater in the developing world, allergic and autoimmune diseases have risen especially in the developed world.

Strachan [6] was among the first to propose that the overall changes in the health status of the developed word population would be associated with an increase in the incidence of allergic diseases, the so-called 'hygiene hypothesis'. The 'hygiene hypothesis' argued that the diminished incidence of childhood infections observed in industrialized countries over the past century impaired the development of Th1 responses, thus increasing the tendency to allergic diseases. There are several experimental studies indicating that an enhanced Th1 response is capable of counterbalancing the predominant Th2 response characteristic of allergic diseases [7, 8]. Support to the 'hygiene hypothesis' came from epidemiological studies showing an inverse correlation between antibody levels to orofecal microbes, such as hepatitis A and *Toxoplasma gondii*, and atopy. A similar correlation was also found between the rise in the incidence of allergies and the decline of cellular reactivity to mycobacteria in Japanese children [reviewed in 7]. However, the immunomodulator effect on

atopy was not reported to all Th1 inducing stimuli. Moreover, there has also been an increase in the incidence of auto-immune diseases, such as rheumatoid arthritis, which clearly do not fit into a Th1/Th2 paradigm [9].

It is of note that helminth infections can impart on the prevalence and/or severity of allergic diseases [7, 8, 10–12]. However, akin to allergic diseases, nematode infections typically induce elevated serum levels of immunoglobulin G1 (IgG1) and IgE in mice (IgG4 and IgE in humans), eosinophilia, intestinal mastocytosis and goblet cell hyperplasia [13]. These responses are controlled mainly by the cytokines interleukin-4 (IL-4), IL-13, IL-5, IL-3 and IL-10, which are indicative of the T helper-2 type (Th2) cell activation. The Th2-type of immune response induced by nematodes has been associated with host protection in most experimental models [13–15], as well as in some studies of human nematode infection, including Strongyloidiasis [16]. Again, the ability of a Th2-predominant disease (helminth infection) to modify another Th2-predominant disease (allergic diseases such as asthma) clearly does not fit into the Th1/Th2 counter-regulatory mechanism.

The protective effect of helminth infection on allergic reactivity has frequently been justified by a possible blocking effect of helminth-induced polyclonal IgE production on mast-cell degranulation [10], i.e. non-specific IgE saturates FcεRI on mast cells, blocking the binding of allergen-specific IgE, inhibiting the cross-link of bound IgE by the allergens, and, consequently, mast cell degranulation and immediate hypersensitivity response to allergens. Recently, a detailed statistical analysis in chronically *Schistosoma haematobium*-infected patients [12] did not confirm the relevance of polyclonal IgE blocking mechanism in the modulation of allergic reaction. Furthermore, it was demonstrated [17] that the number of FcεRI expressed on mast cells or basophils is regulated by circulating IgE levels implying that saturation of binding is not easy. Therefore, polyclonal IgE blocking mechanism as well as the balance between Th1/Th2 immune responses would not explain completely the modulation of allergic reactions in the chronically helminth-infected population. More recently, the presence of immunomodulatory cytokines, especially IL-10, has been associated with a lower prevalence of allergic diseases in groups of individuals with chronic parasite infection. For example, the production of IL-10 by peripheral blood mononuclear cells upon challenge with schistosome antigen clearly correlated with a lowered risk of developing skin reactivity to mite antigens [12]. Similarly, *Schistosoma mansoni*-infected individuals with no allergic manifestations, as assessed by skin prick test, produced greater levels of IL-10 upon stimulation with the relevant allergen. Interestingly, the enhanced IL-10 production was diminished after helminth-specific treatment, demonstrating that parasite infection induced lower levels of Th2 cytokines via the production of IL-10 [18]. These exciting results suggest that chronic parasite

infections affect allergy by inducing regulatory mechanisms, the detailed range and nature of which still needs to be determined.

Although several studies have now shown that a high prevalence of helminth infection in humans may have a protective effect on allergic reactions [10–12, 18] other studies have provided contradictory results. Indeed, some studies have shown that parasitic infections may predispose the host to allergic reactions [19]. It is clear that the association between atopy and helminth infection is more complex than just a matter of Th1/Th2 balance and may be influenced by many factors, including the intensity and continuity of the infection, the infection site and species of parasites [20].

In the most prevalent gastrointestinal nematodes – *A. lumbricoides, Necator americanus, Ancylostoma duodenalis and S. stercoralis* – parasite larva have a lung migration phase and the adult worms establish in the host small intestine. In asthma, perhaps the most important form of allergic inflammation in humans, eosinophilic inflammation of the lung and airway hyperreactivity are characteristic features. Keeping in mind the great incidence and relevance of both nematode infection and allergic airway inflammation to human health, we set out to develop a model of nematode infection to understand the complex association between these two conditions. Particularly, we were interested to understand the mechanisms by which nematode infections modulate airway inflammation and dysfunction in models of allergic asthma. And conversely, our studies try to understand the possible role of allergic airway inflammation and its functional consequences (i.e. airway hyperreactivity) to the ability of a host to deal with parasite infections.

The Model of *S. venezuelensis* Infection in Rodents

S. venezuelensis is a nematode parasite that was isolated from naturally infected wild rats and is easily maintained in laboratory rodents, such as rats and mice. In experimental infection, *S. venezuelensis* larvae have an obligatory migration through the host lungs before establishment into the duodenal mucosa, and the adult worms are eliminated spontaneously from the host after 5 weeks in rats or 2 weeks in mice. Larvae migration through the lungs and worm elimination from intestine induced local eosinophilic inflammation, intestinal mastocytosis and an increase in mucus production by goblet cells as well as increased levels of IgE. The fact that this parasite occurs naturally in rats may provide a more adequate setting to study the host/parasite relationship.

Initial studies evaluated the kinetics of *S. venezuelensis* infection in rats and mice [21, 22]. Most *S. venezuelensis* larvae migrated through the rat lung around 48 h after a subcutaneous infection, and a small number of larvae was still recovered 5 and 7 days postinfection (dpi). In mice, although the maximum

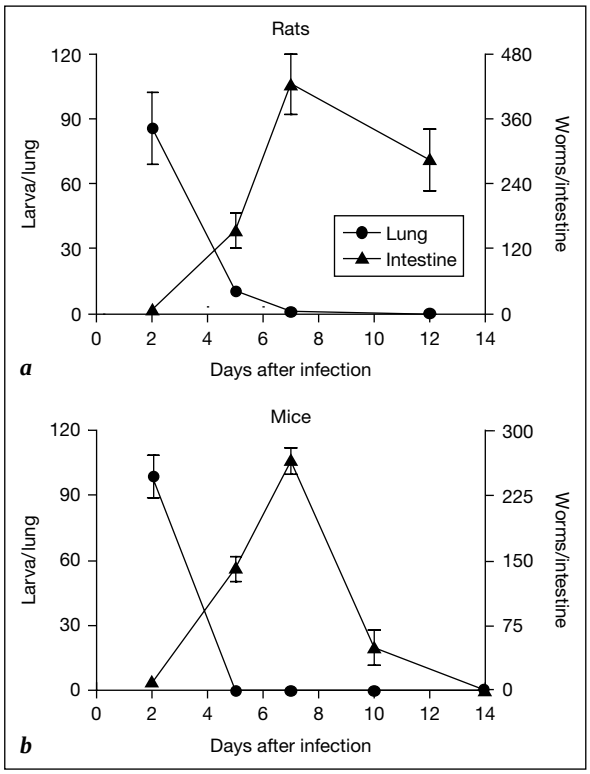

Fig. 1. Kinetics of *S. venezuelensis* infection in (***a***) rats and (***b***) mice. Wistar rats were infected with 1,500 L3 larva and BALB/c mice with 700 L3 larva subcutaneously. At the indicated days, the number of parasites in the lung or intestine was evaluated.

number of migrating larvae in the lungs was also observed at 2 dpi, there were no live larvae in the lungs after 5 dpi. The first few worms were recovered from the small intestine at 2 dpi, reaching maximum around 5–7 dpi in infected rats or mice. Over 50% of adult worms had been eliminated by 10 dpi and no worms could be recovered after 14 dpi in mice. In contrast, the number of adult worms in the small intestine of rats started to decline only at 12 dpi and a few worms were still present after 30 dpi (fig. 1).

S. venezuelensis Infection Induces Eosinophilic Airway Inflammation and Airway Hyperreactivity

Larvae migration induced leukocyte infiltration in the lung tissue and in the bronchoalveolar lavage (BAL) fluid which peaked between 2 and 7 dpi and

disappeared at 12 dpi [21]. During a primary infection, the cells recovered from BAL fluid were mainly mononuclear, but there was an increase in eosinophil numbers at 5 dpi. Eosinophil infiltration was more intense after multiple infections of rats. Fluorescence-activated cell sorter analysis of leukocytes showed that the majority of T lymphocytes present in BAL fluid of 5-day-infected rats were CD4+ T lymphocytes that did not express the CD45RC antigen, suggesting a Th2 profile. At 5 dpi, there were also increased levels of IgE in BAL fluid and an increased concentration of IL-1β and TNF-α in lung tissue homogenates that decreased to basal levels after 12 days. Interestingly, the pulmonary concentration of IL-10 was greater during larvae migration (2 dpi), returning to basal levels at 5 dpi [21].

Histopathological analysis of lung tissue during parasite migration (2 dpi) revealed foci of severe hemorrhage, pulmonary edema and destruction of alveolar wall with mononuclear infiltration at the pulmonary parenchyma. At 5 dpi, hemorrhage was diminished; the inflammation was stronger and with greater number of eosinophils, but the reaction was still focal. The bronchial epithelial and muscle layer became thicker and showed disruption and shedding of epithelial cells, and an increase in the number of goblet cells and mucus production [21]. In multiple-infected rats, the eosinophilic inflammation was especially intense around the bronchial tree and the thickening of the epithelial layer and mucus production was more evident [21]. Interestingly, a similar peribronchial eosinophilic inflammation with thickening of the bronchial epithelial layer and shedding of epithelial cells are characteristics of the pathological changes observed in lungs of asthmatic patients [23].

To determine whether lung inflammation induced by *S. venezuelensis* infection was associated with changes in lung function, the variation of intratracheal pressure in response to increasing doses of acetylcholine was evaluated. The analysis revealed that the lung of infected rats showed airway hyperresponsiveness (AHR) at 5 and 7 days after single or multiple infection and that the AHR had returned to baseline after 12 days [21]. These results were first to show that AHR can indeed occur after infection of a natural host (rat) with one of its natural helminth parasites (*S. venezuelensis*). In our model, the AHR was transient, even in animals constantly exposed to the parasite, and coincident with the peak of lung eosinophilic inflammation, mucus and pro-inflammatory cytokine production and local IgE increase. Airway hyperresponsiveness has also been described in *Strongyloides stercoralis, Toxocara canis, Nippostrongylus brasiliensis* and *Brugia malayi* infected mice. Akin to our work, the latter studies also demonstrate that AHR may occur during parasite migration, although most of the experimental models did not use the natural host of the parasite. In contrast, other studies in the literature that examine AHR in the setting of parasitic infection use parasite antigens, such as *Schistosoma mansoni* egg antigen

or *Ascaris lumbricoides* extract, to immunize and challenge and do not examine AHR during the usual parasite life cycle.

Airway Hyperreactivity Is Shut Off by *S. venezuelensis* Infection but Prevents Parasite Infection

Eosinophilic peribronchial inflammation, excess mucus production, local IgE and bronchial hyperresponsiveness, as induced by *S. venezuelensis* infection, are characteristic changes of the lungs of asthmatic patients [23]. It is debatable whether these pathological, immunological and functional changes seen in the lungs of asthmatic patients could be relevant mechanisms of protection against nematode parasites. To evaluate the possible mutual influence of nematode infection and allergy, the combined effects of experimental allergic airway inflammation, induced by intradermal implantation of heat-coagulated egg white followed by challenge with aerosolized ovalbumin (OVA), and infection with *S. venezuelensis* were evaluated in the rat [24]. The lungs of rats submitted to the experimental allergy protocol showed eosinophilic inflammation that peaked 48 h after the challenge and was accompanied by AHR to an intravenous acetylcholine challenge. In the combined protocol (allergy plus nematode infection), the *S. venezuelensis* infection was given at the time of OVA challenge of immunized rats, therefore the parasite larvae were migrating through the lungs during the peak of OVA-induced eosinophilic inflammation. The combination of allergy with nematode infection resulted in prolonged pulmonary inflammation with increased eosinophil infiltration in bronchoalveolar lavage fluid but not in the lung tissue. In contrast to the increased infiltration of cells, there was a significant reduction of AHR observed 48 h after OVA challenge of the sensitized rats (fig. 2a). Thus, it is clear that during their passage through the lungs, the parasite and/or products released in response to the parasite suppress AHR. The detailed mechanisms by which the parasite switched off AHR are being investigated in our laboratories at present. Nevertheless, the shown ability of IL-10 to suppress allergic airway inflammation and airway hyperreactivity [25] and the increased production of IL-10 during *S. venezuelensis* migration through the lungs suggests that this cytokine may be one of the mechanisms by which the parasite regulates AHR in rats.

In addition to suppressing AHR, the combination of allergy with nematode infection also resulted in a significant reduction in parasite burden, especially during the phase of parasite migration through the lungs (fig. 2b). Eosinophils may play a role in the process of destruction of migrating *Strongyloides spp.* larvae [26]. More recently, El-Marky et al. [27] demonstrated that in IL-5 transgenic mice most of the subcutaneously inoculated infective larvae of

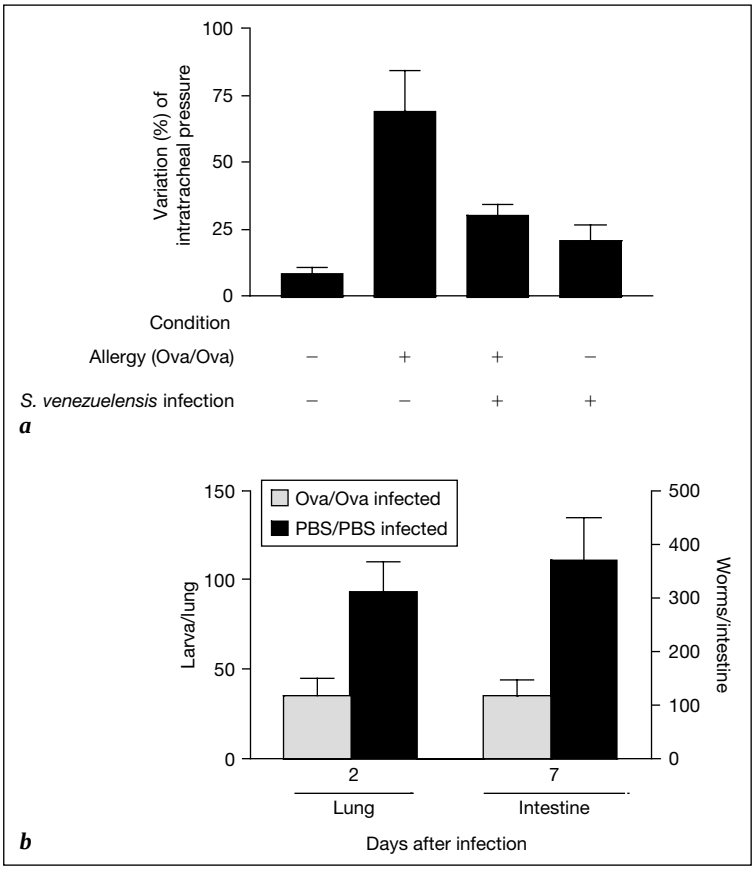

Fig. 2. Effects of *S. venezuelensis* infection on allergen-induced airway hyperreactivity (AHR) (***a***) and, conversely, allergic inflammation on *S. venezuelensis* infection (***b***). In (***a***), notice the clear inhibition by the parasite infection of allergen-induced AHR in sensitized rats. AHR was evaluated 48 h after allergen challenge (ovalbumin – Ova) and parasite infection of sensitized mice. The percent variation of basal intratracheal pressure induced by the injection of acethylcholine (300 μg/kg). In (***b***), notice that allergic airway inflammation and function changes diminish the number of larva retrieved from the lungs and worms from the intestine.

S. venezuelensis were killed during migration, and only a few worms could reach the small intestine. As there was increased eosinophil numbers in BAL fluid after allergen challenge of the immunized and infected mice, it is possible that eosinophils could be one of the effectors responsible for the reduction in the number of *S. venezuelensis* larvae in the allergic rats. Confirmation of the

latter possibility awaits the development of specific tools to block the eosinophil lineage in rats.

More recently, we investigated whether the functional pulmonary response played any role in the ability of the host to deal with *S. venezuelensis* infection. To this end, animals were bronchodilated using a β_2-adrenoceptor agonist during the phase of migration of the parasite through the lungs (24–72 h after infection). Under this experimental condition, the number of worms that reached the intestine was larger in bronchodilated animals [Marcantonio, Negrão-Corrêa and Teixeira, in preparation]. Overall, the latter results suggest that the functional airway response may be part of the ability of the host to deal with nematode infections with a lung cycle. Moreover, the ability of allergic airway inflammation to induce AHR (even if reduced by the parasite) may represent a mechanism to prevent parasite arrival in the intestine. Finally, the ability of *S. venezuelensis* infection to induce molecules which prevent AHR, such as IL-10, may represent a mechanism to evade the enhanced functional airway response to the infection. Of note, there is much IL-10 production during parasite passage through the lungs, which coincides with no AHR. After parasite migration, IL-10 levels drop and AHR is then observed [21].

Role of a Th2 Immune Response for *S. venezuelensis* Elimination from the Intestine

After migration through the lung, *S. venezuelensis* larvae reach the pharynx, are swallowed and penetrated into the small intestine mucosae where they complete their development. Experimental models of nematode infections in mice demonstrate that parasite elimination from the intestine is dependent on CD4+ T cells. Further studies using long-lasting IL-4 inoculations in nematode-infected mice or IL-4-deficient mice have shown that IL-4-dependent mechanisms are essential for the elimination of *Trichuris muris* and *Heligmosomoides polygyrus* infection [13, 14]. However, IL-4 was not necessary for the development of protective immunity against *Nippostrongylus brasiliensis* [13, 14]. More recently, through the use of IL-4 receptor α chain (IL-4Rα) and signal transducer and activator of transcription 6 (STAT6) deficient mice, it became clear that a Th-2 type of immune response was essential for nematode elimination [28]. However, the Th2-related effector mechanisms that mediate worm elimination appear to be very different in each nematode species, possibly reflecting the niche which the parasite inhabits as well as evasive mechanisms employed by each nematode species [13–15, 28, 29]. For example, experimental infection using genetically deficient mice demonstrated that IL-4, but not IL-13, is required for the elimination of *Heligmosomoides*

polygyrus [13], a nematode species that inhabits the small intestinal mucosa. In contrast, IL-13, but not IL-4, is critical for the elimination of *Nippostrongylus brasiliensis* [14, 28], a nematode that lives in the luminal space of the small intestine. Interestingly, both cytokines, IL-13 and IL-4, are required for *Trichuris muris* elimination from the cecum epithelial layer [14] and *Trichinella spiralis* from the small intestine epithelium [28]. An interesting possibility that has not been tested in any detail is that not only the effector mechanisms to eliminate parasites may be different, but the regulatory mechanisms elicited by each infection may also be distinct.

In rodents [21, 22] as well as in humans [16], *Strongyloides* infection induces an immune response which is predominantly Th2 in nature. However, the importance of this Th2 response for the elimination of *S. venezuelensis* from the intestine has not been directly addressed in much detail [14]. Watanabe et al. [30] reported that IL-4-deficient mice infected with *S. ratti* had kinetics of egg elimination very similar to the nondeficient mice, suggesting that IL-4 was not essential for worm elimination. Indirect evidence supporting a role for a Th2 immune response derives from studies demonstrating a role for intestinal mastocytosis in the process of worm elimination [31–34].

More recently we have used gene-deficient mice to examine the role of Th1 and Th2 cytokines during *S. venezuelensis* infection. IL-12-deficient mice, which are unable to produce IFN-γ and, consequently, unable to induce a Th-1 immune response, were capable of eliminating adult worms with very similar kinetics to those of nondeficient mice. The picture was very distinct in IL-4Rα- or STAT6-deficient mice. In the latter animals, there was a great delay in parasite elimination and results showed that large number of *S. venezuelensis* adult worms persisted in the intestine at 14 days after infection [Negrão-Corrêa et al., in preparation]. In wild-type mice, all parasites had been eliminated by day 14 (fig. 1). The latter results are the first direct evidence that the mechanism responsible for the intestinal elimination of *S. venezuelensis* is dependent on IL-4R and STAT6 activation, as previously observed in other GI nematode infections [14, 28]. Moreover, our results also indicated that, similarly to the results reported in *N. brasiliensis* infected mice [28], *S. venezuelensis* adult worm elimination from mice intestine is only dependent on the expression of IL-4R on non-bone marrow derived cells [Negrão-Corrêa et al., in preparation]. In contrast, *T. spiralis*-infected mice depend on IL-4R expression on bone marrow and non-bone marrow derived cells to be able to eliminate intestinal worms from the intestine [28]. This is a further demonstration that although nematode parasites induced a stereotypic Th2 immune response, the effector mechanisms that lead to worm elimination may differ.

The mechanisms responsible for *S. venezuelensis* elimination after activation of the IL-4R/STAT6 pathway are still mostly undefined and under

investigation in our laboratory. However, there is strong experimental evidence suggesting the participation of mast cells and goblet cells in parasite expulsion. The importance of the intestinal mastocytosis in the process of *Strongyloides* spp. elimination was initially reported in W/W^V mice, a mast cell-deficient strain, that were more susceptible to *S. ratti* and eliminated the intestinal worms slower than control mice. A similar effect was also observed in W/W^V mice infected with *S. venezuelensis* [31]. Furthermore, nude mice that are unable to expel *S. ratti* infection restored the intestinal mastocytosis and eliminated most of the parasite after repeated treatment with IL-3. The same treatment did not restore the ability of nude mice to eliminate *N. brasiliensis* infection, even though the IL-3 treatment resulted in intestinal mastocytosis and elimination of concomitant *S. ratti* infection [34]. More recently, the participation of intestinal mastocytosis in the elimination of *S. venezuelensis* was confirmed in IL-3-deficient mice [32] and in mice that lack the p85alpha regulatory subunit of phosphatidylinositol-3 kinase (PI3K), which were deficient in gastrointestinal and peritoneal mast cells and were highly susceptible to *S. venezuelensis* infection [33].

The above studies suggest that mast cells are important for worm expulsion but do not show which mechanisms triggered by mast cells are important. Studies in mice with a deletion of the Fc receptor γ chain and that failed to assemble the high-affinity Fc receptor for IgE (FcεRI) demonstrated that parasites were not efficiently eliminated [35]. It was suggested that mast cell degranulation and the release of sulphated proteoglycans which was not observed in FcεRI-deficient animals was essential to intestinal worm elimination.

S. venezuelensis secretes heparin-binding proteins that are highly adherent and have binding activities to host cells. These adherent molecules were produced by esophageal glands of the parasitic female, were continuously secreted in vivo and adhered to the surface of the intestinal epithelial cells, fixing the worm and allowing invasion. Sulphated proteoglycans released from mast cells [35] or sulphated mucins from goblet cells [36] inhibited the binding of *S. venezuelensis* adhesion substances to intestinal epithelial cells in vitro, and inhibited the invasion of worms into the intestinal mucosa in vivo. Therefore, inhibition of worm binding by some sulphated glycans derived from activated mast cells or goblet cells could be a relevant effector mechanism involved in *S. venezuelensis* elimination from mice gut. It is thus possible that IL-4R/STAT6 activation may induce parasite attrition via the induction of the release of sulphated glycans by mast and/or goblet cells.

More recently, we have examined the ability of an allergic response in the gut (i.e. food allergy) to modify the infection with *S. venezuelensis* in mice. Food allergy was induced by the administration to mice of the allergen (OVA) in

drinking water [37]. The food allergy protocol resulted in the production of anti-OVA IgG1 and IgE, intestinal eosinophilia, mastocytosis and increase in mucus production [37]. This was accompanied by increased vascular permeability of the proximal segments of the small intestine 6 h after oral ingestion of egg white solution and in significant body weight loss throughout the ingestion period [37]. OVA-sensitized and -fed mice that were infected with *S. venezuelensis* had a reduction of over 70% in the number of adult worms in the small intestine 5 and 7 days after infection. Interestingly, the expulsion of parasites was slightly delayed in allergic mice [Silva, Cara and Negrão-Corrêa, unpubl. data]. In its turn, the parasitic infection resulted in heightened anti-OVA IgE, greater mucus production in the intestine and greater weight loss. Nevertheless, there was no overt change in intestinal pathology. The latter results suggest that helminth infection and allergic inflammation have mutual influences not only in the lung but also in the intestine (fig. 3).

Concluding Remarks

Helminth infections are among the most common infections in humans. Although they are rarely the cause of death – though *S. stercoralis* infection may cause complications and death in immunocompromized hosts – helminths represent an enormous infectious burden and a significant source of acute and chronic antigenic stimulation. The usual response to helminth infection is characterized mainly by the activation of lymphocytes with a Th2 predominant phenotype and their ensuing effector arms, including IgE production, mastocytosis, eosinophilia and exacerbated mucus production. This Th2 response is important for the host to limit parasite growth and, possibly, egg production. In addition, it appears that the host is also capable of mounting a functional response, i.e. airway contraction and AHR, that may be relevant for the host to deal with the parasite. In its turn, the helminths induce regulatory mechanisms, such as the enhanced production of IL-10, which may counteract not only the Th2, but also the airway functional response to infection. Thus, the immunomodulatory mechanisms elicited by helminths may not only allow the parasite to evade the host protective immune and functional response and survive for longer time but may also prevent the excessive tissue damage and dysfunction produced by the inflammatory reaction elicited by the parasite. The latter effects clearly favor the host/parasite interaction and a better knowledge of such mechanisms could potentially lead to the development of strategies to control unwanted inflammation in allergic and autoimmune diseases.

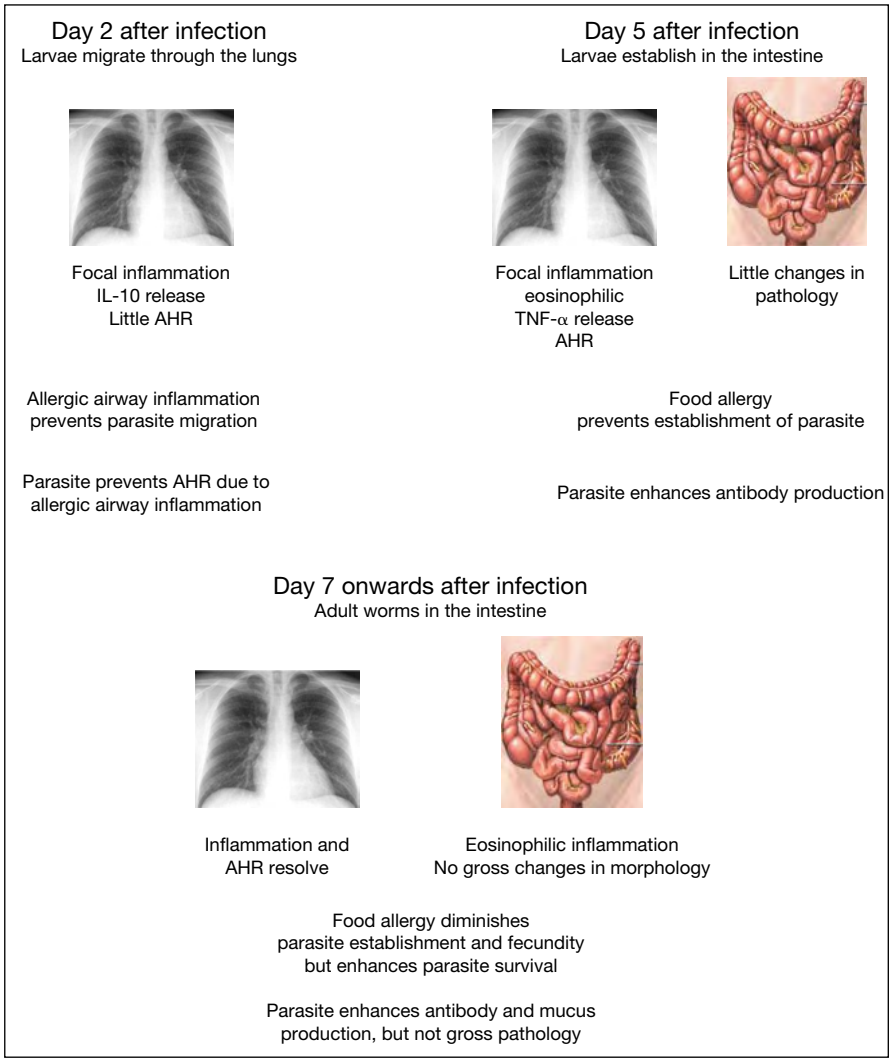

Fig. 3. The mutual influence of allergic inflammation and *S. venezuelensis* infection.

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Human Schistosomiasis Decreases Immune Responses to Allergens and Clinical Manifestations of Asthma

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Abstract

Studies have demonstrated that people living in areas endemic for helminths have a decreased reactivity to skin prick tests to aeroallergens and milder forms of asthma. Hypotheses to explain the inverse correlation between helminth infections and atopy include competition between helminth-induced polyclonal IgE and aeroallergen-specific IgE for high-affinity receptors present on mast cells, increased number of regulatory T cells, and high levels of regulatory cytokines, such as IL-10, produced during helminthic infections. Indeed, cells from asthmatic individuals infected with *Schistosoma mansoni* produce lower levels of IL-5 than asthmatics free of infections. In contrast, IL-10 is more readily produced by allergen-stimulated cells from asthmatics who are infected and is detected only at low levels by cells from helminth-free asthmatics. It is well known that Th2 cytokines are involved in the pathogenesis of allergies and asthma, and some studies indicate that IL-10 is the key cytokine that inhibits the Th2-inflammatory response in allergy. In this chapter we will discuss the association between *S. mansoni* infection, atopy and severity of asthma and possible mechanisms by which individuals living in helminth endemic areas are protected against the development of allergies.

Immunological Response and Clinical Forms of Schistosomiasis

Schistosoma mansoni infection occurs after exposure to contaminated water. Cercariae released by the snail penetrate human skin, lose their tail and change into schistosomula. The schistosomula migrate to the lungs and, in 6 weeks, mature to adult worms and descend to their final habitat, the mesenteric

veins. In this site the female and male adult worms begin sexual reproduction and they release eggs. Once deposited in the host, eggs may remain in the mesenteric veins, be trapped in the intestine, escape to the intestinal lumen or migrate through the portal vein to the liver. Viable eggs in stool can be documented 5–9 weeks after exposure. The immunological response against parasite antigen in schistosomiasis is closely related to pathology. Acute schistosomiasis, a form that occurs 20–60 days after cercariae penetration, is characterized by a strong inflammatory response with very high levels of cytokines such as TNF-α, IL-1 and IL-6. During this phase of the infection adaptive immunity is characterized by a predominant type 1 immune response with high levels of IFN-γ and decreased levels of IL-4 and IL-5 [1]. High levels of pro-inflammatory cytokines have been implicated in the toxemic clinical picture observed during this phase of the disease characterized by fever, weight loss, malaise, abdominal pain and diarrhea. However, this clinical manifestation of the disease is rare because acute schistosomiasis does not occur in individuals living in endemic areas. Severe disease and even death may occur in this phase of the disease. As eggs begin to be eliminated in the stool, clinical recovery may happen even without specific therapy. The strong inflammatory response begins to decrease after patients become asymptomatic. Evolution to the chronic phase is followed by a switching in the adaptive immune response that is now characterized by a predominant type 2 immune response, with high levels of IL-4, IL-5, IL-10 and IL-13 and decreasing levels of IFN-γ [2, 3]. This type 2 immune response is also evident in high levels of total and parasite specific IgE observed during chronic schistosomiasis. The decrease in IFN-γ levels observed in this phase of the disease are in part mediated by IL-10. Although PBMCs from chronically infected individuals express mRNA for IL-4 and produce high levels of this cytokine in vitro, the downregulation of the type 1 immune response does not appear to be modulated by IL-4, since neutralization of this cytokine does not enhance in vitro IFN-γ production in these patients [2]. In contrast, addition of anti-IL-10 monoclonal antibody to cultures stimulated with *S. mansoni* antigen, significantly enhances the levels of IFN-γ. The down-modulation of the immune response observed in patients with chronic schistosomiasis is also documented in newborns from mothers infected with *S. mansoni*, indicating not only that the antigens from *S. mansoni* may sensitize fetal lymphocytes, but also that the cytokine environment (predominant Th2) may influence the immune response observed in newborns from a mother with *S. mansoni* infection. These phenomena also explain the absence of acute schistosomiasis in individuals (including newborns) living in areas that are endemic for *S. mansoni*.

While the majority of the individuals with chronic schistosomiasis will have a mild disease characterized by episodes of diarrhea and constipation,

about 5% of these patients will develop a picture of portal hypertension with increase in the size of the liver and spleen. The pathology of the hepatosplenic form of the disease is predominantly caused by the host immune response to parasite eggs that are laid in the portal venous system, and become trapped in hepatic sinusoids [4]. The granulomatous formation around the eggs leads to liver fibrosis and portal hypertension.

The formation of granulomas around schistosoma eggs is mediated by T cells and, more recently evidence has accumulated showing that cytokines such as IL-4, TGF-β and IL-13 contribute to granuloma formation [4, 5]. A protective role of IFN-γ in liver fibrosis has been shown [6]. In addition to low IFN-γ, low levels of IL-10 and high TNF-a production have been associated with liver fibrosis [6]. More recently, evaluating patients without hepatosplenomegaly but with various degrees of liver fibrosis classified by ultrasonography, revealed that increased IL-5 and IL-13 production by T cells was associated with degree III hepatic fibrosis [7]. Moreover, these cytokines also were increased significantly in patients who exhibited progressive hepatic fibrosis after 1 year without therapy. These human observations are supported by studies in mice that have pointed out an important role of IL-13 in the development of liver fibrosis [8]. Although IL-4 and IL-13 share the same receptor and many biological activities, there are functional differences between these two cytokines. While granuloma formation was partially reduced in IL-4-deficient mice, blocking the IL-13 and IL-4 receptors in these animals almost completely abrogated granuloma development and liver fibrosis [8].

IL-10 is able to decrease granuloma formation in schistosomiasis and it is produced in low levels in patients with hepatosplenomegaly. The diminished pathology seen with IL-10 is probably related to the ability of IL-10 to down-regulate macrophage activation, decrease class I and class II MHC expression and reduce activation of Th1 and Th2 cells. The immunomodulatory effects of IL-10 will be discussed in detail later in this chapter.

Down-Modulation of Type 1 Immune Response in *S. mansoni* Infection

There is evidence both in vitro and in vivo that *S. mansoni* infection down-regulates the production of type 1 cytokines and also decreases cytotoxic responses. To evaluate the ability of *S. mansoni* infection to modulate the immune response to an unrelated antigen, patients infected with *S. mansoni* and noninfected controls without evidence of exposure to tetanus antigen (absence of antibodies to this antigen) were immunized with tetanus toxoid and the in vitro production of IL-4 and IL-5 was evaluated in tetanus toxoid-stimulated

PBMCs [9]. While uninfected controls produced both IFN-γ and IL-4, patients with *S. mansoni* infection produced high levels of IL-4 and low levels of or no IFN-γ. There was an inverse correlation between parasite load and IFN-γ levels. Moreover, patients with a low degree of infection had mRNA for IFN-γ and produced small amounts of this cytokine, while patients with a high parasite load had no mRNA for IFN-γ.

Evidence that *S. mansoni* decreases cytotoxic T cell and humoral immune responses comes from studies in BALB/c mice infected with vaccinia virus expressing the GP160 protein of the human immunodeficiency virus (HIV) [10]. In contrast to animals infected only with the vaccinia virus, which produced type 1 cytokines, animals co-infected with *S. mansoni* secreted minimal amounts of IL-2 and IFN-γ. Moreover, CD8+ T cells of these animals had little or no cytotoxic activity against fibroblasts transfected with the GP-160 of HIV. As a consequence of the decreased anti-viral cytotoxic response, viral elimination was reduced in these animals. In humans, a similar down-modulation of the type 1 immune response has been observed in patients co-infected with *S. mansoni* and hepatitis C virus (HCV). While 73% of patients infected with HCV alone demonstrate a lymphocyte proliferative response to viral antigens, lymphocyte proliferation was only observed in 8.6% of patients co-infected with HCV and *S. mansoni* [11]. It is known that the appearance of cytotoxic T cells against viral antigens is associated with the elimination of the virus in the acute phase of HCV infection [11]. Consequently, co-infection with *S. mansoni* changes the clinical course of HCV in humans. While individuals only infected with HCV had a decrease in viremia following the acute phase of the disease, high viral load was observed in patients co-infected with HCV and *S. mansoni*. Virus maintenance is related to development of chronic hepatitis C. While 5 of 15 (33%) patients with HCV alone developed chronic hepatitis, all 17 patients who had HCV and schistosomiasis developed chronic hepatitis [11].

Other evidence that *S. mansoni* can change the immune response during viral infection comes from studies in patients co-infected with human T cell lymphocytotropic virus (HTLV-1) and *S. mansoni*. HTLV-1 is a retrovirus that predominantly infects CD4+ T cells. In these cells, the regulatory gene tax of the HTLV-1 transactivates several genes related to the immune response such as IL-1, IL-2, IL-2 receptor, GM-CSF and IL-15 genes. As a consequence, spontaneous lymphocyte proliferation and production of high levels of type 1 cytokines are observed in individuals infected with HTLV-1. Cytotoxic CD8+ T cells specific to tax protein are generated but are not able to clear the virus. While the majority of individuals infected with HTLV-1 are asymptomatic, it is estimated that a small proportion of infected individuals will develop HTLV-1-associated myelopathy or tropical spastic paraparesis (HAM/TSP) or the adult T cell leukemia/lymphoma (ATLL). In comparison with HTLV-1 carriers, patients with

HAM/TSP produce significantly more IFN-γ and TNF-α and have an increased frequency of cells secreting these cytokines. The activation of both CD4+ and CD8+ T cells with production of pro-inflammatory cytokines plays an important role in the pathogenesis of HAM/TSP. In these patients, HTLV-1 proviral load is high and macrophages and T cells secrete chemokines and express adhesion molecules which contribute to both CD4+ and CD8+ T cells crossing the blood-brain barrier. The production of cytokines by these activated T cells leads to tissue damage in the spinal cord and the development of myelopathy.

In areas where HTLV-1 infection and schistosomiasis are endemic, such as Brazil and Africa, the impact of the association of these infections in the immunological response and in the clinical expression of the diseases has been evaluated. It has been shown that HTLV-1 can modify the immunological response to *S. mansoni* antigens by decreasing IL-4, IL-5 and IL-13 production and decreasing IgE levels [12]. However, pathology in patients co-infected was attenuated [12]. Co-infected patients do not develop the hepatosplenic form of schistosomiasis and only 5% of them had liver enlargement.

One important aspect of co-infection of HTLV-1 with *S. mansoni* is the ability of schistosomiasis to modify the immune response observed in HTLV-1 and the documented inverse association between co-infection with *S. mansoni* and development of HAM/TSP. While helminthic infection was observed in 27% of individuals that are HTLV-1 carriers, the prevalence of *S. mansoni* in patients with myelopathy associated to HTLV-1 was 1%. This difference could not be attributed to decreased exposure of patients with myelopathy to contaminated water. In fact, all individuals had lived in the area endemic for *S. mansoni* for a long period of time and all had denied exposure to contaminated water as adults. One possible explanation is that *S. mansoni*, by down-modulating the type 1 immune response observed in HTLV-1 and by decreasing proviral load, reduces the neurological disease associated with T cell activation and secretion of pro-inflammatory cytokines.

Regarding the immune response, spontaneous IFN-γ production by PBMC in vitro was significantly lower in patients co-infected in comparison to those who only had HTLV-1. Moreover, in patients who were dually infected, there were a lower number of $CD4^+$ T cells expressing IFN-γ and a higher number of T cells expressing IL-10 [12]. Part of the down modulation of IFN-γ production observed in patients co-infected with HTLV-1 and *S. mansoni* was decreased by neutralization of IL-10. Therefore, the modulatory action caused by schistosomiasis in the immune response in patients infected with HTLV-1 is at least partially related to schistomiasis-induced IL-10.

Development of HAM/TSP and ATLL in patients infected with HTLV-1 is associated with dysregulation of the immune response and high proviral load. As helminth infection decreases cytotoxic activity against cells expressing viral

antigens [10], it could be expected that down-modulation of the immune response in patients dually infected with *S. mansoni* and HTLV-1 could cause an increase in the proviral load. However, co-infection with *S. mansoni* reduces the proviral load suggesting that helminths may inhibit HTLV-1 transcription. As the spread of the virus is accelerated by the activation of T cells [13], it is possible that low proviral load in patients co-infected with HTLV-1 and helminths may be due to the down-regulation of the immune system observed in these patients.

Of interest are recent studies looking at the influence of helminth infections on the development of auto-immune diseases. The inflammatory response, critical in the pathology of autoimmune disease, can be regulated by the immune response to helminths. *S. mansoni* infection, for instance, has been implicated in protection against autoimmune diseases. While the pathogenesis of auto-immune diseases mediated by T cells involves the Type I immune response with production of IFN-γ and TNF-α, chronic *S. mansoni* infection induces a strong type 2 immune response with increased production of IL-10.

The down-regulation of the type 1 response by *S. mansoni* infection was supported by an experimental study, which showed that infection with this parasite prevented the development of the autoimmune disease, insulin-dependent diabetes mellitus in genetically susceptible non-obese diabetic (NOD) mice [14]. In this study, inoculation of *S. mansoni* eggs into 5-week-old NOD mice totally inhibited the development of disease. T cells from diabetes-protected mice made IL-10 after re-stimulation in vitro with *S. mansoni* antigen [15], implicating that this cytokine is involved in the down-modulation of the Th1 response that prevents diabetes in these animals.

S. mansoni infection also decreases tissue damage and clinical manifestations of other Th1-mediated auto-immune diseases such as multiple sclerosis and Crohn's disease. Using a mouse model to study experimental autoimmune encephalitis (EAE), a multiple sclerosis-like disease, it was demonstrated that infection with *S. mansoni* delays the onset of the disease and decreases inflammation in the central nervous system [16]. Attenuation of the clinical course of EAE was followed by a reduction in the synthesis of pro-inflammatory mediators, such as IFN-γ, TNF-α and NO, by spleen and central nervous cells in vitro, while the levels of IL-4 and IL-5 in plasma were, as predicted, higher in the parasite-infected group [16].

Crohn's disease is also mediated by an overly active Th1 inflammatory response and it is characterized by dysmotility of the gut as a result of chronic intestinal inflammation. In the trinitrobenzesulfonic acid (TNBS) murine and rat model of colitis, colonic inflammation is due to an infiltration of IFN-γ-producing CD4+ T cells. Exposure to eggs of *S. mansoni* protects mice from development of TNBS colitis [17]. In humans with Crohn's disease and ulcerative

colitis, induction of a Th2 immune response by administration of eggs from the porcine whipworm, *Trichuris suis,* lead to remission in 86% of cases [18].

Together these data demonstrate that *S.mansoni* infection is able to down modulate the type 1 immune response to various infectious agents. The consequence of these changes is quite variable. While the association of *S. mansoni* with HCV leads to the development of worse liver pathology, the association of *S. mansoni* with HTLV-1 may decrease rates of progression to HAM/TSP. Moreover, attenuation or even complete protection from development of T cell mediated auto-immune diseases, such as diabetes mellitus, Crohn's disease and EAE, have been documented in mouse models.

Immune Response in Atopy

Atopy is a term used to describe an immune-based inherited condition that makes individuals more likely to have allergic diseases such as asthma, rhinitis and atopic dermatitis. Atopic diseases can be classified in two distinct forms: (1) IgE-mediated allergic diseases – the extrinsic or also called allergic form, or (2) the variant diseases that occur in the absence of sensitization and low levels of IgE, i.e. the nonallergic or intrinsic forms of atopy.

The diagnosis of asthma is based on the patient's medical history, physical examination, and laboratory results. Symptoms include wheezing, shortness of breath, chest tightness and cough, particularly at night and in the early morning. The initial events of the acute phase of allergic reactions are characterized by bronchospasm and edema, which results from allergen-triggered mast cell degranulation. The late phase of allergy is characterized by an inflammatory process that involves eosinophil infiltrate and cytokines released by T cells, beyond mast cells mediators. There is now strong evidence that airway inflammation is a predominant underlying problem in patients with asthma, and it has been suggested that ongoing inflammation may lead to airway injury and remodeling.

The ability of cytokines to induce and perpetuate inflammation in asthma results from activation of transcriptional factors, such as nuclear factor-κB activator protein 1, nuclear factor of activated T cells and a number of the family of signal transduction-activated transcriptase (STAT) factors, which are proteins that bind to the promoter regions of genes.

The actions of Th2 cytokines in promoting allergic inflammation can be summarized as follows: IL-4 regulates isotype class switching in B cells to IgE synthesis, IL-5 stimulates eosinophil growth, activates these cells and prolongs eosinophil survival. IL-13 has a number of actions similar to IL-4, because these cytokines share the IL-4R alpha chain. Besides stimulation of IgE

production, IL-13 and IL-4 can induce expression of CD23, class II MHC molecules, adhesion molecules on endothelial cells (vascular cell adhesion molecule 1) and chemokine production. This facilitates migration of the inflammatory cells from the circulation into the lamina propria, the epithelium and airway lumen. These cytokines also activate mast cells and eosinophils and inhibit pro-inflammatory gene expression (IL-1, TNF-α and IL-6) by monocyte-macrophage populations. Because of these functions, IL-4, IL-5 and IL-13 play a pivotal role in airway responsiveness, mucus hypersecretion, and subepithelial fibrosis, which participate in the pathogenesis of asthma. In contrast to IL-4, IL-13 does not exert certain functions, such as T cell proliferation and the ability to drive the T cell response toward a Th2 phenotype. This is probably because T cells do not express functional IL-13 receptor.

Although type 2 CD4+ cells have been implicated in the pathogenesis of asthma, it is predominantly Th1-activated cells, which produce IFN-γ and TNF-α, that contribute to the inflammation and tissue damage in asthma. In a mouse model of airway hyporesponsiveness, it was demonstrated that administration of IFN-γ-producing cells resulted in airway inflammation, reinforcing the idea that the regulation of the immune system in allergic disease does not result from the simple imbalance between Th1 and Th2. Moreover, in HTLV-1 infection, a condition characterized by a strong type 1 immune response with production of high levels of IFN-γ, asthma may occur, indicating that in individuals who do not modulate their immune response, both exacerbated type 1 and type 2 responses co-exist.

Influence of Parasite Infection on the Development of Atopy and Asthma

In addition to the downregulation of type 1 immune responses involved in the pathogenesis of autoimmune diseases, parasites have also been shown to influence the development of type 2-mediated allergic diseases. The prevalence of allergic diseases has increased in developed countries in the last two decades. The International Study for Asthma and Allergy in Childhood (ISAAC) and the European Community Health Respiratory Survey (ECHRS) showed an increased prevalence of asthma and rhinitis in the western industrialized countries, when compared to developing countries. Environmental factors, such as poor sanitation and hygiene that result in a high prevalence of childhood infections, have been implicated as protective against the development of allergic diseases. Among infectious agents, helminths are able to modulate the immune response, preventing the development of allergies. Studies performed by Lynch et al. [19] have both demonstrated that people living in an endemic area for

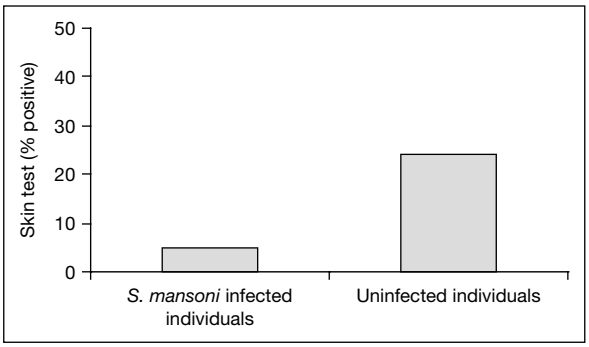

Fig. 1. Skin test reactivity in individuals with or without *Schistosoma mansoni* infection [22].

Ascaris lumbricoides have a decreased reactivity to allergens in the immediate hypersensitivity skin prick tests, and that treatment with antihelminthic drugs resulted in increased frequency of positive skin prick tests and aeroallergen-specific IgE production. Other studies have also shown that infections with geohelminths inhibit allergen skin prick test reactivity among subjects living in endemic regions [20, 21]. Supporting this idea, we evaluated whether immediate hypersensitivity reactions and aeroallergen-specific IgE levels are influenced by the presence of *S. mansoni*, taking into account the potential influence of age and gender on this association [22]. We showed that the proportion of patients with a positive skin test for aeroallergens was approximately five times greater in the uninfected group than in the group with high *S. mansoni* parasite burdens living in the same area of Caatinga do Moura, Brazil (fig. 1). There were no statistically significant differences in the total and *Dermatophagoides pteronyssinus* antigen 1 (Der p1) specific IgE levels between the two groups. Patients without helminthic infection were 6.3× more likely to have atopy (defined as a positive test for at least one of antigens) compared to patients with a high parasite load. This association did not change significantly after adjusting for the potential effects of age and gender [22]. It was also demonstrated, in a case-control study, that people from a *S. mansoni* endemic area are highly exposed to dust mites, and that despite exposure to allergens, there were significantly less positive skin prick tests in asthmatics from the *S. mansoni* endemic area than in those from a rural nonendemic area [23].

Although there is a consensus that helminthic infections decrease skin reactivity to aeroallergens [19–22], and reduce the risk of asthma development [24, 25], there was no well-controlled study showing that helminthic infections interfere with the severity of asthma. To test the hypothesis that infection with

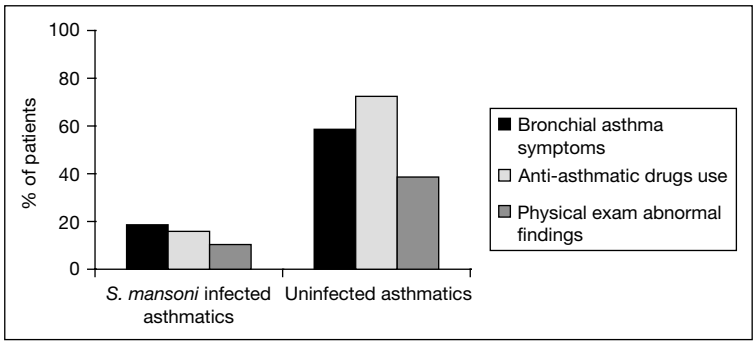

Fig. 2. Severity of asthma during a one-year follow-up study in asthmatic individuals with or without *Schistosoma mansoni* infection [23].

S. mansoni interferes with asthma severity, Medeiros et al. [23] performed a case-control study in a rural endemic area of schistosomiasis in Brazil. An ISAAC-based questionnaire was used to identify individuals with a history of asthma in the endemic area. Age- and sex-matched asthmatic controls from an urban non-endemic area were also selected using the same questionnaire. It was demonstrated that people from the endemic area had a more severe asthma as assessed by: (a) a questionnaire that included questions about presence of asthmatic symptoms, such as, cough, wheezing, and dyspnea; (b) use of anti-asthmatic drugs, and (c) physical examination (fig. 2). This study showed that individuals from a *S. mansoni* endemic area, who were equally exposed to aeroallergens, home pollutants, climate conditions and of the same socioeconomic status had a milder course of asthma in a 1-year follow-up, compared to asthmatics not living in a *S. mansoni* endemic area.

The inhibition of the cutaneous response to aeroallergens and the decrease in asthma severity observed in individuals from helminth endemic areas could be explained by: (1) competition between helminth-induced polyclonal or specific IgE and aeroallergen specific IgE for the high affinity IgE receptors present on mast cells; (2) production of helminth-specific IgG4 antibodies that could compete with specific IgE to the antigen and therefore inhibit IgE-mediated responses, and (3) high levels of regulatory cytokines produced during helminthic infections, such as IL-10 and TGF-β, that could suppress the immune response to non-related antigens. A study conducted in Brazil [22] showed that, in spite of a lower prevalence of positive skin prick tests among individuals with high *S. mansoni* parasite load, there was no significant difference in total and Der p 1-specific IgE production between infected subjects with low or high parasite load, which does not support the first hypothesis.

Other published data have also not found a relationship between levels of IgE and protection against allergy [21, 24]. The hypothesis that IgG4 competes for the allergen and inhibits allergic responses is not well evaluated; however, negative skin prick test responses in subjects infected with helminths was not associated with a higher production of IgG4 [21]. It has been shown that IL-10 stimulates IgG4 differentiation, and it seems that this cytokine represents the most important mechanism of protection of helminth-infected subjects against allergy.

The way the immune system modifies the Th2 response to allergens probably depends on the cytokine environment. During infections the cytokine environment can be polarized into a Th1 or Th2 type response. Viruses and intracellular bacteria drive the immune response to a polarized Th1 type, while chronic helminth infections lead to a Th2 response. Studies in this field suggest that both agents that drive Th1 and those that drive Th2 responses can prevent atopy. In this sense there are two different explanations to the low prevalence of atopy in developing countries: (1) the modulation of Th2 responses involved in allergy by a microbe-induced Th1 response, the original 'hygiene hypothesis' [26], and (2) the recently described 'counter-regulation mechanism', in which both Th1 and Th2 responses can be modulated by regulatory T cells [27]. The basis of the hygiene hypothesis is that microbial infections that drive Th1 responses are essential to a balanced immune response. Therefore, conditions that facilitate microbial infections, such as children from a family with large number of siblings and those with early day-care attendance, could protect against allergies. On the other hand, those children with a low exposure to infections and submitted to frequent antibiotic treatment are more susceptible to developing allergies. Conflicting data in the literature do not confirm the hygiene hypothesis.

The counter-regulation mechanism is based on studies that suggest that, instead of Th1 vs. Th2, it may be the induction of a strong regulatory T cell network that leads to production of modulatory cytokines such as IL-10 and TGF-β, and development of regulatory T cells that prevent the development of both Th1-mediated autoimmune disease and Th2-mediated allergic disease. It has been proposed that Th2 cells develop as an abnormality of T regulatory (Tr) cell development, possibly as a result of inadequate production of IL-10 or enhanced IL-4 and IL-13 production, resulting in the development of allergy and asthma [28]. Indeed, helminth-related protection from allergy does not result from changes in the Th1/Th2 imbalance. It is instead related to the development of regulatory mechanisms and production of IL-10 [21, 29]. Even microbes that stimulate Th1 responses and have been implicated in the hygiene hypothesis, can upregulate the production of the anti-inflammatory cytokine IL-10 [30, 31] and this could be the mechanism underlying the 'hygiene

hypothesis'. Based on the counter-regulation mechanism, the low frequency of allergic and auto-immune diseases observed in developing countries could be due to microbe-induced regulatory mechanisms, and, if so, the decreased infectious and antigenic pressure of the westernized lifestyle has deprived people of this important counter-regulatory feedback [27].

It is well known that helminth infections are associated with a strong Th2 immune response, and there are studies suggesting that IL-10 produced during *Schistosoma* infection down regulates the immediate immune response in atopic individuals living in schistosome-endemic areas [21, 29].

Evaluating the immune response of asthmatic individuals infected with helminths, including *S. mansoni*, living in an endemic area, compared to uninfected asthmatic subjects, we observed that PBMCs of asthmatic individuals infected with helminths, after re-stimulation in vitro with Der p1, produced lower levels of Th2 cytokines such as IL-5 and IL-4, and higher levels of IL-10 in comparison with asthmatic uninfected individuals (fig. 3) [29]. There was a positive association between the levels of Der p1-induced IL-10 and *S. mansoni* parasite load in those patients [29]. The addition of recombinant human IL-10 to PBMCs cultures of noninfected individuals, whose cells produce IL-5, resulted in downregulation of IL-5 production. Furthermore, treatment of asthmatics living in an endemic area with albendazole and praziquantel leads to a decrease in IL-10 production and exacerbation of asthma symptoms. Supporting this study, injection of recombinant murine IL-10 along with OVA in BALB/C mice prevented the release of IL-5 in the peritoneal fluid [32].

These above-mentioned studies indicate that the decreased atopy and diminished manifestations of asthma are due to downregulation of the type 2 immune response mediated predominantly by IL-10. It cannot be ruled out, however, that IL-10 can modulate the immediate hypersensitivity response through downregulation of FCε receptor expression on mast cells and basophils, or deactivate these cells preventing release of inflammatory mediators.

IL-10 is an anti-inflammatory cytokine produced by monocytes/macrophages, Th2 cells and, as recently demonstrated, Tr cells such as T helper 3 (Th3) cells, Tr1 cells, or CD4+CD25+ cells. These Tr cells have been isolated from mice and human T cells grown in the presence of IL-10 [28], and they are able to, directly or through production of IL-10 or IL-10 and TGF-β, inhibit the allergic immune response [33–35]. Regulatory effects of IL-10 in allergic diseases include: reduction of pro-inflammatory cytokine production by macrophages; down-modulation of IgE-triggered mast cell cytokine production, and reduction of IgE synthesis and a switch toward IgG production by B cells [36]. IL-10 also reduces eosinophil survival and chemokine production, which inhibits eosinophil recruitment to the site of allergic inflammation [37].

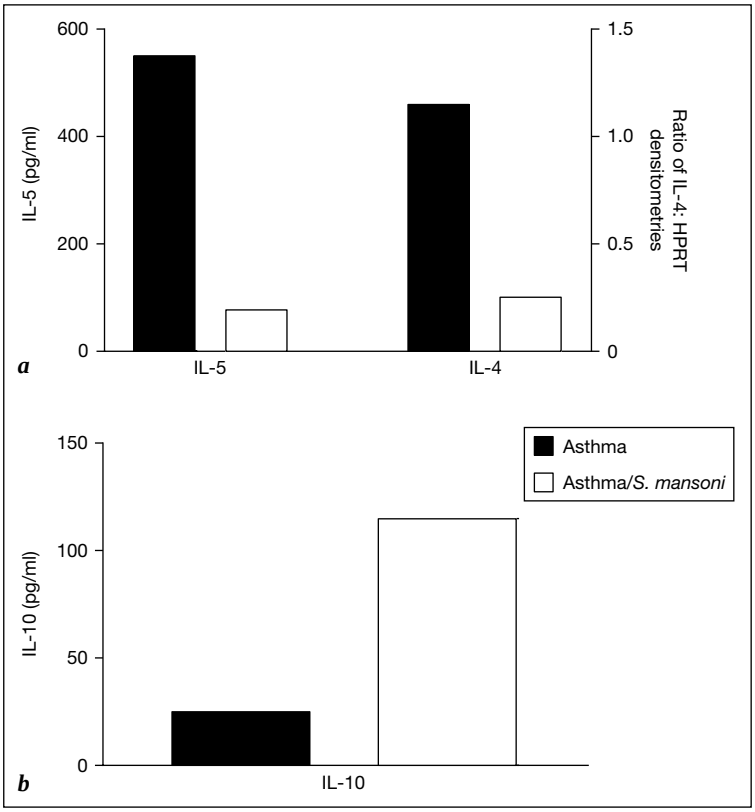

Fig. 3. ***a*** Levels of IL-5 (pg/ml) in *D. pteronyssinus* Ag1-stimulated PBMC cultures and the ratio of IL-4: HPRT densitometries by reverse-transcription polymerase chain reaction in PBMCs from asthmatic patients with or without *Schistosoma mansoni* infection. ***b*** Levels of IL-10 (pg/ml) in *D. pteronyssinus* Ag1-stimulated PBMC cultures of asthmatic patients with or without *Schistosoma mansoni* infection [29].

Other possible mechanisms by which IL-10 inhibits allergic responses include the ability of this cytokine to inhibit the mast cell release of histamine and other mediators [38]. These could therefore be mechanisms behind the inhibition of the skin prick test reaction in helminth-infected individuals and the modulation of asthma severity in individuals infected with *Schistosoma*.

Tr cells represent an important source of IL-10, and they probably occur in chronic helminth infected subjects, whose T cells produce substantial amounts of IL-10. Moreover, IL-10 associated with a Tr response seems to mediate the parasite-specific hyporesponsiveness observed in helminth-infected individuals, since PBMCs of these individuals produced high levels of this cytokine,

and neutralization of IL-10 in cultures stimulated with parasite antigen reversed the T cell hyporesponsiveness [2].

These recent publications support a link between IL-10 and protection against allergic diseases and raise interest in studies concerning helminth antigens with the capability of inducing IL-10 production. Several *S. mansoni* candidate antigens have been identified and some of them are protective against parasite infections in mouse models. These antigens include Sm97-paramyosin [39], irradiation-associated vaccine antigen (Sm62-IrV-5) [40], triose phosphate isomerase (Sm28-TPI) [41, 42], *S. mansoni* 23-kDa antigen (Sm23) [43], 14-kDa *S. mansoni* polypeptide (Sm14) [44], glutathione-S-transferase (Sm28-GST) [45, 46], a fraction of *S. mansoni* soluble adult worm antigenic preparation named PIII [47], and a single antigen obtained from PIII, p24 [48]. Some of these antigens are able to elicit IL-10 production by human T cells. It was also demonstrated that the oligosaccharides produced by *S. mansoni*, lacto-N-fucopentose is capable of inducing IL-10 production in vitro by human cells [49].

Although currently available therapies are effective in controlling asthma symptoms and limiting exacerbations in the majority of patients, there is a subset of patients who develop severe asthma with decreased lung function, lack of responsiveness to therapy, or frequent exacerbations. The IL-10-dependent mechanisms by which asthmatic subjects infected by *S. mansoni* have a less severe asthma is still under study and elucidation of these mechanisms could create new options for prevention and treatment of allergic diseases and asthma.

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Proteases in Helminth- and Allergen-Induced Inflammatory Responses

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Abstract

Proteolytic activity is a central biochemical property that endows molecules with intrinsic allergenicity. Thus, the cysteine protease of dust mite, Der p1, the aspartic protease of cockroach, Bla g 2, the serine protease of *Aspergillus fumigatus* and the bacterial subtilisins are all major allergenic molecules responsible for the increase in asthma and atopic conditions worldwide. These proteases induce Th2-driven inflammatory responses in the airways by disrupting the epithelial cell junctions so that these, and other molecules, gain access to, and alter the function of, underlying cells of the innate immune system (dendritic cells, mast cells, basophils and macrophages) and B and T cells. Helminth parasites secrete proteases to gain entry into their hosts, and to feed on and migrate through tissues. Their action leads to tissue damage and the activation of inflammatory responses dominated by elevated IgE, eosinophilia and Th2 cells, much like allergenic responses. In certain situations, such as in acute infections (especially with zoonotic helminths), proteases secreted by helminths may sensitise individuals to allergens. However, the anti-inflammatory responses observed in chronic helminthiases, involving IL-10 and TGFβ, that are primarily responsible for controlling immune-mediated damage to the host that is initiated by secreted proteases, coincidentally protects against similar inflammatory damage by allergens.

What makes an antigen an allergen and how do these induce allergic reactions? The answers to these questions are critical to the understanding of the nature of molecules that initiate and sustain allergic inflammation and to the design of strategies to prevent this condition. Our current assessment of the potential for a protein to be allergenic focuses on three main aspects: (1) inherent allergenicity, which refers to the likelihood of an immunoglobulin E (IgE)

antibody or T-cell response developing to a particular molecule; (2) cross-reactivity, which refers to the ability of an IgE clonotype or a T cell clone previously induced by one allergen to react with another molecule, and (3) clinical symptoms, i.e. the manifestation of the allergic response, such as eczema, rhinitis, conjunctivitis, asthma and anaphylaxis [1].

Clinical and functional data have been extensively reported for common allergens for many years. More recently, in an attempt to identify the structural signatures of these allergens, a number of databases have been generated that contain molecular information, including primary sequence, secondary and tertiary structure and T cell/IgE binding epitopes, for known allergens (table 1). Researchers can use basic bioinformatic tools to interrogate these databases for common 'allergen' motifs and to predict the potential allergenicity of unknown or query molecules, much like the standard 'blasting' of general protein databases. Indeed, the present recommendation for the prediction of allergenicity and allergic cross reactivity sanctioned by the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) is a scheme based on an assessment of sequence similarity between the query protein and known allergens. Thus, the query protein is considered potentially allergenic if it has (a) >35% sequence similarity over an 80 amino acid segment, or (b) an identity of six or more contiguous amino acids when compared with established allergens [1].

While sequence comparisons may reveal similarities in contiguous residues (linear epitopes) they clearly fail to disclose structural characteristics of proteins that may be essential in the induction of IgE or in the determination of IgE cross-reactivity. Since surface exposure is important for certain epitopes of allergens to induce IgE, structural analysis tools are also required to identify secondary or tertiary structures that may impact on allergenicity. However, extensive analyses of allergenic proteins for which the protein folds are known, or can be predicted on the basis of homology, indicate that allergens have few, if any, structural features in common [1]. Nevertheless, most allergens are grouped into a small number of rather general structural classes: (1) antiparallel β-strands; (2) antiparallel β-sheets associated with one or more α-helices; (3) α+β structures in which the α- and β-structural elements are not intimately associated; (4) α-helical, and (5) parallel β-strands in combination with an α-helix linking the two strands (allergens with these structural arrangements seem to be underrepresented) [1].

The lack of a characteristic structural feature between allergens indicates that factors other than sequence similarity and 3-dimensional conformation are a prerequisite for a protein to exhibit allergenicity. The accumulation in the databases of allergens that possess proteolytic activity may be leading to the conclusion that this is one central biochemical property that confers a protein

Table 1. Allergenic proteases from various sources

Allergen	Species	Biochemical nature	Accession No.
Mites			
Der p 1	*Dermatophagoides pteronyssinus*	cysteine protease	U11695
Der p 9		serine protease	AF409110
Der f 1	*Dermatophagoides farinae*	cysteine protease	X65196
Blo t 1	*Blomia tropicalis*	cysteine protease	AF277840
Eur m 1	*Euroglyphus maynei*	cysteine protease	X60073
Animals			
Fel d 1	*Felis domesticus*	protease	*X62477*
Fungi			
Asp f 5	*Aspergillus fumigatus*	metalloprotease	Z30424
Asp f 10		aspartic protease	X85092
Asp f 13		serine protease	M99420
Asp f 18		serine protease	P87184
Asp fl 1	*Aspergillus flavus*	serine protease	AF137272
Asp fl 13		serine protease	
Asp n 18	*Aspergillus niger*	serine protease	AAA32702
Asp o 13	*Aspergillus oryzae*	serine protease	CAA35594
Cur l 1	*Curvularia lunata*	serine protease	
Epi p 1	*Epicoccum purpurascens*	serine protease	P83340
Pen ch 13	*Penicillium chrysogenum*	serine protease	AAF23726
Pen ch 18		serine protease	AAF71379
Pen c 1	*Penecillium citrinum*	serine protease	
Pen c 2		serine protease	AF098517
Pen c 13		serine protease	
Pen o 18	*Penecillium oxalicum*	serine protease	AAG44478
Tri r 4	*Trichophyton rubrum*	serine protease	AF282514
Bacteria			
Alcalase	*Bacillus subtilis*	serine protease	
Esperase	*Bacillus licheniformis*	serine protease	
Savinase		serine protease	
Insects			
Api m 7	*Apis mellifera*	serine protease	AY127579
Bom p 4	*Bombus pennsylvanicus*	protease	A56338
Bla g 2	*Blattella germanica*	aspartic protease	U28863
Pol d 4	*Polistes dominulus*	serine protease	P81656
Foods			
Act c 1	*Actinidia chinensis*	cysteine protease	P16785
Ana c 1	*Ananas comosus*	cysteine protease	AAK54835
Cuc m 1	*Cucumis melo*	serine protease	D32206
Car p 1	*Carica papaya*	cysteine protease	AAB02650
Gly m 1	*Glycine max*	thiol protease	P24337

Compiled from allergen-specific data sources: www.allergen.org, www.allergenonline.com, http://fermi.utmb.edu/SDAP/index.html and www.allergome.org.

with the ability to generate IgE and Th2-type cytokine responses. Also emerging in the literature is the idea that proteases, both endogenous and exogenous/environmental, are critical in the regulation of the immunological environment at epithelial surfaces, and in the pathophysiology of allergic respiratory disease [2]. Proteases are major components amongst the profile of antigens secreted by helminth parasites during infection and perform a number of essential functions, including modulation of the host immune response [3]. Could these enzymes be key to the relationship between allergy, atopy and parasitic infections?

Proteases Are Major Allergens Derived from Various Organisms

Of the diverse biochemical agents associated with classified allergens, proteolytic enzymes appear to be particularly potent in allergy-susceptible individuals. These allergens are generally endoproteases, i.e. they cleave peptide bonds within the protein structure, and most belong to the cysteine, aspartic or serine class of proteases (table 1). Endoproteases are ubiquitous in nature and hence it is not surprising that proteolytic allergens are found in many organisms, both plant and animal.

The significance of protease activity in allergy was first highlighted more than a decade ago when it was discovered that the major allergen from the house dust mite (HDM), *Dermatophagoides pteronyssinus* (Der p 1), was a cysteine protease. In mites, proteases such as Der p 1 are involved in food digestion and found in faecal pellets from where they become aerosolized and inhaled. A dose-response relationship can be demonstrated between the levels of Der p 1 inhaled, the prevalence of sensitization and the risk of asthma [4]. Der p 1 is the most immunodominant dust mite allergen involved in the induction of IgE-mediated hypersensitivity and is now considered the most common cause of allergen-linked asthma worldwide [4]. However, because of the increasing attention given to allergies in recent times, other allergens, such as the aspartic protease Bla g 2 of the cockroach, are growing in clinical relevance [5]. As much as 25–37% of asthmatic children in the United States are allergic to Bla g 2 [5].

Bacterial-derived proteases used in the manufacture of detergent products were also among the first identified allergens and are associated with occupational outbreaks of rhinitis and asthma. IgE-mediated respiratory allergies correlate with exposure to bacterial enzymes, such as the serine protease subtilisins (alcalase, savinase and esperase) (table 1). Surveillance of enzyme-specific IgE

antibodies before the onset of allergic symptoms has minimized the risk of occupational allergy in the detergent industry [6].

Airborne spores of *Aspergillus* and *Penecillium* fungi are commonly implicated in allergic disease and are considered important causative agents of extrinsic bronchial asthma [7]. Extracts from the dermatophyte fungus, *Trichophyton rubrum*, also induce both immediate and delayed-type hypersensitivity reactions. The causative agents are now believed to be active proteases, predominantly of the serine class but also aspartic and metallo-proteases that are liberated by these fungi [7].

Food allergic reactions are causing increasing concern as approximately 5% of children worldwide under the age of 4 years exhibit IgE-mediated food allergic reactions. Moreover, food hypersensitivity has been related to moderate or severe atopic dermatitis in 35% of children with these skin symptoms. Some of the components of food that cause sensitization are serine and cysteine proteases, for example, those present in melon, kiwi and papaya fruit [8].

How Do Proteases Act as Allergens?

Considerable advances have been made in elucidating the mechanisms by which proteolytic enzymes may function as allergens (the majority of investigations have used Der p 1 as the model allergen). A review of the main literature reveals that allergens may intervene at various steps (or even several) in the allergic inflammatory cascade including: (a) the degradation of epithelial barrier cells to permit passage of the allergen throughout tissues; (b) cleavage of receptors on the surface of innate immune cells eg. dendritic cells; (c) cleavage of receptors on B and T cells; (d) activation of mast cells via protease-activated receptors (PARs), and (e) alteration of the protease/anti-protease balance of mucosal epithelia.

Disruption of the Epithelial Barrier

The primary risk factor for the development of allergic sensitization is the delivery of allergen across the mucosal epithelium. Paracellular channels of the epithelial layer are normally sealed by tight junctions, macromolecular assemblies of proteins that form contiguous rings at the apices of epithelial cells. Der p 1 has been shown to degrade the transmembrane proteins occludin and claudin involved in tight junction adhesion and sealing resulting in increased permeability of the bronchial epithelium [9]. This digestive action may facilitate the passage of allergens across the mucosa and enhance the accessibility to antigen presenting cells residing beneath the epithelial barrier.

Modulation of Dendritic Cell (DC) Function

Passage across the mucosal surface would bring the allergen in direct contact with dendritic cells (DC), key players in the development of allergic inflammation. A pivotal role in the activation and function of DC is played by the CD40 surface ligand. When DC are exposed to Th1 inducing antigens, such as LPS, CD40 expression becomes elevated and production of IL-12 increases in response to CD40 engagement [10]. However, DC matured in the presence of proteolytically active Der p 1 produce significantly less IL-12 in response to LPS stimulation and the balance of IFNγ and IL-4 production by T cells is modulated such that a Th2 response is promoted [10]. This alteration in the T cell cytokine balance results from proteolytic cleavage of CD40 by Der p 1, as inactive Der p 1 has no affect on DC [10].

Modulation of B and T Cell Function

Der p 1 can influence peripheral blood T cells to promote a pro-allergic Th2 type response by cleaving CD25, the 55-kDa α-subunit of the IL-2 receptor [11]. Since this receptor is pivotal for the propagation of Th1 cells, removal of CD25 by Der p 1 leads to diminished proliferation and reduced secretion of IFNγ in response to mitogenic stimulation. There is a consequential polarisation of T cells towards a Th2 phenotype, with increased secretion of IL-4 and IL-13 (cytokines responsible for inducing IgE synthesis). In addition, Der p 1 cleaves CD23, which is involved in the negative feedback regulatory cycle of IgE synthesis, from the surface of cultured human B cells and thus potentially disrupts this mechanism to cause excessive IgE synthesis and subsequent development of the allergic phenotype [12].

Activation of Mast Cells Via Protease Activated Receptors

The production of mediators of allergic inflammation from mast cells follows the antigen-mediated aggregation of IgE that is bound to high affinity Fc epsilon receptor I (FcεRI) on the cell surface. However, the activation of mast cells by allergens can also occur *via* a non-IgE-mediated pathway, and proteases from mites, fungi and insects are among those molecules that can generate allergic inflammation in this way [2]. For example, when the serine protease (Der f) from the HDM *Dermatophagoides farinae* is administered into the airways of mice it induces the degranulation of mast cells and up-regulation and secretion of a number of pro-allergic cytokines, including IL-4, IL-6, IL-9 and IL-13, without IgE aggregation [13]. It has been suggested that this mast cell activation is initiated by cleavage of cell surface protease activated receptors (PARs) [2].

PARs are G protein-coupled transmembrane receptors that mediate cellular responses and are activated by cleavage of an N-terminal domain [2, 14]. Four

PARs have been identified; PAR-1 is expressed on neutrophils, platelets, endothelial cells and fibroblasts, while PAR-2 is found on T cells, neutrophils, epithelial cells, endothelial cells, smooth muscle cells and neurons. PAR-1, -3 and -4 are activated by thrombin, while PAR-2 is activated by trypsin [2]. Many allergenic proteases have similar specificities to the mammalian serine proteases thrombin and trypsin, and therefore allergenic proteases may function by activating PARs. This activation leads to G-protein signalling cascades generating transcriptional responses through ERK (extracellular signal related kinases), mitogen-activated kinases and NFκB and ultimately to the production of chemokines and cytokines that enhance IgE production and airway hyperresponsiveness [2, 14].

Der p 1 can induce IL-6 and IL-8 cytokine release from bronchial epithelial cells via the activation of PAR-2 receptor [15] and the serine proteases of HDM, Der p 3 and Der p 9, cleave PAR-2 on lung epithelial cells causing the activation of phospholipase C which is the initial step in the transcription of cytokine genes [16]. Similarly, the cockroach protease allergen, Bla g 2, activates PAR-2 on bronchial epithelial cells resulting in the activation of ERK and the subsequent induction of IL-8 transcription [17]. Whether these allergens also modulate PAR-3 or PAR-4 remains unknown, although given the involvement of PAR-4 in the induction of IL-6 and IL-8 secretion this has been suggested [2, 17].

Alteration of the Protease/Anti-Protease Balance

Mucosal epithelia are protected from damage by endogenous proteolytic enzymes, like elastase, by the presence of anti-proteases such as α_1-antitrypsin, elafin and secretory leukocyte proteinase inhibitor (SLPI). Maintaining the balance of proteases and anti-proteases at the epithelial matrices is critical to protecting lung tissues and an upset in this balance results in conditions such as emphysema and asthma [18]. Clinical studies indicate that subjects with a deficiency in α_1-antitrypsin are more prone to developing asthma. Furthermore, the administration of exogenous proteases such as papain, proteinase 3, human cathepsin B and human cathepsin L into mouse lungs results in the development of many features of pulmonary disease that is attributed to the inactivation of anti-proteases [18]. Recently, Der p 1 was shown to cleave and inactivate several anti-proteases including α_1-antitrypsin, elafin and SLPI [18]. Inactivation of these in vivo would modulate the elastase/anti-elastase balance in favour of elastase and result in the upregulation of IL-8 from lung epithelial cells and a shift in the immune environment to one that facilitates the development of a pro-inflammatory allergic response [19].

Squamous cell carcinoma antigen (SCCA) proteins are cross-class serine and cysteine protease inhibitors found in the peripheral blood of bronchial

asthma patients and are induced by IL-4 and IL-13 [20]. SCCAs inhibit Der p 1 protease activity and are produced in the lung in response to the immunomodulatory effects of this allergen. These inhibitors have been suggested to specifically target exogenous cysteine proteases (and possibly other proteolytic allergens) to protect the lungs against allergic reactions [20]. On the other hand, it is noteworthy that some protease inhibitors, such as the cystatins from cat skin and potato and an aspartic protease inhibitor from potato [www.fermi.utmb.edu/cgi.bin/SDAP], have been identified as allergens and, therefore, altering the protease/anti-protease balance in either direction may leave the lung-epithelium open to events that lead to allergy.

Proteases Provoke Allergenic Responses to Non-Peptidolytic Molecules

All of the processes outlined above whereby proteolytic allergens may induce allergic inflammation depend on the enzyme being functionally active. The requirement for protease activity in the induction of allergic inflammation has been demonstrated extensively through the use of protease specific inhibitors such as E-64, phosphoramidon and antipain. For example, complete inactivation of protease activity in *Aspergillus oryzae* serine protease allergen prevented both airway inflammation and Th2 activation in an experimental murine model of asthma [14]. Moreover, selective inhibition of Der p 1 protease activity by the synthetic peptide based inhibitor, PTL11028, resulted in the suppression of both airway hyper-responsiveness and inflammation and correlated with reduced levels of allergen-specific IgE in the blood of rats [21].

It is well known that many atopic patients develop an allergic reaction to antigens that lack protease activity and hence this property would not seem to be an exclusive requisite for allergenicity. On the other hand, this does not rule out the possibility that these individuals were concomitantly or previously exposed to a proteolytic allergen. Of importance in this regard is that serine protease allergens derived from *Aspergillus* sp. are capable of conferring allergenic potential, as judged by the recruitment of IL-4 producing cells and induction of antigen specific IgE, to otherwise innocuous antigens, such as ovalbumin, applied to the respiratory tract [14]. Furthermore, co-injection of Der p 1 enhanced the IgE antibody response to chicken egg ovalbumin [22] and promoted the Th2 response to *Schistosoma mansoni* Sm28-GST by reducing IFNγ responses and antigen specific IgG2a [23]. Therefore, proteases may destabilise or alter the microenvironment within target tissues making them more susceptible to pro-allergic inflammation and IgE responses which are then driven by non-peptidolytic molecules.

Do Parasites Exacerbate or Protect against Allergy?

Immune responses to helminth parasites are similar to those observed in allergy in that they are typified by a polarised Th2 immune response, involving high levels of IL-4, IL-5 and IL-13 accompanied by mastocytosis, eosinophilia and potent IgE production [24]. This would suggest that a helminth infection predisposes an individual to allergic reactions; indeed, in a number of infections including anisakiasis [25], enteric infection with the dog hookworm [26] and echinococcosis [27] increased allergic reactivity is a symptom of infection. On the other hand, field studies suggest that helminth infections may, in fact, protect people from allergies. For example, children in the Gabon who are infected with *Schistosoma haematobium* exhibit a 63% lower risk of developing reactivity to HDM: removal of the parasites by drug treatment results in increased atopic responses [24, 28]. In South America, the presence of intestinal worms such as hookworm, *Ascaris* and *Trichuris*, all of which induce high IgE levels, also correlate with lesser skin reactions to HDM [24, 28]. However, a study in Venezuela noted that individuals with light helminth infections exhibit raised allergic reactions which were relieved following drug treatment, whereas those with high infection were protected from atopy which became exacerbated on drug treatment [24, 28].

Helminth parasites have complicated life cycles and go through various developmental stages in their host before establishing chronic infections that can last a long time (many years even). At the same time as skewing the immune system toward a Th2-driven response, they have a general suppressive effect on the host immune system that prevents their elimination and reduces immune-mediated tissue damage. It has been suggested that cytokines of the anti-inflammatory network, particularly IL-10 and transforming growth factor-β (TGF-β), that are produced in response to continual stimulation of the immune system by parasite antigens, are pivotal to regulating the damage they cause and that, coincidentally, these have a bystander protective affect against allergic reactions [24]. In support of this idea, individuals that are prone to allergies tend to have lower IL-10 levels while people with *S. haematobium* and other helminths have raised IL-10 levels which decrease with drug therapy and, in turn, results in increased atopy [28].

In contrast to anthropophilic infections of humans, zoonotic helminth infections, such as with the fish parasite *Anisakis simplex* and the dog hookworm *Ancylostoma caninum*, are often of short duration. A relative absence of suppressor cytokines are observed to these zoonoses, probably because humans are non-permissive and unadapted hosts, and consequently are more likely to evoke allergic responses [25, 26]. Therefore, whether a parasitic infection predisposes or protects an individual against allergies appears to be influenced by

parasite species, whether infection is a zoonosis or not, parasite load, stage of infection, length of infection and host genetic factors (including ability to produce IL-10).

Helminth Parasites Secrete Proteases

Helminth parasites synthesise proteases of all four major mechanistic classes, (cysteine-, serine-, aspartic and metallo-proteases), although the relative importance of each class varies between parasites [3]. Proteases often represent a major component of the parasite total protein production; for example, 4–5% of ESTs characterised for *S. mansoni* encode proteases (cysteine and aspartic), whereas almost 10% of the ESTs described for the related flukes *Clonorchis sinensis* and *Fasciola hepatica* encode cysteine proteases [29]. Proteases are also abundant in parasitic nematode transcriptomes; for example, 17% of *Haemonchus contortus* gut-derived ESTs encode cathepsin B-like enzymes, large gene families encoding cysteine proteases are found, and filariae and both cysteine and aspartic proteases are highly expressed in hookworms. The functions of these proteases are varied, but include essential roles in parasitism such as facilitating entry and penetration of host tissues, digestion of host tissue for nutrients, tegumental (trematodes) and cuticular (nematodes) turnover and immunoregulation. To perform these functions proteases are actively secreted or excreted by the parasite, usually from their guts or surface, and often in copious amounts [3].

Proteases are essential for parasites that move or migrate through tissues and, therefore, it is easy to understand how these would be delivered directly into host tissues (notably mucosal barriers) during the acute stages of infection. After reaching maturity, many helminths reside in spaces outside the circulation or main visceral tissues, for example in the intestines, lungs or bile ducts. However, these parasites generally feed on the walls of these tissues or blood drawn through them and, therefore, proteases still gain access to the host circulation. In short, helminth-infected individuals are continuously exposed to parasite-secreted proteases during acute and chronic infection.

Can Parasite Proteases Sensitise Individuals to Environmental Proteases *Via* Cross-Reactive IgE?

A number of reports have shown that excretory/secretory (ES) products of helminths collected from in vitro cultures can induce Th2 responses, involving enhanced levels of IgE, when injected into mice [24, 28]. Proteases are associated

with these ES products and there is evidence that these can directly induce IgE responses. For example, using affinity-purified IgE antibodies from patients with acute tropical pulmonary eosinophilia, transpeptidases from *Brugia malayi* and *Wuchereria bancrofti* were identified as major filarial allergens [30]. Similar methods were used to isolate two allergens secreted by *Paragonimus ohirai*, which were subsequently found to be cysteine proteases [31]. Furthermore, cysteine proteases secreted by *Nippostrongylus brasiliensis* [32], *Onchocerca volvulus* [33], *Ancylostoma caninum* [34] and *S. mansoni* [35] have also been shown to be among the target antigens to which IgE antibodies associated with Th2 immune responses are reactive. Therefore, parasite protease-specific IgE is induced by helminth infections, however, it is not known whether this antibody is capable of sensitising individuals to protease allergens in the environment.

Many parasite cysteine proteases and common allergenic proteases are members of the same clan – clan CA of the C1 family of cysteine proteases – and are structurally related [3]. Using the current recommendation for the prediction of allergenicity by WHO/FAO, we compared the primary sequences of cathepsin L and cathepsin B cysteine proteases from a number of helminths, including *S. mansoni*, *Fasciola hepatica* and hookworms, to allergens within the SDAP allergen database (http://Fermi.utmb.edu/SDAP). In summary, our analyses revealed that the overall similarity in amino acid sequence between the parasite and common allergenic cysteine proteases is relatively low (data not shown); the highest match found was between the parasitic cathepsin Ls from *F. hepatica* and *S. mansoni* and other cysteine protease allergens in foods such as kiwi fruit (Act c 1, 38%), papaya (Car p papain, 36%) and soybean (Gly m 1, 35%), and the dustmite allergen, Der p 1 (30%). Moreover, this similarity is dispersed throughout the primary sequences except for one region of eight identical amino acids, from residues 22–29 in mature Der p 1 (fig. 1). While this match fulfilled the criteria of allergenicity as defined by the WHO/FAO, the regions of high identity/similarity fall within the active site groove of the proteases and would not be accessible to binding free or mast cell-bound IgE.

Analysis of tertiary structure has been used to determine whether allergens have common or conserved features that are recognised by the immune system with particular interest in mapping linear IgE epitopes. While there does not appear to be a consensus allergen structure [1], comparative molecular modelling of a number of clan CA cysteine proteases indicated that they display a putative IgE binding epitope, previously identified on Der p 1, although this varied in shape and degree of accessibility depending on the protease. The core of the epitope consists of a centrally located conserved tyrosine (residue 154 in Der p 1) flanked on both sides by accessible amino acids [36]. While the corresponding regions in *S. mansoni* and *F. hepatica* cathespin Ls (fig. 1) and

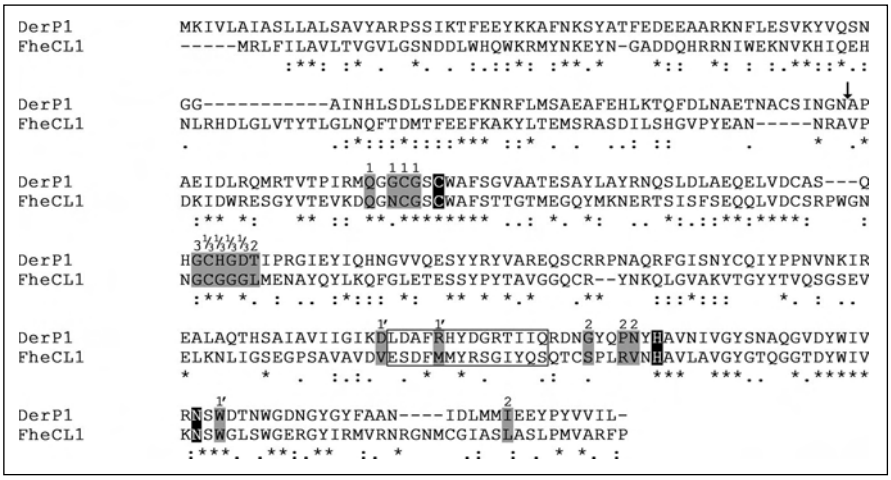

Fig. 1. Comparison of Der p 1 with *F. hepatica* cathepsin L1 primary sequences. Sequences were aligned using ClustalX 1.81. The cleavage point between the propeptide and mature regions of Der p 1 is indicated by an arrow, while the Der p 1 IgE epitope [36] is indicated by a box. Residues of the catalytic triad (Cys, His and Asn) that are situated in the S1 subsite of the active site are indicated in white text in black background, while residues implicated in other subsites are shaded in grey (subsite designations are numbered above these residues). Degree of conservation is indicated by the bottom row: identical (star), highly similar (two dots) and weakly similar (one dot). GenBank accession numbers for the sequences are as follows: Der p 1 (P08176), *F. hepatica* cathepsin CL1 (U62288).

other helminth cysteine proteases (not shown), do possess the conserved central tyrosine residue, we found that there are few similarities in the flanking amino acids.

In conclusion, it appears that the similarities between parasite and allergen cysteine proteases are confined to regions that are not accessible to antibody binding, and, therefore, it is not likely that parasite proteases induce IgE that sensitises individuals to other protease allergens. The results of an analysis of helminth serine and aspartic proteases yielded the same result. Although hookworm aspartic protease, *Ac*-APR-1, is listed as the only parasite protease allergen (www.allergenonline.com), this is based on its structural similarity to allergenic aspartic proteases, such as cockroach Bla g 2, rather than on a direct demonstration of its ability to induce allergic responses. At present, there is no clinical data showing that parasite protease-specific IgE cross-reacts with HDM, which contains both cysteine and serine proteases, and enhances a patients reactivity in skin-prick tests.

Parasite Peptidolytic Activity May Sensitise Individuals to Allergens

As discussed above, functional activity is necessary for the induction of allergic inflammation by fungal, mite and insect peptidolytic allergens. Given that parasite and allergenic proteases exhibit conservation in the active sites, which would endow them with similar/overlapping substrate specificities, the potential of parasite proteases to induce sensitivity to environmental allergens may be related to their action on certain cell types or immune responses that are also targeted by allergens. This mode of sensitisation would not depend on the activation of cross-reactive IgE. Parasite proteases can modify host immune responses in various ways – as described below.

Degranulation of Mast Cells and Basophils

Mast cells and basophils both possess high-affinity receptors for IgE (FcεRI) which when cross-linked by bivalent or multivalent antigens are activated to release various mediators, including IL-4 and IL-13, that influence IgE production and the pathophysiology of allergies [2]. Basophils are also elevated in the blood of people infected with hookworm (*Necator americanus*) and filariae (*B. malayi*), and are considered a major source of IL-4 that serves to amplify Th2 responses [37, 38]. Recently, basophils that accumulated in tissues of mice, mainly lung and liver, during an infection with *N. brasiliensis*, were shown to be a principle source of IL-4. However, stimulation of these basophils was independent of FcR cross-linking [39, 40]. Both the secreted cysteine proteases of *N. americanus* and the cysteine protease allergen Der p 1 have been shown to induce basophils to degranulate and secrete IL-4 and IL-13 even in the absence of IgE. Importantly, the action of these proteases on basophils was dependant on their proteolytic activity [41].

Modulation of T Cell Responses

The secretion of proteases by helminth parasites into the circulation ensures that their effects are systemic and it is likely that they contribute to the potent induction of Th2 responses that are observed in these infections. Our studies with *F. hepatica* cathepsin L cysteine proteases have shown that an intravenous injection of active proteases can suppress Th1 responses to a respiratory infection of *Bordetella pertussis* and delay clearance of the bacterial pathogen [42]. These proteases also suppressed the development of Th1 responses to a *B. pertussis* vaccine or keyhole limpet haemocyanin (KLH) given intra-peritoneally [43]. The capacity of cathepsin L to downregulate Th1 responses differs to that of Der p 1, since cathepsin L is unable to cleave the T cell receptor CD25 (but like Der p 1, it does not cleave various other T cell receptors including CD3, CD4, CD7, CD8, CD18, CD28, CD47 and CD69).

Nevertheless, a systemic suppression of Th1 responses may predispose individuals to Th2 responses when exposed to allergens.

Antigens delivered remote from the lungs can influence the reactivity of these tissues to allergens, an example being the experimental induction of allergy to chicken egg ovalbumin. When administered intra-pulmonarily, the antigen induces a tolerogenic response in the lungs, but when administered following priming of the immune response with intra-peritoneal injections of the antigen in alum it induces all the hallmarks of allergy [2, 14]. By analogy, the secretion of parasite proteases may prime the lungs to respond to other proteases, and to non-protease molecules, that would not otherwise induce allergy.

Recruitment and Activation of Alternatively Activated Macrophages

The alternative activation of macrophages is important in the development of allergic inflammation, particularly asthma [44]. Fizz1, a recognised marker for alternatively activated (AA) macrophages, was first identified in secretions from inflamed alveolar epithelium and was implicated in stimulating fibrosis during pulmonary inflammation [45]. The expression of another marker, Ym1, is up-regulated in allergy and is a known chemotactic agent for eosinophils [46]. Interestingly, studies in both humans and mice have demonstrated the recruitment of AA macrophages during a variety of helminth infections, including *B. malayi* [47], *N. brasiliensis* and *Litomosoides sigmodontis* [48], with similar expression profiles (i.e. up-regulated Fizz1 and Ym1) to those observed in allergy. In both disease states AA macrophages have been implicated in the development of the Th2 immune environment, IgE elevation and eosinophilia.

Recently, we demonstrated that infection of mice with larvae of *F. hepatica*, or repeated injection of worm ES products, induced the recruitment and alternative activation of macrophages in the peritoneal cavity and the development of potent Th2 immune responses [49]. As shown in figure 2, the induction of the AA macrophage markers Ym1, Fizz1 and Arginase1, and of the Th2 cytokine IL-4, can be observed in bronchial lavage cells isolated from BALB/c mice that were given a single intra-pulmonary dose of purified *F. hepatica* cathepsin L1. Since inactive mutant cathepsin L1 had no effect on the lungs these studies show that exogenous proteases can set up a pro-inflammatory environment involving AA macrophages in this tissue.

Are Helminth Proteases Allergens?

Using the criteria of the WHO/FAO many helminth proteases could be listed as allergens since they exhibit significant identity with known allergens.

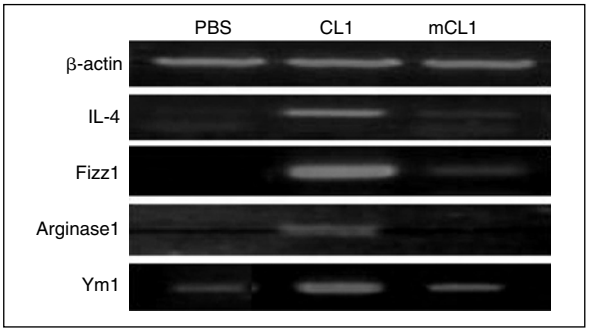

Fig. 2. Alternative activation of macrophage in lungs following administration of cathepsin L protease. BALB/c mice were given 50 μg of recombinant *F. hepatica* cathepsin L1 (CL1), inactive cathepsin L1 (mCL1) or PBS intra-tracheally. Twenty-four hours later bronchoalveolar lavage fluids were obtained by injection and aspiration of 0.5-ml volumes (total 4–5 ml) of warm RPMI 1640 medium via cannulation of the trachea. Cells were recovered by centrifugation and resuspended in Tri-reagent for extraction of total RNA. Markers of alternatively activated macrophages (Ym1, Fizz1, Arginase1) and for β-actin and IL-4 were analysed by RT-PCR. The data shown is from a single mouse, and is representative of findings from 4 mice (the expression of each gene was similar in each mouse).

While in a number of helminth infections anti-protease IgE has been detected in patient serum this may simply be part of the overall Th2-driven immune response to these parasites and, as pointed out here, would not be expected to cross react with environmental allergens. Furthermore, there is no clinical evidence indicating that helminth protease induce IgE-mediated allergic-like responses (e.g. skin reactions involving mast cell degranulation, asthma) during infection. Hence, the WHO/FAO criteria for identifying allergens may not be suitably applied to parasite proteases.

The existence of a diverse range of protease allergens of various mechanistic classes (cysteine-, serine-, aspartic- and metallo-proteases) points to the idea that the critical biochemical property for intrinsic allergenicity is 'proteolytic activity' (in its broadest sense rather than in a peptide-specific sense). Therefore, both the HDM cysteine protease, Der p 1, and the cockroach aspartic protease, Bla g 2, proteases with distinct peptide bond preferences, can induce allergic responses in the lung that lead to an asthmatic condition. The common event that would initiate the allergic response would simply need to be the degradation of the protein molecules that bind the lung epithelial cells together. Disruption of the lung epithelium would allow these proteases access to the underlying cells of the innate immune system, e.g. dendritic cells, mast cells, basophils, macrophages, and to B and T cells. As discussed in this

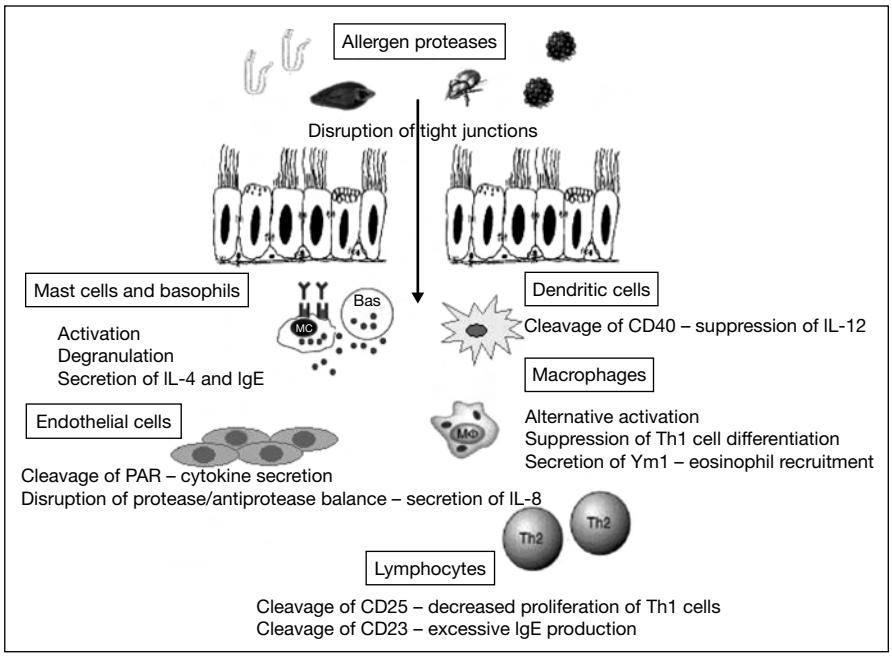

Fig. 3. Schematic representation of events that that lead to protease-induced inflammatory responses.

review, proteases could modify the activity of these cells by cleaving surface receptors and altering intracellular signalling pathways, and this could be exacerbated by upsetting the protective protease/anti-protease balance at the lung surface (fig. 3). Additionally, proteases would facilitate access for many other molecules in allergenic substances, such as HDM faecal antigens, to host immune cells which may enhance inflammatory reactions in the lungs. While it is well documented that proteases themselves can act as allergens, a recent study showed that adding active proteases (in this case *Aspergillus fumigatus* serine protease) to non-allergenic proteins can precipitate an allergic response [14].

So, could helminth proteases cause allergic reactions? The very fact that these molecules have proteolytic activity would suggest that they can behave in a similar way to allergens. Like allergenic proteases, helminth proteases contribute to development of a Th2-type immune environment by degrading host tissues so that these and other molecules can gain access to the underlying cells of the immune system. Allergic responses induced by helminths are generally associated with acute infections, and are particularly pronounced in zoonotic

infections that induce potent Th2 responses. Take for example, the often life-threatening allergic responses in people that ingest fish contaminated with *A. simplex*. Allergic responses coincide with the degradation of the intestinal wall of the host by larvae, facilitated by proteases released from the parasite [25]. The parasite secretions induce a potent Th2 response with the production of IL-4 and IL-5, elevation of specific and total IgE, the infiltration of eosinophils and hyperplasia of intestinal mastoid tissue. Patients become positive to skin prick tests using *A. simplex* antigen. Thus, the hallmarks of gastro-allergic anisakiasis (GAA) are similar to allergic responses in the lungs.

But could parasite proteases sensitise individuals to protease allergens in the environment? Helminth proteases, if delivered into the lung, would set up a pro-inflammatory environment (as described in this review). Some helminths, for example hookworms, that pass through the lungs during their migration in the mammalian host break through the alveoli and are then coughed up and swallowed into the gut. To get through into the air spaces, they secrete proteases which induces wheezing or productive cough at this stage of infection. However, in most chronic infections, with the exception of helminths that penetrate lung tissues, proteases are not delivered at this site but are usually liberated into tissues such as the liver and intestinal wall or directly into the circulation. Nevertheless, the continual passage of low levels of helminth protease through the lung *via* the circulation may be sufficient to sensitise this tissue making it more susceptible to exogenous protease activity and to other airborne molecules. Proteases could prime the draining lymph nodes to a Th2-driven pro-inflammatory response. This may be mediated by the blocking of Th1 cytokine production, as we have shown that an intraveneous injection of cathepsin L cysteine protease suppresses IFNγ production in the spleen, hepatic and lung lymph nodes [42, 43].

Conclusion

The argument concerning the association between helminth infection and the incidence of allergy in the developed and developing worlds is complicated by the fact that many factors, both parasite- and host-derived, influence the clinical manifestations of allergy. However, regulation of the immune responses to chronic helminth infections by anti-inflammatory cytokines, including IL-10 and TGF-β, which function to prevent excessive immune-mediated host tissue damage, is suggested to have a bystander dampening effect on allergic responses (these cytokines may block the degranulation of mast cells and possibly basophils, and the activity of T cells) [24, 28]. In situations of acute infections, especially with zoonotic helminth parasites, or where low parasite

burdens exist, allergy and atopy is more pronounced because of the relative absence of anti-inflammatory cytokines. Given that the damage induced by helminth parasites is closely linked with the secretions they produce, a main component of which is proteases, it may not be surprising that mechanisms designed to defend against their action are also effective against immune mediated damage evoked by allergenic proteases.

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Mechanisms Underlying Helminth-Induced Th2 Polarization: Default, Negative or Positive Pathways?

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Abstract

Since the initial description of Th1 and Th2 subsets in the 1980s, there has been enormous progress in identifying the molecular events and the transcriptional factors that regulate Th differentiation in response to a specific stimulus (e.g. antigen dose, co-receptors, cytokines). Although TCR cross-linking and engagement of co-stimulatory molecules are necessary for activation of CD4+ lymphocytes, these two events do not appear in themselves to explain Th1/Th2 commitment. Among pathogens, helminths are the main examples of Th2-cell inducers in both humans and experimental models. This review will focus on our recent findings on the requirements for Th2 polarization by the parasitic trematode *Schistosoma mansoni*. In particular, we will address the ongoing controversy as to whether Th2 development depends on positive vs. negative vs. the absence of signals from antigen-presenting cells. In addition, we will discuss the similarities between the pathways involved in parasite- and allergen-induced Th2 differentiation.

The primary function of the immune system is to provide host defense against pathogens. To mount an effective immune response against a diverse range of microorganisms with distinct strategies of invasion and survival, the immune system must employ two of its main features: the ability to recognize foreign antigens and the plasticity. The clonotypic receptors expressed on T and B lymphocytes provide the molecular basis for the first, while the second property is assured by the induction of different CD4+ T cells that elicit and orchestrate distinct immune effector mechanisms. The cellular and molecular basis for the latter has been provided by the concept of Th1 and Th2 subsets introduced by Mosmann et al. [1] in 1986. They described two different phenotypes of CD4+

T lymphocytes characterized by the secretion of specific and distinct subsets of cytokines: Th1 cells secrete IFN-γ and TNF while Th2 produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. Subsequent studies have demonstrated that Th1/Th2 lymphocytes represent a final stage of a multistep differentiation process of naïve Th cells [2]. Both Th1- and Th2-type immune responses include potent cell-mediated and humoral components but the effector cells and antibody isotypes involved are different. Th1 cells are responsible for the activation of macrophages to a microbicidal state, the support of CD8+ antiviral effectors, and the induction of IgG antibodies that mediate opsonization and phagocytosis. In contrast, Th2 cells stimulate the growth and differentiation of mast cells and eosinophils as well as the production of antibody isotypes including IgE that can mediate their activation.

The biological heterogeneity of parasitic agents and the chronic infections they often elicit offer important advantages for investigating the function and regulation of Th subsets in mice [3] as well as humans [4]. Th1 vs. Th2 polarization can be manifested in response to the same pathogen in genetically different hosts or be induced by different types of parasites in the same host. While the immune response to helminth infections is invariably characterized by secretion of Th2 cytokines, infections with intracellular protozoa and bacteria often induce a highly polarized Th1 response. This strong correlation between Th polarization and the nature of the invading pathogen may reflect the evolutionary pressure for the selection of specific defense mechanisms that promote host/pathogen co-existence.

Successful immune responses against invading microorganisms can turn detrimental when directed against self-antigen or environmental antigens. In most cases, autoimmunity manifests as a Th1-type response that results in uncontrolled destruction of the host itself due to the production of inflammatory cytokines [5]. On the other hand, allergic reactions against environmental non-infectious antigens are characterized by Th2 responses that closely resemble those observed during helminth infections [6].

In this review we will concentrate on factors that govern Th2 responses in helminth infections with special emphasis on the murine *Schistosoma mansoni* model and, where relevant, discuss the similarities between the pathways involved in parasite- and allergen-induced Th2 differentiation.

The Immune Response during Infection with *Schistosoma mansoni*

Like many other parasitic helminths, *S. mansoni* has a complex life cycle with a definitive mammalian and an intermediate invertebrate host [6]. Moreover,

different developmental stages occur in the mammalian host with the infective cercariae transforming into migrating schistosomula that differentiate into adult male and female worms. The mature worms take residence in the portal vasculature and females produce eggs that need to transit the intestinal wall and be excreted in order to perpetuate the life cycle of the parasite. The majority of the eggs, however, become trapped in hepatic sinusoids and trigger granuloma formation.

During the prepatent period of infection, the first 5 weeks following exposure to cercariae, the host immune response to worm antigens is weak and primarily Th1 in nature [6]. After the time of egg deposition, which takes place ~5 weeks postinfection, the immune response changes both quantitatively and qualitatively [6]. First, the intensity of the egg-specific immune response rapidly surpasses the response against worm antigens, because while the parasite does not multiply in the mammalian host, mature worm pairs produce 300 eggs/day throughout their extended life-span in the host. Second, a highly polarized Th2 response is established as a consequence of this new antigenic load. When injected in naïve mice *S. mansoni* eggs or soluble egg antigens (SEA) have an intrinsic ability to promote development of Th2 cells [6, 7]. The continuous production of Th2 cytokines, in particular the profibrotic factor IL-13, induces collagen deposition at the tissue sites of egg granulomas that leads to the development of fibrosis and ultimately results in severe liver pathology and organ malfunction [9]. Nevertheless, the host clearly profits from its decision to mount an egg-specific Th2 response rather than a Th1 response, because mice that can generate only Th1 responses suffer from cachexia and rapidly succumb to infection following egg deposition [6].

Role of IL-4 in *S. mansoni*-Induced Th2 Polarization

The list of immunomodulatory factors that have been described to influence the development of Th1 and Th2 cells over the past decade is extensive and complex (fig. 1). In the case of a Th2 response, while each of the factors listed can trigger Th2 polarization in particular settings, the presence of IL-4 at the time of CD4+ lymphocyte priming has by far the most dominant effect on the differentiation of Th2 cells. Thus, the simplest explanation for the selective induction of a Th2 response by *S. mansoni* eggs would be their ability to trigger IL-4 secretion from one of the cell types of the innate immune system. Despite extensive investigation, the contribution of IL-4 production by professional antigen-presenting cells (e.g. dendritic cells) to Th2 development has not been firmly established. In contrast, it is well documented that various types of granule-containing cells (e.g. basophils, eosinophils, mast cells, non-B non-T cells)

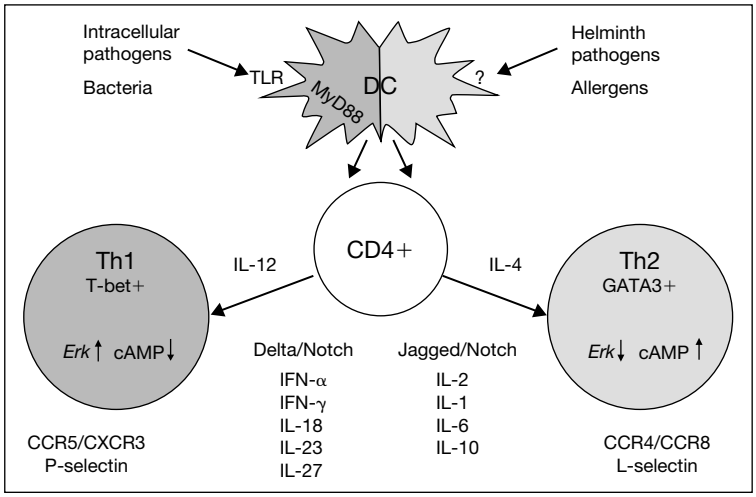

Fig. 1. Factors influencing Th1/Th2 polarization. When exposed to parasite extracts from pathogens known to induce polarized Th1 and Th2 responses DC are able to promote differentiation of the corresponding Th phenotype. In addition, multiple cytokines, chemokines, costimulatory molecules, and intracellular signals have been described to selectively drive commitment towards one or the other phenotype.

possess depots of preformed IL-4 that are rapidly released upon degranulation [9–13]. Moreover, a number of these cell types have been implicated as major sources of IL-4 production during helminth infection in vivo [9, 10]. For example, Sabin et al. [14] have shown that in mice injected with *S. mansoni* eggs, mast cells elicit the recruitment of IL-4-producing eosinophils. However, egg-induced Th2 responses were not compromised in IL-5-deficient mice, which are unable to develop eosinophilia [14]. Similarly, Th2 cytokine production and serum IgE levels in infected FcεRI-deficient mice were comparable to those observed in wild-type animals, despite the inability of non-B non-T cells in the former animals to secrete IL-4 in response to egg antigens [12]. Natural killer (NK) T cells have also been implicated as a possible accessory source of IL-4. The initial conclusion that mice lacking NKT cells mount normal egg-specific Th2 response [15] has been recently challenged by the observation of Faveeuw et al. [17] that *S. mansoni*-infected mice deficient in CD1d, the NKT cell-specific restriction element, display a reduced Th2 response. Together the above results suggest a scenario in which multiple sources of IL-4 play redundant roles in Th2 polarization during *S. mansoni* infection. Finally, there is also compelling evidence from studies in IL-4 chimeric mice [18] that CD4+ lymphocyte-derived IL-4 may be sufficient for successful Th2 development.

The key evidence for the limited role of accessory IL-4 in *S. mansoni*-induced Th2 differentiation came from experiments in mice with genetically engineered null mutations in genes encoding IL-4, IL-4R or the signaling molecule STAT-6. In direct agreement with the independent reports that demonstrated both in vivo and in vitro that conventional IL-4-producing CD4+ lymphocytes can develop in the absence of IL-4R signaling [18, 19], we showed that IL-4R- or STAT-6-deficient animals when infected with *S. mansoni* develop significant numbers of SEA-specific Th2 cells [20] despite the presence of an expanded population of Th1 cells. Importantly, this residual Th2 response was still able, through the action of IL-13, to trigger significant egg-associated liver immunopathology in *S. mansoni*-infected IL-4-deficient mice [21].

While IL-4R/STAT-6 signaling is not essential for priming of IL-4+ CD4+ T lymphocytes, IL-4 remains undeniably important in Th2 phenotypic maturation [18, 20]. This is because IL-4R signaling acts indirectly to enhance the frequency of Th2 lymphocytes by dampening the concomitant development of Th1 cells, as well as directly to influence/skew the fate of activated bystander CD4+ lymphocytes. Similarly, it has been shown that the primary response to another Th2-eliciting parasite, *Nippostrongylus brasiliensis*, is IL-4-independent, while the secondary response requires the cytokine [18]. In addition, IL-4 is not essential for Th2 polarization in some models of allergen-induced airway hyperreactivity [22]. Taken together, these studies clearly demonstrate that IL-4-independent Th2 development occurs in vivo and can be easily detected in response to helminth Th2 polarizing agents, and thus may reflect an initial step in CD4 T cell subset diversification that precedes the phase in which IL-4 plays a dominant role.

S. mansoni-Induced Th2 Polarization Requires DC: Evidence against a Default Pathway

The list of the factors known to influence Th1/Th2 polarization (fig. 1) manifests as a symmetrical set of pairs of pro-Th1 and pro-Th2 elements. Moreover, many of the factors display a cross-regulatory function in which they augment the development of one Th subset and suppress the other. Such antagonism can be considered an efficient process for polarizing the immune response towards the appropriate effector type. The groundbreaking studies on this issue focused on immunity to the protozoan parasite *Leishmania major*, which is notable for its ability to induce protective Th1 responses in C57BL/6 and other inbred mouse strains and exacerbated Th2 responses in BALB/c mice. In this system, neutralization of IL-12 in infected C57BL/6 mice resulted in the development of a Th2 response and ensuing disease, whereas neutralization of

IL-4 in infected BALB/c mice promoted a Th1 response and resistance [23]. These findings led to the view that the absence of a positive signal for Th polarization allows the response to default to the opposing pathway.

The default concept was rapidly accepted as a model of Th2 differentiation since it could circumvent the requirement for exogenous IL-4. According to the 'default hypothesis' Th1 or Th2 effector choice is determined by the presence or the absence of a signal that is required for Th1 priming, with IL-12 being the major candidate. IL-12 is produced by dendritic cells (DC), which due to their unique capability to present antigens to naïve CD4+ lymphocytes and maintain stable MHC-peptide complexes, are considered to be the most effective antigen-presenting cells. Consistent with the default hypothesis, the results from different laboratories agree that no significant production of IL-12 can be detected after DC exposure to extracts from various helminth parasites, including SEA. Moreover, *S. mansoni*-infected IL-12-deficient mice display a pattern of Th2 polarization indistinguishable from wild-type animals [24]. Conversely, the administration of exogenous IL-12 together with *S. mansoni* eggs completely blocks Th2 development and induces a typical Th1-type response [25].

Nevertheless, with more detailed studies specifically designed to test the default model of Th2 differentiation, it became increasingly apparent that this simplistic view, while applicable in some settings (e.g. murine *L. major* infection), cannot account for Th polarization in general. For example, while most non-helminth pathogens promote Th1 development by means of selective mechanisms that trigger IL-12 secretion by DC, no default to a Th2 cytokine profile was observed when Th responses to these pathogens were analyzed in an IL-12-deficient setting [7].

Perhaps the most compelling arguments against the default model for Th2 polarization have come from experiments employing in vitro models for CD4+ lymphocyte priming in the presence of DC conditioned with pathogen extracts. These studies clearly demonstrate that DC do not deliver only peptide/MHC complexes, but also provide signals that bias Th1/Th2 differentiation [26]. The active role of DC in SEA–mediated Th2 polarization was first demonstrated in an elegant study by MacDonald et al. [28] in which a Th2 response was induced in mice by injecting SEA-conditioned bone-marrow-derived DC. Using an in vitro bystander assay with OVA-specific transgenic CD4+ T lymphocytes, we later showed that SEA-treated CD11c+ $B220^{-}$ splenic DC are able to promote Th2 differentiation in vitro independently of direct TCR triggering by SEA [28]. In the absence of SEA, when optimal TCR cross-linking (signal 1) and engagement of costimulatory molecules (signal 2) are provided, Th cells proliferate extensively, but do not acquire a Th2 phenotype, a finding in direct disagreement with the default model. However, in the presence of SEA-conditioned DC a large proportion of CD4+ cells became Th2 cells, demonstrating a

requirement for a specific third signal in Th2 polarization. Conversely, when SEA was replaced with an extract from a pathogen known to induce Th1 responses, CD4+ lymphocytes acquired a Th1 phenotype [28]. Importantly, in agreement with the IL-4-independent Th2 priming observed during *S. mansoni* infection discussed above, SEA-exposed DC isolated from IL-4-deficient mice were able to trigger Th2 polarization both in vivo [29] and in vitro [28]. Importantly, neither B cells [28] nor macrophages [SS and DJ unpublished data] were able to substitute for DC in mediating SEA-driven Th2 polarization.

Taken together, these results support the concept that both Th1 and Th2 commitment requires a specific third signal provided by DC and that these signals may be distinct from those provided by the polarizing cytokines IL-12 and IL-4.

S. mansoni Th2 Polarization As A Negative Pathway: Down-Regulation of DC Functions

A critical issue concerns the nature of the third signals provided by DC that drive CD4+ T lymphocytes to selectively express Th1 vs. Th2 cytokine response patterns. DC represent heterogeneous cell populations and one of the initial hypothesis was that activation of different subsets of DC leads to differential Th1/Th2 commitment. Work by Madonado-Lopez et al. [31] proposed that murine CD8α+ and CD8α− DC subsets instruct the development of Th1 and Th2 cells, respectively. Similarly for human cells, monocyte-derived DC were found to induce Th1 differentiation in an IL-12-independent fashion, whereas DC derived from plasmacytoid cells induced Th2 differentiation even in the presence of anti-IL-4 antibodies [31]. Nevertheless, even if such intrinsic preferences may exist in the absence of pathogen-derived signals, the requirement for different DC subsets is not absolute and their influence can be overridden by appropriate external stimuli. Indeed, SEA is able to exert a Th2-polarizing effect on a wide variety of DC including bone-marrow derived DC [28], murine CD8α+ and CD8α− DC [28, SS and DJ unpublished data] as well as monocyte-derived human DC [32].

Comparative analysis of DC conditioned by pathogen extracts known to prime Th1 and Th2 responses have revealed clear differences in their state of activation. In the former case DC rapidly upregulate expression of numerous co-stimulatory molecules, cytokines and chemokines [33]. In contrast, in many cases Th2 pathogens trigger only minimal responses in DC. This conclusion is supported by results from microarray-based analyzes demonstrating that in the presence of Th1 stimuli large numbers of genes are upregulated in DC, while exposure to SEA or other helminth Ag results in minimal gene expression [34, 35]. Lack of full activation is a common feature of DC stimulated by

different helminth pathogens (e.g. *Brugia malayi* [34], *N. brasiliensis* [36]). A somewhat different view has emerged from the work of Trottein et al. [38] who showed that whole *S. mansoni* eggs (as opposed to SEA extract [35]) induce bone marrow-derived DC to produce IFN-β that in turn upregulates the expression of a large family of IFN-inducible inflammatory mediators, although the in vivo relevance of this pathway for Th2 development has yet to be determined.

DC are able to recognize invading microorganisms through a series of pattern-recognition receptors that are specific for common, highly conserved motifs found in pathogens. The best-studied family of these surface molecules is the Toll-like receptors (TLR) [38]. There have been two major reasons for considering the involvement of TLR in the selective polarization of Th cells by DC. First, the pathogens recognized by TLR include many bacteria, protozoa, viruses and fungi known to promote Th1 responses. Second, DC triggered through TLR express many of the functions that one would predict to be involved in Th1 commitment. When experimentally tested the association between Th1-biased responses and TLR-dependent signaling was readily confirmed. Conversely, when assessed for its role in Th2 responses, the contribution of TLR signaling appeared to be minor if any. To perform these analyzes, mice deficient in MyD88, a critical adapter molecule for most TLR, were employed and it was shown that MyD88 is required for Th1 polarization by type 1 pathogen extracts (e.g. *Toxoplasma gondii*), but not for the generation of SEA-induced Th2 response in vivo [7] and in vitro [28].

In order to better understand the effects of DC conditioning for Th1 vs. Th2 priming, we analyzed the phenotype of DC exposed simultaneously to SEA and an extract from the Th1 pathogen *T. gondii*. Unexpectedly, we found that SEA induces a down-regulation of DC responsiveness to Th1 stimuli as measured by both expression of costimulatory molecules and chemokines [28]. These observations are in agreement with a later report by Kane et al. [36], in which the inhibitory effect of SEA on TLR-mediated activation of DC was analyzed at the protein as well as the gene expression level. Similarly, murine DC exposed to excretory/secretory extracts from *N. brasiliensis* are profoundly suppressed in their capacity to produce bioactive IL-12 [36]. Furthermore, ex-vivo studies with the infective larval stage (L3) of *B. malayi* demonstrate down-regulation of genes involved in antigen processing and presentation in human DC [39].

Taken together, these findings plus the observation that in the absence of Th2-promoting factors unstimulated mature DC do not promote Th2 polarization strongly argue that down-regulation of DC function is critical for Th2 cell development. Interestingly, different non-microbial agents known to down-regulate DC functions, such as IL-10 [40] and PGE_2 [41], have been shown to

promote Th2 effector choice. At present there is little information of how different allergens affect DC functions. Nevertheless, results from one of the best-characterized models involving Der p 1, the major house dust mite allergen, suggest that a decreased rather than an increased state of DC activation is associated with Th2 polarization [42]. Moreover, pollen-associated phytoprostane allergens, known to augment Th2 polarization, were recently shown to inhibit IL-12 production by DC [43].

S. mansoni Th2 Polarization As A Positive Pathway: Selective Activation of DC

While the correlation of DC activation state with development of Th1 vs. Th2 effectors is an attractive model, the direct cause and effect relationship has yet to be established. The ability of Th2 stimuli to suppress DC functions induced by Th1 stimuli in general, rather than IL-12 secretion in particular, might be a prerequisite condition that would decrease the sensitivity for 'undesired' Th1 responses and at the same time allow the recognition of a weak positive signal by CD4+ cells required for Th2 polarization. Indeed, factors able to positively influence the Th2 response have also been identified. For example, in order to efficiently prime a Th2 response in vivo, SEA-primed bone-marrow DC need to express CD40 [44] although this requirement does not appear to be essential for Th2 polarization during *S. mansoni* infection [45]. In common with human DC exposed to SEA [32], murine DC pretreated with *N. brasiliensis* excretory antigens upregulate the expression of the maturation marker OX40L [36]. However, when OX40-deficient mice were infected with *N. brasiliensis*, a Th2 response comparable to infected wild-type mice was observed [46]. While OX40L/OX40 as well as ICOSL/ICOS interactions have been proposed to play a critical role in Th2 commitment, their primary role in vivo appears to be to sustain the effector phase of an inflammatory Th2 response rather than mediate polarization itself.

Although TLR engagement clearly leads to activation of DC and subsequent Th1 polarization, there have been several recent reports describing TLR involvement in the induction of helminth-induced Th2 responses. The schistosome-associated sugar LNFPIII that in multimeric form is able to promote Th2 polarization appears to require the expression of TLR4 [47], although the lectin receptor DC-SIGN has been identified as its primary receptor on DC [48]. Interestingly, TLR4 has also been implicated in Th2 development in the OVA-induced model of asthma involving low dose LPS [49], as well as in the ability of the filarial nematode phosphorylcholine-containing secreted product, ES-62, to trigger DC [50]. While not directly demonstrated to participate in the process

of Th2 polarization, double-stranded RNA from schistosome eggs was shown to preferentially activate TLR3 [51]. In addition, under certain experimental conditions Th2 response induction by flagellin from *Salmonella typhimurium* requires TLR5 expression [52]. Nevertheless, in all these conditions TLR engagement appears to trigger only a partial signal leading to neither full DC activation nor optimal expression of proinflammatory cytokines. This partial DC stimulation is further supported by the findings that TLR2 ligands, which in general are known to induce a lower degree of DC activation when compared with ligands for other TLR ligands, can preferentially trigger Th2 polarization [53]. Since in most of the studies described above the downstream signaling pathway was not examined, and in one setting TLR lacking a functional intracytoplasmic domain remained active [50], it is currently unclear whether TLR signaling is required for Th2 polarization or if TLR are simply required for facilitating contact and/or internalization by DC of some (e.g. less immunogenic) but not all Th2-promoting agents.

The DC surface molecules specifically induced by Th2 stimuli remain largely uncharacterized. Recent reports by Maekawa et al. [55] and Amsen et al. [56] suggest that selective expression of either Delta or Jagged molecules on DC may play a critical role in Th effector choice. Delta ligands are induced by Th1 stimuli downstream of TLR/MyD88 signaling and are involved in Th1 polarization. In contrast, expression of Jagged ligands does not involve TLR activation and promotes Th2 polarization. Importantly, Jagged constitutes an instructive signal for Th2 differentiation that is independent of IL-4. In addition to increasing levels of GATA3, the key transcription factor involved in Th2 differentiation, the interaction between Jagged and its receptor Notch expressed on CD4+ cells promotes Th2 differentiation by directly regulating transcription of the IL-4 gene. The role of Jagged in Th2-type parasitic infection is currently a subject of intensive investigation.

While the nature of the positive signal(s) on DC conditioned for Th2 priming has not yet been critically identified it is clear that such DC express a specific phenotype distinct from that of either the tolerogenic DC that cause CD4+ unresponsiveness or DC in a neutral state of activation that induce CD4+ cell activation but not their differentiation.

How Do SEA-Conditioned DC Promote IL-4 Secretion in CD4+ Cells?

A major difference between the processes of Th1 vs. Th2 commitment is the degree of their dependence on the instruction by non-CD4 T cells. Development of Th1 cells is not only actively driven by fully activated DC, but

in addition, fully mature Th1 responses require persistent production of Th1 polarizing cytokines (e.g. IL-12, IL-18, IL-23, IL-27), all of which are exclusively produced by activated APC and not by CD4+ lymphocytes. In contrast, once Th2-primed DC induce an initial burst of IL-4 in a few activated Th lymphocytes, the subsequent amplification and stabilization of the Th2 response is self-sustained within the CD4+ T cell population by autocrine IL-4. Thus, a key question in Th2 polarization is to understand what factors and circumstances trigger early IL-4 production by activated Th precursor cells.

Using an in vitro model for priming murine CD4+ cells, we demonstrated that the Th2-promoting activity of SEA-conditioned DC is associated with a reduced frequency of Th lymphocytes exiting the first division of the cell cycle despite a comparable level of cell activation [28]. When the latter was mimicked by drug-induced arrest after the S phase of the first cycle in peptide-stimulated CD4+ cells, a specific induction of Th2 cells was observed [28]. Similarly, when the in vivo kinetics and amplitude of the OVA-specific primary T cell response induced in the presence of an extract from *Ascaris suum* was analyzed, the immunomodulatory effect was associated not only with a Th2 bias of the antigen-specific response but also a reduced proliferative capacity of the antigen-specific responder T cells [56]. These findings suggest that the early burst of IL-4 in CD4+ lymphocytes may result from a temporary delay in initial T cell cycling. Importantly, however, even when a temporary delay in initial T cell cycling is induced, fully activated DC as a result of stimulation through TLR, will completely abolish the capacity of CD4+ lymphocytes to differentiate into Th2 cells and will instead promote the generation of Th1 cells [28]. These findings again reiterate the critical importance of the state of activation of DC as the dominant and overriding factor in Th differentiation.

While the role of CD4+ T cell hyporesponsiveness on Th effector choice is very difficult to test in vivo, correlative evidence can be readily obtained in vitro. For example, in the absence of parasite-derived signals Th polarization in vitro is influenced by the DC:T cell ratio with lower ratios favoring Th2 development [57]. When the DC:T cell ratio is constant, low doses of antigen promote Th2, whereas intermediate doses lead to Th1 development [58]. Interestingly, low-dose antigen was found to induce a similar delay in initial CD4+ cell cycling as that observed with SEA-exposed DC and in the presence of optimal dose of antigen [SS and DJ unpublished data].

Initially, the evidence that Th2 differentiation results from suboptimal DC/CD4 interactions was used to support the default model of Th2 differentiation in which the absence or merely existence of a signal, favors a Th2 phenotype. However, based on our current understanding of the state of activation of DC with Th2 inducing potential, we would argue that helminth parasite extracts play an active role in decreasing the intensity of the interaction at the DC/CD4

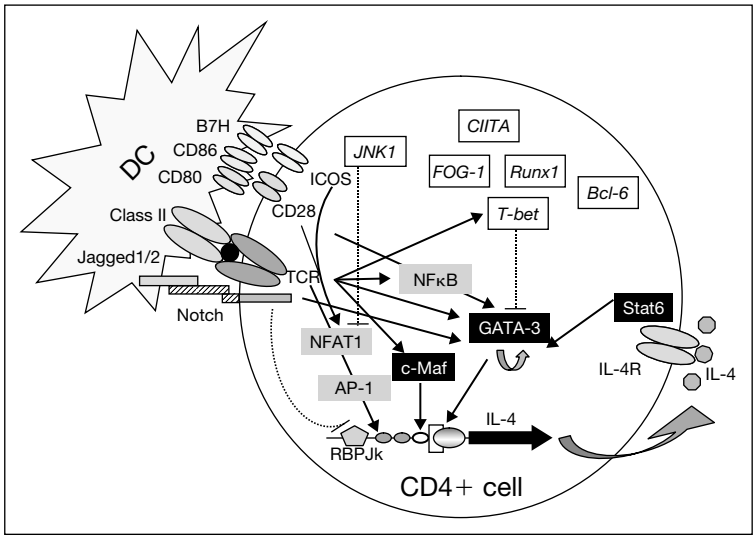

Fig. 2. Molecular pathways involved in the expression of the IL-4 gene in activated CD4+ T cells. Transcription of the IL-4 gene is controlled by multiple positive and negative elements. Although the strength of TCR signal together with costimulatory molecules may play an important role, the transcriptional factors induced by TCR ligation (gray rectangles) are not Th2 specific. Among the Th2-specific transcriptional factors c-Maf, GATA-3, and Stat-6 (black rectangles), GATA-3 is the master switch for Th2 development. It is expressed in resting Th cells and can direct IL-4-independent Th2 development through autoactivation. At the same time multiple control elements, such as T-bet, FOG-1, Runx1 (open rectangles) negatively regulate GATA-3, and together with other suppressors of Th2 development (e.g. Bcl-6 and JNK1) prevent uncontrolled IL-4 expression. While the nature of the signals provided by Th2-inducing DC are still unknown, they may involve Notch activation that directly induces GATA-3 as well as conversion of the RBPJk suppressor into an IL-4 gene activator. The initial burst of IL-4 in turn mediates amplification and full stabilization of Th2 effectors through the Stat-6-dependent pathway. Thus, the regulation of the IL-4 gene represents a fine balance between multiple positive and negative signals.

interface regardless of whether this is achieved by negative or positive signals from DC. Although at present this concept has not been confirmed in vivo during helminth infection, it is supported by a study involving mutants in the Tec kinase family that play an important role in TCR signaling [59]. Despite a profound defect in TCR signaling, which is manifested in severely impaired CD4+ T cell proliferation, mice deficient in both Itk and Rlk kinase mount normal Th2 responses to *S. mansoni* eggs in vivo [59].

Since IL-4, a major Th2 polarizing factor, is also a growth factor for Th cells, the hypothesis that delayed cell progression leads to early IL-4 expression

appears contradictory. However, it is important to point out that at present both positive and negative factors are implicated in the control of IL-4 gene expression (fig. 2). The activation of CD4+ lymphocytes by TCR cross-linking and engagement of costimulatory molecules is followed by progression through S phase which is a prerequisite for the demethylation of the chromatin that is necessary to make the IL-4 locus accessible to transcriptional factors [60]. Importantly, GATA-3, present at low levels in resting Th cells, can induce Th2 differentiation even in the absence of IL-4R signaling [61]. However, spontaneous expression of GATA-3 is negatively controlled by a whole set of inhibitory regulatory transcriptional elements such as ROG, FOG-1, Runx and T-bet [62, 63]. How signals received from DC are networked with signals from the TCR and subsequently reflected in the expression level of these different transcription factors during cell division is not clear. Our current data suggest that a decreased rate of Th cell proliferation may promote GATA-3 activity. Interestingly, the immunodominant epitopes characterized for some allergens (e.g. Bos d 2) are suboptimal for human T cells and induce weak proliferative responses [64]. Such poor TCR stimulation has been suggested to be a factor accounting for Th2 polarization in general [65].

Conclusions

Recent advance in DC immunobiology have dramatically improved our understanding of Th2 commitment as it relates to helminth pathogens. However, many key issues still remain unresolved. Of critical importance is the characterization of helminth molecule(s) with Th2-inducing activity and their corresponding DC receptors and signaling pathways. Also, with the new in vitro systems at hand, it will be important to carefully analyze the events occurring at the DC/CD4 T cell interface that determine subsequent Th2 differentiation. Although distinct in some aspects, allergens and helminth-driven Th2 polarization appear to share many important features. Continuous progress in both of these two research areas is important and vital to our ultimate understanding of the biological significance of the Th2 response as well as for the rationale design of interventions targeting this pathway that underlies both allergic and helminth-induced diseases.

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Regulation of Dendritic Cell Function by Pathogen-Derived Molecules Plays a Key Role in Dictating the Outcome of the Adaptive Immune Response

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Abstract

There is increasing awareness that dendritic cells (DCs) can interpret pathogen-inherent signals and play a pivotal role in polarizing Th cell differentiation. Polarized Th1 responses are induced by DCs, which respond to pathogen-derived TLR ligands to mature and produce IL-12 and related cytokines that are instrumental in Th1 cell outgrowth. In contrast, DCs exposed to SEA (soluble egg Ag from the helminth parasite *Schistosoma mansoni*) retain a (modified) immature phenotype and induce Th2 responses. In addition to providing positive signals for Th1 cell development, DCs activated to mature by TLR-engagement also provide a potent negative signal that prevents the development of Th2 cells. Production of this signal is dependent upon a MyD88-dependent signaling pathway in DCs. In contrast, exposure of DCs to SEA severely limits their ability to respond to inflammatory TLR ligands such as LPS and CpG. Thus as part of their pathogen-specific response programs, DC can exert negative as well as positive signals for Th response polarization. These effects may have powerful and systemic effects on disease outcome.

During infection with schistosomes, and most other parasitic helminths, the dominant T helper cell response is Th2-like [1]. Production of IL-4 and IL-5 by these cells explains the elevated IgE and eosinophil levels that characterize helminth infections. There are very important host-protective attributes conferred by this type of response [2]. Th2 cells are capable of orchestrating granuloma formation and of helping egg Ag-specific antibody production, both of which are essential for the prevention of hepatotoxic effects associated with

egg deposition in the liver. Moreover, Th2 responses ameliorate potentially lethal inflammatory events that can occur during infection. Lastly, Th2 cells are implicated in the development of resistance to infection. It should be noted that there are also negative consequences of Th2 responses, not least of which in schistosomiasis is the potential for severe fibrosis stimulated by another Th2 cytokine, IL-13 [3]. There is considerable ongoing interest in elucidating the mechanisms by which the immune system is able to recognize helminth parasites and respond with the appropriately polarized immune response. Here we discuss recent developments in our understanding of the role of dendritic cells (DCs) in this process. While there are several subsets of DCs, clearly distinguishable by surface markers expression, most of our discussion will reflect the available data, and focus on CD11b+CD11c+, so called myeloid, DCs.

Dendritic Cells and Their Role in Th Cell Activation

DCs are specialized highly phagocytic cells that are found in most tissues [4]. They continuously sample their environment and proteolytically degrade acquired proteins into peptides that can be complexed with MHC class II (MHCII) molecules for display at the cell surface for recognition by CD4 T cells. By virtue of the fact that they express a panel of pattern recognition receptors (PRRs), most especially Toll-like receptors (TLRs) [5] and C-type lectins [6], that recognize pathogen-specific molecular patterns, DCs are able to sense infectious organisms. TLR-ligation generally leads to changes in expression of a large number (hundreds) of genes that result in significant changes in DC function and behavior [5]. This process is called maturation, since the cells mature from being poorly capable, to being highly capable, of activating naïve Th cells. This reflects the fact that the genes upregulated by TLR ligation include MHCII and costimulatory molecules such as CD80/CD86, which by ligating T cell receptor and CD28, respectively, together provide the required signals 1 and 2 for T cell activation. In addition, maturing DCs change the panel of chemokine receptors that they express such that their migration from tissues to draining lymph nodes is promoted, and begin to produce a panel of cytokines that have both local effects on innate effector cells, as well as on the biology of responding CD4+ cells. Paramount amongst these, in terms of our discussion, is IL-12, a cytokine that plays a pivotal role in promoting the development of Th1 cells [7]. In summary then, TLR-activated DCs are particularly adept at carrying Ag from peripheral tissue sites of infection to LNs where they can meet and provide activation and polarization signals for Ag-specific T cells [4].

In contrast to TLRs, ligation of C-type lectins does not induce DC maturation [6]. Most evidence suggests that the primary function of these PRRs is to increase Ag uptake efficiency by acting as Ag-recognition receptors; in this way DCs are able to accumulate foreign versus self proteins most effectively and thus more efficiently present peptides derived from non-self. However, recent data indicate that the C-type lectin DC-SIGN antagonizes TLR-signaling, and could be playing an important role in the interaction of schistosome Ag with DCs [8] (see below).

Th Response Polarization

Simplistically, Th responses can be considered to polarize into those dominated by IFNγ-producing Th1 cells, or IL-4/IL-5/IL-13-producing Th2 cells. Of great importance in this process are the cytokines IL-12 and IL-4 [9]. DCs, macrophages, and neutrophils are the major physiological producers of IL-12 [10]. The participation of these cells in innate responses to inflammatory pathogens prior to, or coincident with, the initiation of adaptive Th responses can create an environment rich in IL-12, which promotes the outgrowth of Th1 cells. Other cytokines such as IL-18 and IL-27, which can be made by DCs, and IFNγ, which is a major product of NK cells as well as of Th1 cells themselves, support this pathway [7]. Thus, non-Th cells that are involved in innate immunity produce factors that promote polarization of the adaptive T cell response. For Th2 response development IL-4 rather than IL-12 is a crucial polarizing factor. The cellular source of the IL-4 important for Th2 response development is generally unclear, but it is likely provided by the responding Th cells themselves rather than by accessory cells. Additional to central roles for IL-12 and IL-4, there is evidence for the participation of type I interferons and specific costimulatory events including the ligation by CD80 on DCs of CD28 on CD4 cells for the development of Th1 cells, and for IL-6 and the delivery of costimulation by CD86 or OX40L in Th2 response development. However, unlike the case of IL-4 and IL-12, the relative importance of these additional factors in Th1/Th2 decision process varies greatly from model to model (reviewed in [11]). Nevertheless, the fact that DCs can be the source of many of the cytokines and costimulatory signals implicated in Th polarization suggests that the differential expression of these factors by DCs could account for their ability to direct Th1 vs. Th2 response development. Thus there has been considerable interest in comparing the ability of different types of Ag to induce DC maturation, and in attempting to understand whether this reflects in any way preferential ligation of different PRRs [12].

The Interaction of SEA with DCs

Interest in the role of DCs in Th2 response induction during schisotosomiasis intensified when it was discovered that bone marrow derived DCs pulsed in vitro with a soluble extract of schistosome eggs (SEA) induced SEA-specific Th2 responses when injected into mice [13], and that SEA could condition human DCs to polarize Th responses in a Th2 direction in vitro [14]. This contrasted strikingly with the result of pulsing DCs with bacterial Ag, where the induced Ag-specific Th response was Th1-dominant [13, 14]. Moreover, SEA is able to condition splenic DCs to drive Th2 polarization of DO.11.10 OVA-specific TCR-transgenic CD4 cells in vitro [15]. These results suggest that DCs play a decisive role in dictating the polarization bias of the Th response, and that contact between DCs alone and SEA is sufficient for SEA to elicit Th2 responses. Thus detailed analyses of the outcome of the interaction between DCs and SEA should allow us to understand the underlying basis of Th2 polarization in schistosomiasis and, possibly, related diseases.

Initial targeted analyses of expression of the maturation markers MHCII, CD80, CD86, CD40, OX40L, IL-6, IL-12, IL-15, IL-18 suggested that DCs are not induced to mature by SEA [13]. Thus, it can be envisaged that SEA induces Th2 responses simply by default, owing to its failure to elicit IL-12 production. While attractive, this explanation is, for reasons that will be enlarged upon later, overly simplistic and likely to account only partly for the Th2-response inducing characteristics of SEA. An alternative view, first proposed conceptually by Kapsenberg and coworkers [16], is that SEA stimulates the expression by DCs of a 'third signal' (in addition to the first and second signals provided by MCHII/peptide and costimulatory molecules, respectively) that gives positive instruction for Th2 development. Recent analyses of SEA-pulsed DCs have attempted to identify the nature of such a third signal.

Broad scale unbiased assessments of changes in gene expression induced by exposure to SEA revealed that it does induce changes in expression of a small number (<30 when stringent statistical parameters are used), the majority of which are not affected by the TLR4 ligand *Escherichia coli* LPS-induced maturation process [17]. Based on reported functions of the SEA-affected genes, it is currently unclear how any of them might confer upon DCs the properties known to accompany exposure to SEA, such as the ability to induce Th2 responses. Nevertheless, more detailed analyses of these third signal candidates are clearly warranted.

Not least because it is generally a signal for IL-12 production, TLR-ligation is considered to condition DCs to induce Th1 polarization [12]. The fact that SEA fails to elicit the major changes in DC behavior induced by the widely investigated TLR ligands LPS, CpG or, in our own hands, heat-killed

Propionebacterium acnes, fits with this view [17]. Moreover, MyD88, a major component of the signaling pathways elicited by most TLRs, is not required for SEA to induce Th2 responses in vivo or in vitro, but has been shown to be essential for Th1 response induction [15, 18]. Thus we, and others, postulated the simple viewpoint that SEA does not contain a TLR-ligand, and that this in part accounts for the relatively immature status of DCs exposed to SEA, and for the ability of these DCs to induce Th2 responses. Recent findings require that this view be revised. Several reports indicate that TLR-ligation is integral to the conditioning of DCs for Th2 response induction. For example, the TLR2 ligands *Porphyromonas gingivalis* LPS [19], synthetic bacterial lipopeptide (Pam3cys, [20]) and schistosome egg lyso-phosphatidylserine (which is not present in SEA) [21] promote the ability of DCs to induce Th2 responses. The effects of ligation of DC TLR2 by Pam3cys, and exposure of DCs to SEA, have been shown to have much in common, both leading to sustained phosphorylation of ERK1/2 in the absence of phosphorylation of other MAPKs, p38 and JNK [22]. One downstream target of ERK1/2 is c-fos, and increased levels of this transcription factor were demonstrated in SEA and Pam3cys pulsed DCs. Remarkably, RNAi-mediated loss of c-fos expression in DCs resulted in a population of cells capable of making IL-12 in response to SEA in these experiments [22]. Thus there is evidence that SEA suppresses IL-12 production via activating an ERK-dependent pathway upstream of c-fos. Whether SEA-induced IL-12 production in the absence of c-fos reflects an altered outcome of ERK phosphorylation or the effects of a coincident activation pathway, induced by SEA and normally suppressed by c-fos, is unclear at present, but of considerable interest.

In separate experiments, a key glycan component (lacto-n-fucopentaose III) of SEA was shown to initiate sustained ERK phosphorylation via a TLR4-dependent process, supporting the view that TLR-initiated ERK activation is key to the generation of the third signal for the induction of Th2 responses by DC, but implicating TLR4 rather than TLR2 as the receptor for SEA [23]. These data are consistent with the reported ability of low-dose *E. coli* LPS to promote Th2 response development in an asthma model [24].

Eggs and SEA Induce Different Responses – An Unexpected Finding

Both eggs and SEA induce Th2 responses, and it is thought that the physiologically/immunologically relevant components of eggs are those that are secreted in vivo by living eggs, and are contained within the SEA extract. Thus, it seemed reasonable to assume that the response of DCs to SEA and to living

eggs would be similar. However, microarray analyses revealed that, in contrast to SEA, live eggs induce in DCs a signaling pathway leading to the expression of an array of genes including IL-12 p40, type I IFN and, as a result of autocrine/paracrine activation, IFN-stimulated genes [25, 26]. These responses have been found to reflect MyD88-dependent and independent signaling via TLR2 and TLR3 [26]. While the ligand for TLR2 remained undefined, that for TLR3 turned out to be dsRNA. This is the first evidence for a physiologically relevant non-viral source of dsRNA ligand for TLR3 [26] (the authors suggest that egg-derived dsRNA may reflect functional RNA interference and/or retrotransposons in schistosomes). While it remains unclear how the DC activation pathway initiated via TLR2/TLR3 signaling plays into the preferential induction of Th2 responses by eggs, it is logical to assume that these manifestations of the exposure of DCs to eggs contribute to the potent antigenicity of this stage of the schistosome.

SEA Inhibits TLR-Initiated DC Maturation

In analyses of the effects of SEA on DCs a major recent finding is that SEA has profound suppressive effects on signaling initiated by classically inflammatory TLR-ligands. Indications of this have been provided by studies in which DCs have been co-pulsed with SEA plus *E. coli* LPS [27], *P. acnes* [28], or soluble *T. gondii* antigen (STAg) [15]; in each case activation by the inflammatory stimulus is substantially inhibited by SEA. We have found that reduced responsiveness to TLR-ligands in part reflects the fact that SEA augments TLR ligand-induced production of IL-10 [17]. However, analyses of IL-10−/− DCs revealed distinct IL-10-independent suppressive effects of SEA. IL-10 independent mechanisms are evident in the suppression of TLR ligand-induced MAPK and NF-κB signaling pathways. Although initial analyses focused on the ability of SEA to inhibit IL-12 production, or costimulatory molecule upregulation, detailed microarray based analyses have a significant effect of SEA on the expression of over 100 LPS-regulated genes [17]. These findings indicate that SEA exerts potent anti-inflammatory effects by directly regulating the ability of DC to respond to proinflammatory TLR ligands.

How SEA is able to suppress TLR-initiated signaling is the focus of ongoing research. One possibility, based on the work outlined above, is that inhibition of inflammatory TLR-ligand initiated signaling is regulated by SEA-mediated ligation of TLRs (such as TLR2) that initiate anti-inflammatory pathways. A second possibility is based on recent reports that the C-type lectin DC-SIGN recognizes the major Lewis x (Galbeta1,4(Fucalpha1,3)GlcNAc) glycan epitope of SEA [29]. This is of considerable interest, since binding of

the *Mycobacterium tuberculosis* component manLAM to DC-SIGN has been shown to inhibit the production of IL-12, and promote the production of IL-10 elicited by the TLR4 ligand *E. coli* LPS [8]. Thus DC-SIGN ligation confers an anti- rather than pro-inflammatory phenotype on DCs. The ability of DCs to acquire SEA via DC-SIGN may also go some way towards explaining why SEA is such a strong antigen. Of considerable interest, given these findings, is that the unusual α_3-fucose-containing sugar modifications on SEA play a crucial role in Th2 response induction [30]. This is illustrated by the findings that non-polarizing antigens modified with LNFPIII gain the ability to induce Th2 responses [31]. Thus the post-translational modifications that allow egg proteins to bind to DC-SIGN are the same as those that confer the ability to induce Th2 responses, suggesting cause and effect.

TLR-Ligand-Activated DCs Provide Negative Instruction for Th2 Response Polarization

In many instances, the absence of IL-12 is not sufficient to allow Th2 response development. Immunizations with *Toxoplasma gondii* or with *M. avium* induce strong Th1 responses in WT mice but fail to stimulate Th2 responses in IL-12−/− animals [18]. Moreover, DCs pulsed with *P. acnes* induce a polarized Th1 response in WT mice, yet *P. acnes*-pulsed IL-12−/− DCs fail to induce a Th2 (or Th1) response when injected into IL-12−/− mice [32]. On the basis of these findings we hypothesized that some Th1 response-inducing pathogens would induce strong IL-12-independent negative signals for Th2 response development [32]. Data from co-cultures of DCs pulsed with ovalbumin (OVA) plus or minus TLR-ligands (LPS, CpG, *P. acnes*), plus OVA-specific $CD4^+$ cells from OTII TCR-transgenic mice, have provided evidence to support this hypothesis. In these cultures, OVA-pulsed DCs stimulate the development of discrete populations of IL-4-producing (Th2) and IFNγ-producing (Th1) CD4+ cells. However, OVA-pulsed, TLR-ligand-stimulated DCs profoundly suppress Th2 cell outgrowth even under conditions where IL-12, IFNγ, or other potential inhibitors such as IL-18, IL-23 or IL-27, are absent or are blocked [32]. Production of the negative signal in this system is dependent upon a MyD88-dependent signaling pathway in DCs. This is striking in light of the fact that MyD88−/− mice have been shown to develop Th2 responses after immunization with *T. gondii* and *Mycobacteria* antigens [18]. The facts that SEA: (1) fails to initiate DC maturation analogous to that observed in, for example, *P. acnes*-activated DCs; (2) is able to induce Th2 responses in the absence of MyD88, and (3) inhibits the ability of *P. acnes*, LPS and CpG, (as well as other TLR-ligands) to induce DC activation, are consistent with the

notion that SEA (and other Th2 antigens?) promotes Th2 response polarization in part by preventing TLR-ligands from stimulating the production of inhibitory signals that block Th2 cell development.

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Glycans Modulate Immune Responses in Helminth Infections and Allergy

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Abstract

Infections of humans and animals by parasitic helminths share key features with atopic diseases, such as allergic asthma. Both diseases lead to the induction of high levels of Th2-type cytokines associated with abundant IgE production and eosinophilia. This immunological association has raised strong interest in the nature of the molecules that promote Th2 and regulatory T cell responses, and the molecular mechanism. Complex carbohydrates are potent inducers of Th2 responses, and carbohydrate antigens (Ags) can stimulate the production of different classes of glycan-specific antibodies (Abs), including Th2 associated IgG but also non-specific IgE. In this review we focus on the immunological responses towards glycan Ags derived from allergens and parasitic helminths, especially schistosomes. Biological effects of carbohydrate Ags are dependent on recognition of these Ags by carbohydrate-binding proteins (lectins). Cell-surface C-type lectin receptors (CLRs), such as DC-SIGN, L-SIGN, the mannose receptor, macrophage galactose binding lectin, and other lectins, such as the soluble collectins and galectin-3, recognize particular glycan Ags of schistosomes and allergens, which may contribute to orchestrate Th2 associated adaptive responses. Remarkably, schistosomes express 'self glycan' Ags that are recognized by CLRs on DCs, whose principal function is thought to capture self-glycan Ags and generate regulatory T-cells to induce tolerance to these Ags. By expressing such self-glycan Ags, schistosomes may deceive the host immune system to their own benefit. The host protects itself against too much damage by down-regulating helminth-induced Th2 immune responses, and may thus simultaneously be protected against excessive Th2 cell-mediated allergic responses.

Infections by parasites that cause malaria, African trypanosomiasis, leishmaniasis, lymphatic filariasis, and schistosomiasis are a major worldwide

health concern. Among them, parasitic worm (helminth) infections rank as a major cause of morbidity and suffering especially in the developing countries. A striking feature of parasitic helminths is that they trigger strong T helper type 2 (Th2) immune responses in their hosts. Moreover, the worms modulate and evade the host immune response, to enable their survival, migration and development in different host tissues.

Infections of parasitic helminths share key features with allergic diseases such as allergic asthma. Both helminth infections and allergic diseases lead to the induction of high levels of Th2-type cytokines associated with abundant IgE production and eosinophilia. The clinical outcomes of helminth infections and allergy, however, are clearly different, since in helminth infections allergic symptoms are generally lacking. Remarkably, many studies show an inverse relationship between helminth infections and atopic reactivity, which suggests that helminth infections can protect against allergic diseases. A heavy and continuous immune stimulation, such as occurs in chronic helminth infections, may lead to the generation of a strong regulatory anti-inflammatory network. It is thought that in individuals with chronic helminth infection, regulatory T cells down-regulate excessive immune responses to protect the host, not only against parasite-induced, but simultaneously against excessive allergen-induced, inflammatory reactions [1, 2].

The immunological association between helminth infections and atopic diseases has raised strong interest in the nature of the molecules that promote Th2 and regulatory T cell responses, and the molecular mechanism of the polarized immune response. An interesting class of molecules in this respect is the complex carbohydrates, which show similarity and cross-reactivity between many living organisms. Increasing evidence indicates that carbohydrates are potent inducers of Th2 responses [3, 4]. Carbohydrates are built up from a limited number of monosaccharides to yield complex structures with a tremendous diversity in composition and linkage types between the sugar residues. In addition, the carbohydrate residues may be further modified by aglyco substituents, such as O-phosphorylcholine, O-methyl, and O-sulfate groups. Despite this diversity, many glycan Ags are shared between different organisms that are phylogenetically very distant. High mannose type N-glycans, for example, are widely observed in eukaryotes, including humans, worms, and plants (fig. 1; table 1). But glycan abundance even for high mannose-type N-glycans can be different; such glycans on mature glycoproteins are not abundant in human glycoproteins, but they are more common in many plant allergens and parasite glycoconjugates. Plants, helminths, and other invertebrates such as insects and snails share ‘common’ glycan determinants that are not found in humans (table 1). Such glycan Ags, as well as non-human ‘species-specific’ glycan Ags, are highly immunogenic and represent a major focus for the host immune response in helminth infections. It is therefore expected that immunogenic

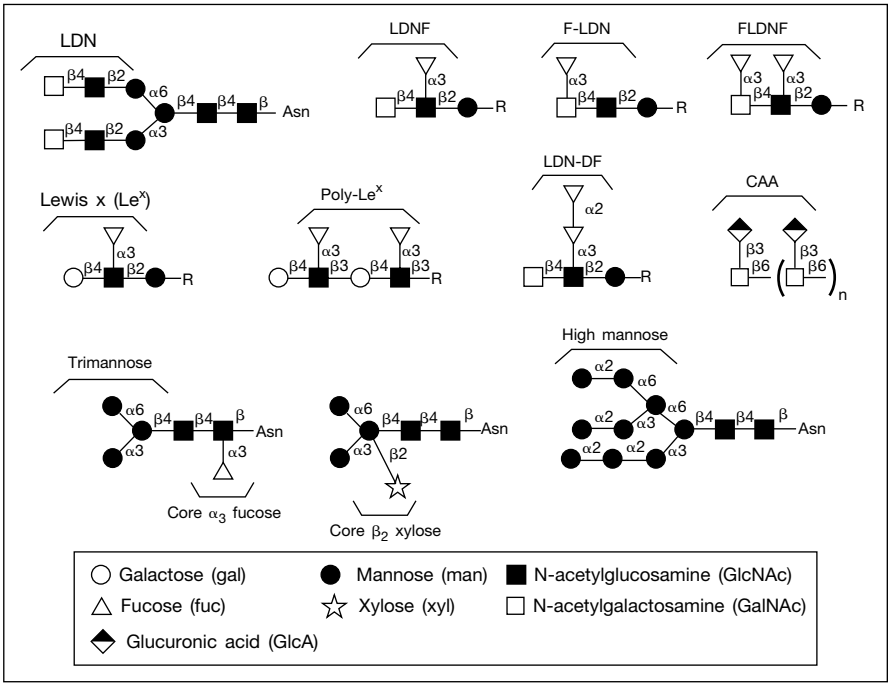

Fig. 1. Structures of antigenic glycans expressed in schistosomes. Many different glycan antigens are found in glycoconjugates from *Schistosoma spp*. and they are depicted here in symbol form. The common names for the Ag determinants are shown.

helminth- and allergen-derived glycan Ags that drive Th2-mediated immune responses contribute to an immunological association between helminth infections and atopic diseases. Of particular interest is the interaction of allergen- and helminth-derived glycan Ags with dendritic cells (DC). Through cell-surface C-type lectin receptors (CLRs), DC recognize specific glycan Ags [5] and this may be of crucial importance for the subsequent polarization of DC towards DC subsets with a Th2 or regulatory phenotype.

Glycan Ags in Schistosomes

Schistosomes are blood flukes (trematodes) and have a complicated life cycle, requiring an intermediate freshwater snail host. Cercariae released by the snail in water can penetrate the skin of a vertebrate, and transform into *schistosomula* that migrate via the lungs to the veins of the abdominal cavity. Male and

Table 1. Glycan Ags on glycoconjugates from phylogenetically unrelated species, and their recognition by lectins

	S. mansoni	Nematodes	Insects	Snails	Plants	Human	Recognition by human CLR
LDN	+	+	+	+	–	+	galectin-3 hMGL
LDNF	+	+	+	+	–	+	DC-SIGN hMGL
LDN-DF	+	–	–	–	–	–	–
F-LDN	+	–	–	+	–	–	SP-D
Le^x	+	+	–	–	–	+	DC-SIGN
Le^a	–	–	–	–	+	+	DC-SIGN
Trimannose	+	+	+	+	+	+	–
Core α_3Fuc	+	+	+	+	+	–	–
Core β_2Xyl	+	+	–	+	+	–	–
High mannose	+	+	+	+	+	+	DC-SIGN MR

All lectins are human, except for galectin-3 that is hamster-derived. The recognition of the glycan Ags by the lectins is dependent on the multivalency of presentation on the protein. Glycans not found in humans are immunogenic.

– = Not detected or not known; + = found in at least one species of the group.

female *worms* mate and produce eggs that become lodged within host tissues, causing pathology. Some eggs escape from the body via feces or urine and eventually reach the water, where the miracidia hatched from the eggs then can complete the cycle by infecting a compatible snail host.

Schistosoma mansoni and other parasitic helminths generate a large array of glycan Ags that in schistosomes include the Lewis x (Le^x) Ag, poly-Le^x, lacdiNAc (LDN), fucosylated LDN sequences (LDNF, LDN-DF, and F-LDN), core α_3-fucose, core β_2-xylose, and glucuronic acid-containing CAA (fig. 1). Many of these Ags occur in O- and N-glycans of glycoproteins and in glycolipids and secreted polysaccharides (fig. 2). Some of these Ags are shared by many different helminths. For example, N-glycans with the core α_3-fucose are expressed by the ruminant nematode *Haemonchus contortus*, and trematodes (e.g. *Schistosoma* species) [6–8], but this antigen is also present in plants and insect glycoproteins [9, 10] (table 1). The LDN and LDNF structures are also found in other parasitic helminths, such as *Fasciola hepatica, Dirofilaria immitis* and *H. contortus*, whereas the Le^x structure may be more restricted in expression and has been found in schistosomes and the bovine parasitic nematode *Dictyocaulus viviparous* [7, 8]. Some Ags, such as Le^x, LDN and LDNF, are expressed by all developmental stages of schistosomes, including newly

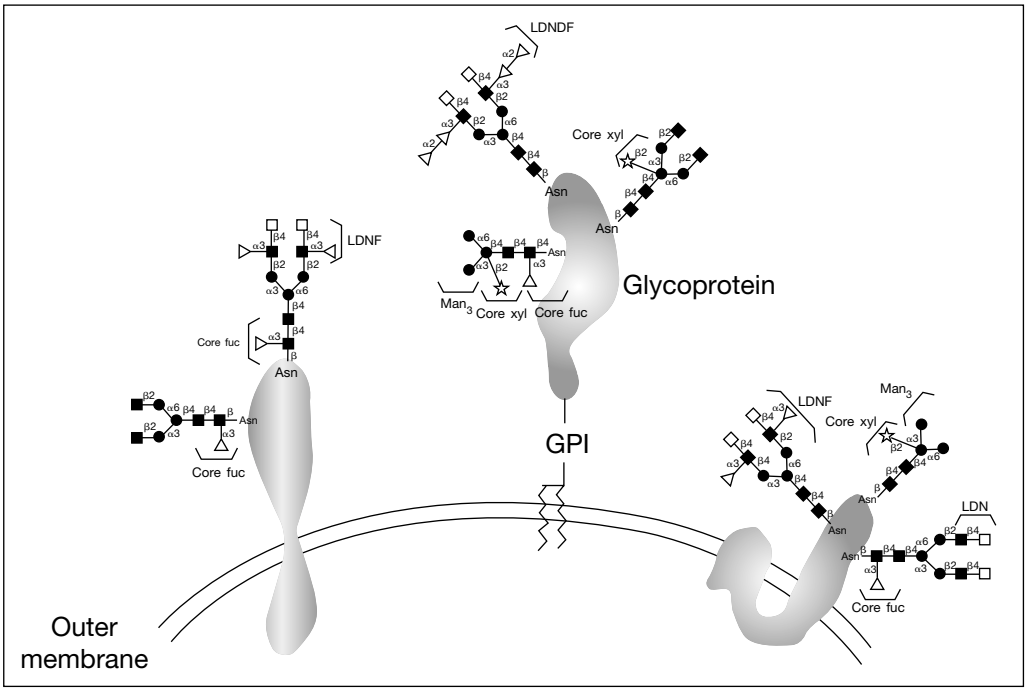

Fig. 2. Representation of the surface of *S. mansoni.* Glycoproteins can express different glycan molecules that each can carry different glycan antigens. Here only N-glycans are shown, but a variety of glycan antigens can also be expressed in O-linked and lipid-linked glycans.

transformed schistosomula. Sialic acid is a major component of many vertebrate and microbial glycoconjugates; however, helminths, including the free-living nematode *Caenorhabditis elegans*, appear to lack sialic acid-containing glycans, presumably due to a lack of genetic machinery for generating sialic acid and incorporating it into glycan structures.

Schistosome Glycan Ags Generate High Levels of Anti-Glycan Abs in Infection

Many early studies documented the existence of immune responses to glycan Ags expressed by schistosomes. The general immunogenicity of schistosomes has long been known, since skin penetration and infection by the free swimming cercariae induce ‘swimmer’s itch’, a cercarial dermatitis in humans usually associated with bird schistosome infections (e.g. genus *Trichobilharzia*)

in previously sensitized individuals. The cercarial glycocalyx is highly immunogenic with immunomodulatory activity [11], but this glycocalyx is lost during transformation into schistosomula and new glycan Ags are generated by the developing worms in their hosts. Some of the immunogenic schistosome-derived glycans associated with production of Abs are shown in figure 1. One of the first defined Ags in schistosome infections was the Le^x Ag (also called CD15 and the stage-specific antigen-1 or SSEA), which is expressed in all schistosome species [6, 12]. The Le^x Ag and poly-Le^x are also found in the secreted circulating cathodic antigen (CCA) [13]. The discovery of Le^x expression in schistosomes and immune responses to it in humans and animals were at first surprising, since the Le^x Ag is widely expressed in human leukocytes. Thus, it is likely that the mode of Le^x presentation by the parasite is substantially different from the host, thus stimulating immune response, but the immune mechanism underlying this response is not well understood.

The soluble egg Ags (SEA) of *Schistosoma mansoni* contain an array of complex glycoconjugates, including LDN, LDNF, and Le^x Ags, and are highly immunogenic in infected animals. While impractical as a vaccine, SEA has shown the potential for glycan Ags to provide protective immunity. For example, BALB/c mice intranasally vaccinated with SEA conjugated to soluble cholera toxin B subunit (CTB) demonstrated significantly reduced liver granuloma formation and mortality following challenge infection [14]. As an immunization model for schistosomiasis, some researchers use radiation-attenuated (RA) schistosome cercariae, which are sterile but can undergo significant maturation in infected animals. Early studies showed that vaccination of mice with RA-attenuated schistosome cercariae induced unspecified anti-carbohydrate responses. Recent studies in chimpanzees exposed repeatedly to RA-attenuated schistosome cercariae found that the major immune responses (both IgM and IgG) generated in the animals were primarily against glycan Ags (e.g. LDN, LDNF, Le^x, FLDN, LDN-DF) [15, 16]. Many of these Abs cross-reacted with soluble Ags from larvae (schistosomula), adult worms, and eggs. These findings are consistent with many other studies showing that in humans, primates, and rodents, the schistosome-derived glycan Ags elicit generation of all isotypes of immunoglobulin – IgA, IgM, IgG, and IgE – and all subclasses of IgG, including IgG1 [8, 13, 17]. *S. mansoni*-infected patients generate mainly IgM, but also IgG and IgA, against LDN, LDNF, and Le^x, although immune responses to Le^x are lower than responses to LDN and LDNF and less specific to schistosome infections [8]. *Schistosoma japonicum*-infected individuals develop IgG to LDN-DF, while *S. mansoni*-infected individuals express primarily IgM to the Ag. By contrast, both IgG and IgM to LDN-DF are expressed in *Schistosoma haematobium*-infected individuals [17]. Thus, immune responses to glycan Ags are a dominant feature of human infection by schistosomes.

Helminth- and Allergen-Derived Glycan Ags Induce Th2 Responses

Several lines of evidence indicate that carbohydrates as complex structures linked to carrier molecules induce Th2 mediated immune responses, both in allergy and in helminth infections. Carbohydrates expressed on Cry j 1, the major allergen of *Cryptomeria japonica* pollen that causes the most prevalent allergic rhinitis or pollinosis in Japan, were demonstrated to play a major role in promoting Cry j 1-specific Th2 responses in vitro, although they were not major targets as T cell epitopes [18]. While this study did not investigate the contributions of individual glycan Ags, it is known that glycan Ags on Cry j 1 include biantennary complex type N-glycans containing the highly immunogenic core α_3-fucose and core β_2-xylose Ags, along with fucosylated residues in outer branches [19]. Core α_3-fucose and/or core β_2-xylose Ags also occur on invertebrate allergens and within glycoconjugates of pathogenic helminths (fig. 1, 2) [7, 9, 10, 20, 21]. Bee venom phospholipase A2 carries an N-glycan containing the core α_3-fucose Ag [10], and several T cell clones have been identified from bee venom-sensitized individuals that proliferate in response to honey bee PLA2 but not to its non-glycosylated variants, providing evidence for the involvement of N-glycans in T cell recognition [22].

A glycoprotein fraction purified from *S. mansoni* egg extracts including the core α_3-fucose and core β_2-xylose Ags, was shown to generate a strong Th2-biased cellular response in a murine model of immunization with pulsed DC [4] that was largely dependent on the carbohydrate components. In addition, anti-core α_3-fucose and anti-core β_2-xylose IgG1 (a Th2-associated isotype), but not IgG2b (a Th1-associated isotype) Abs, have been found in a murine schistosome infection. These data also show that complex carbohydrates in soluble egg Ags (SEA) of *S. mansoni* contribute to the Th2 immune responses, but they are not conclusive about the nature of the glycans that trigger the Th2 polarization.

In a murine schistosome model, both SEA-containing glycans, as well as glycans carrying the Le^x glycan Ag, effectively induce a Th2 response [3, 23]. Nasal lymphocytes from mice sensitized with native SEA produced the Th2 type cytokines IL-4, IL-5 and IL-10 when restimulated in vitro with native SEA, in contrast to results obtained with SEA treated with periodate, which causes oxidation and destruction of most glycan structures. In addition, a neoglycoprotein containing the Le^x antigen induced a strong Th2 response in BALB/c mice. The neoglycoprotein was over 1,000-fold more potent in inducing Ab production as compared to the non-glycosylated protein-carrier, an effect that was dependent on the fucose-residue of Le^x. In these studies Le^x itself did not function as an epitope for either IgG or IgE, but its conjugation with protein was essential for the adjuvant activity [23]. Remarkably, it was also found that protein-linked glycans from the non-pathogenic nematode *C. elegans* are able

to trigger production of Ag-specific IL-4 in mice, demonstrating that the induction of a biased Th2 response is not restricted to parasitic helminths [24].

Glycan Ags also play a key role in induction of granuloma formation in murine schistosomiasis. Granulomatous inflammation in schistosomiasis is a Th2-cytokine mediated host defence mechanism that serves to protect organs against the harmful SEA. Earlier studies showed that sensitization with Le^x resulted in an increased cellular response towards SEA-coupled beads implanted in the liver and to the formation of granulomas [25]. Using a similar model-system of implantation of Ag-coated beads to mimic schistosome eggs, it was found that both SEA- and KLH-coated beads that share several glycan Ags can directly induce a granulomatous response [26].

Thus, a wide variety of glycan Ags can induce Th2 responses, suggesting a common mechanism that remains to be established. Evidence is accumulating that the glycan-mediated Th2 response is the result of a specific, rather than a default pathway. This became especially apparent in recent studies showing that mouse DC co-pulsed with schistosome SEA and the bacterium *Proprionebacterium acnes* (Pa), and transferred into wild-type mice, induced concurrent Pa-specific Th1, but not Th2, responses and SEA-specific Th2, but not Th1, responses [27]. These results indicate that the SEA-specific-Th2 response is induced even in a Th1 stimulated setting. In summary, these data suggest a specific Th2-inducing pathway for glycans that depends on recognition of the glycan Ags by specific carbohydrate-binding proteins on immune cells, probably in concerted action with other receptors.

Immunogenic Glycan Ags Shared between Helminths and Allergens Are IgE Epitopes

The strong glycan-mediated Th2 immune responses associated with the production of anti-carbohydrate Abs include the production of anti-carbohydrate IgE, and/or significant amounts of non-specific IgE [3, 4]. The presence of anti-carbohydrate IgE is of particular interest, since several glycan Ags are shared between allergens and pathogenic helminths and elevated IgE is a hallmark for both helminth infections and atopic diseases.

Cross-reactivity of IgE Abs to many plant, arthropod, and mollusc extracts has been observed for over 20 years and 'cross-reactive carbohydrate determinants' (CCDs) were identified [28]. The immunogenic core α_3-fucose and/or core β_2-xylose glycan Ags (fig. 1; table 1) have been demonstrated to be the major CCDs for IgE found in sera of patients allergic for plant substances, honeybee venom or snails. IgE Abs from sheep infected with the nematode *H. contortus*, and mice infected with *S. mansoni* bind to core α_3-fucose and/or

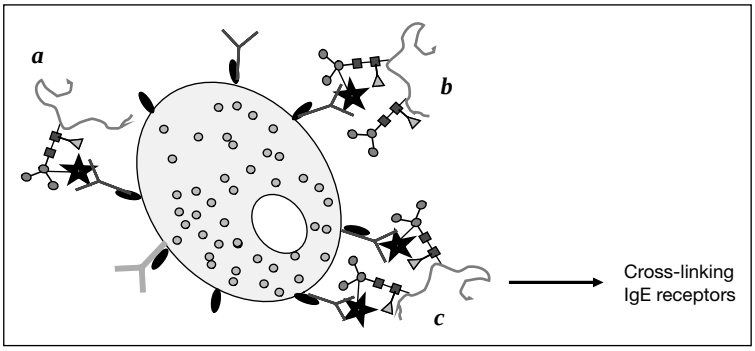

Fig. 3. Binding of glycoproteins carrying the 'CCD' core β_2-xylose to host immune cells via core β_2-xylose specific IgE. Cross-linking of IgE receptors can occur when one glycoprotein carries multiple glycan molecules carrying a core β_2-xylose Ag. **(*a*)** Direct binding of a glycoprotein in having a single glycan. **(*b*)** Direct binding of a glycoprotein with multiple glycans. **(*c*)** Direct multivalent binding of a glycoprotein with multiple glycans.

core β_2-xylose glycan Ags from plant glycoproteins [20]. These data suggest that CCDs may contribute to the observed immunological association between helminth infections and allergy. Several other common glycan Ags may similarly act as IgE epitopes thereby causing cross-reactivity between helminths and allergens. IgE Abs specific for the LDNF Ag, which is found in helminths, snails and insects, have been demonstrated in *S. mansoni*-infected mice [8]. Other potential CCDs are shown in table 1.

The clinical relevance of the carbohydrate-specific IgE Abs is unresolved. While some studies found poor biological effects of CCD IgE, other studies showed that anti-glycan IgE from allergic patients can trigger mediator release from mast cells [29]. The latter findings indicate that the CCD IgE has sufficient affinity to induce cross-linking of IgE receptors. It is known that the structural properties of the cross-linking Ag are of crucial importance to induce biological properties. The potential of a CCD-Ag to cross-link IgE receptors is dependent on the presence of multiple glycan chains carrying this CCD within one protein molecule (fig. 3). Glycoproteins are usually very heterogeneous; they may contain different amounts and types of glycan Ags (fig. 2), and the binding affinity and biological activity of the CCD IgE may be sensitive to small structural differences. Therefore, it is unlikely that anti-CCD IgE, directed toward helminth CCDs, for example, would cause extensive mast cell degranulation upon encountering allergens carrying the same CCDs on a different protein-carrier. It will be important to carefully design future studies using defined allergenic CCD glycoforms to elucidate the role of the 'CCD IgE' in helminth infections and allergy.

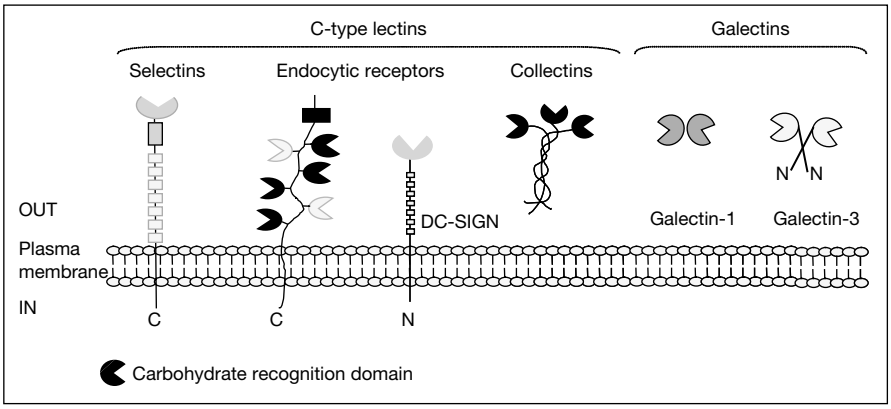

Fig. 4. Organization of the carbohydrate recognition domains (CRD) within cellular and soluble C-type lectins, and the soluble galectin-1 and -3. Cell-surface lectins can be type I, or type II transmembrane proteins. Multivalent binding of ligands can be achieved by the involvement of multiple CRDs within one molecule. Alternatively, molecules carrying one CRD can multimerize, such as in the case of galectin-3. DC-SIGN is thought to form tetramers [adapted from 'C-type lectins'; in: Essentials of Glycobiology. New York, Cold Spring Harbor Laboratory Press].

Glycan Ags Regulate Immune Responses via Interaction with Host Lectins

While much remains to be learned about the mechanisms of the immunomodulatory effects exerted by glycan Ags, it seems likely that the effects are initiated by specific recognition of the pathogen- or allergen-derived glycans by host receptors. Recognition of glycans is mediated by a specialized family of host receptors that are lectins (also termed lectin receptors and carbohydrate-binding proteins), which bind specific glycan Ags through one or more carbohydrate recognition domains (CRDs) (fig. 4). Different classes of animal lectins, such as the Ca^{2+}-dependent C-type lectins, galectins, and siglecs, are distinguished based on highly conserved amino acid sequences within their CRDs that can define their carbohydrate-binding properties. High affinity binding by some carbohydrate-binding proteins is enhanced through avidity by multivalent binding of the glycan molecule by multiple CRDs within one lectin, or by clustering of lectin molecules carrying a single CRD (fig. 4). Below we will review the importance of lectins for carbohydrate recognition within the innate immune system focussing on helminth infections and allergy, and for functional reasons we will discriminate between cell-surface bound lectins and soluble lectins (fig. 4).

Cell-surface lectins are abundantly expressed by innate immune cells such as macrophages and DC [5]. These lectins capture glycosylated pathogens and allergens for presentation to T cells, and are pivotal for the activation of diverse signalling pathways that in concert with other stimuli orchestrate the development of adaptive immune responses [30]. Important groups of soluble lectins that contribute to innate immunity are the families of carbohydrate-binding proteins termed collectins and galectins. Such lectins can diffuse into tissues and/or enter the blood circulation. They can bind to various glycosylated pathogens or allergens, but they can also bind to host cells in the vicinity, and trigger functions such as phagocytosis or cytokine production.

C-Type Lectins on Antigen-Presenting Cells Recognize Glycan Ags

DC recognize invading pathogens using pattern recognition receptors, including CLRs and Toll-like receptors (TLRs). TLRs recognize pathogen-associated molecular patterns, such as nucleic acids or lipids, and these interactions lead to DC maturation and induction of pro-inflammatory cytokines. CLRs contain one or multiple CRDs that specifically recognize glycan ligands in a Ca^{2+}-dependent manner [31]. Interaction of CLRs with carbohydrate Ags induces internalization of the Ags, but by itself does not lead to DC-maturation. Many CLRs on DC have been implicated in Ag capture and endocytosis. Receptor-mediated internalization may facilitate a more efficient presentation of captured Ags as compared to soluble Ags [5]. Upon internalization via CLRs, captured Ags reach endosomal and subsequently lysosomal compartments, where loading onto MHC class II molecules can occur. In addition, Ag recognition by CLRs can result in presentation by glycolipid Ag-presenting CD1 molecules, as well as in cross presentation on MHC class I molecules.

It is currently felt that the principal function of CLRs is to induce tolerance to ‘self glycan’ Ags, by uptake of such Ags and subsequent generation of regulatory T cells. Many pathogens carry glycan Ags that are similar to self-glycans, or mimic self-glycan Ags and thus interact with CLRs. Pathogen-DC interaction may lead to simultaneous stimulation of TLRs and CLRs, and the balance between TLR and CLR stimulation may be crucial for steering the final immunological response toward predominantly Th1 or Th2, and in regulating induction of suppressor T cells now termed T regulatory cells or Treg [32].

It is likely that important clues to understand the molecular mechanisms underlying the specific glycan-mediated Th2-biased immune responses in helminth infections and allergy will be found in interactions of helminth glycan

Ags with innate immune cells. The switch from a Th1 to Th2 response in rodents upon schistosome infection takes place soon after egg-laying commences, when the eggs secrete heavily glycosylated egg Ags (SEA) [33]. In mouse models, DC pulsed with SEA potently stimulate Th2 responses both in vivo and in vitro, while failing to undergo a conventional maturation process [33]. In an in vitro assay, SEA induced the development of human monocyte-derived DC into effector DC that promoted the development of Th2 cells via enhanced expression of OX40 ligand. The lack of a conventional DC maturation in the above-mentioned studies, in combination with data that show the capacity of glycan Ags to bias strong Th2 responses [3, 4, 23], suggest an involvement of CLRs on DC in induction of Th2 responses in schistosome infections, and possibly also allergy.

Recognition of Schistosome- or Allergen-Derived Glycan Ags by Antigen-Presenting Cells

Recently, many CLRs have been identified on the cell-surface of Ag-presenting cells such as DC and macrophages [5]. Below we summarize the data that support the interaction of human Ag-presenting cells with schistosome- and/or allergen-derived glycan Ags and their possible functional consequences.

Schistosome Egg Glycan Ags Interact with DC-SIGN

DC-SIGN (Dendritic-cell specific ICAM-3 grabbing non-integrin, CD209) is a human type II transmembrane C-type lectin that contains only one C-terminal CRD (fig. 4) and is abundantly expressed on immature DC. DC-SIGN has affinity for glycoconjugates containing mannose, N-acetylglucosamine, and fucose and interacts with many pathogens [32, 34]. DC-SIGN strongly interacts with schistosome-derived SEA glycoprotein extracts. This interaction in vitro could be substantially inhibited by monoclonal Abs (mAbs) recognizing Le^x and LDNF, two major glycan Ags within SEA, whereas a combination of anti-LDN and anti-LDNF mAbs further reduced binding [35]. Recently we showed that DC-SIGN also binds to schistosomal cercarial glycolipids [Meyer et al., unpubl. results], indicating that DC-SIGN can recognize both natural occurring schistosome glycoproteins and glycolipids. Analysis of the carbohydrate specificity of DC-SIGN in vitro in glycan-arrays showed that DC-SIGN weakly interacts with the trisaccharides Le^x and LDNF [36]. A 5-fold increase in binding of DC-SIGN was found toward biantennary N-glycans containing these epitopes [unpubl. results]. In addition, glycans containing poly-Le^x constitute a preferred ligand compared to the trisaccharide Le^x [37]. Biantennary N-glycans containing Le^x and LDNF, as well as more complex

N-glycans carrying repeating Le^x determinants (poly-Le^x), have been demonstrated in adult schistosomes [8, 13], and may constitute the natural schistosome glycoprotein ligands for DC-SIGN. Molecular modeling of Le^x, LDNF and Le^y glycan ligands into the CRD of DC-SIGN indicates that these glycan ligands all similarly interact with DC-SIGN, suggesting that they may trigger similar functions. It has been reported that DC-SIGN can bind also Le^a Ags and high mannose glycans [36, 38] that are abundant on plants, including several allergens [9]. It will be interesting to study their interaction with DC-SIGN, since DC are key players in allergic respiratory diseases by inducing a Th2 response [39].

L-SIGN Binds Schistosome Egg Glycan Ags

A human homologue of DC-SIGN named L-SIGN or DC-SIGNR, is found on a subset of endothelial cells in lymph node (LNEC) and liver sinusoid endothelial cells (LSECs) [40]. Since LSECs function as a liver-resident Ag presenting cell population, L-SIGN may play a role in the recognition and uptake of glycosylated Ags that are secreted by schistosome eggs trapped in the liver of infected hosts. Indeed, CHO cells expressing L-SIGN bind to SEA [38]. Despite the high sequence identity between DC-SIGN and L-SIGN, the lectins bind a different subfraction within SEA. Both lectins recognize Le^a, Le^b and Le^y, but unlike DC-SIGN, L-SIGN does not bind the Le^x antigen [38]. L-SIGN shows a higher affinity for mannose than DC-SIGN [36], and this may indicate that high mannose-type N-glycans within SEA constitute L-SIGN ligands, a hypothesis that we are currently exploring.

Interaction of Schistosome Glycan Ags with hMGL

The macrophage galactose-type lectin (MGL, also called DC-ASGPR or HML) is another member of the CLRs, and is expressed on immature DC and macrophages in skin and lymph node in both man and mouse [41]. A thorough analysis of the glycan-binding properties of human MGL (hMGL) by the glycan-array showed an exclusive specificity of the lectin for glycans with a terminal α- or β-linked GalNAc [42]. CHO cells expressing hMGL, but not the parental CHO cells, showed binding to schistosome SEA. The α-GalNAc specific *Helix pomatia* lectin, which recognizes α-GalNAc, binds poorly to SEA [Van Die, unpublished results], suggesting that α-GalNAc is not a major hMGL ligand within SEA. The binding of hMGL to SEA, however, could be substantially blocked by both mAbs against the LDN and the LDNF glycan Ag, whereas a combination of anti-LDN and anti-LDNF mAbs further reduced binding [42]. These data demonstrate that LDN and LDNF glycan Ags, which both contain a terminal β-linked GalNAc residue, constitute ligands for hMGL within SEA.

The Mannose Receptor Recognizes Ags from Schistosome Eggs and House Dust Mite Der p 1

The mannose receptor (MR, CD206) is a type I transmembrane protein with an extracellular domain that consists of eight C-type lectin domains (CRDs) and a cysteine rich N-terminal domain. The MR is a pinocytic and phagocytic receptor that preferentially targets mannose-containing structures, although it can also bind oligosaccharides containing a terminal GlcNAc or fucose. CRDs 4 and 5 of the MR are minimally required for binding to mannose, GlcNAc- and fucose-terminating oligosaccharides. The MR has been shown to interact with many pathogens [34]. Recently, it was shown that several glycoproteins within schistosome SEA interact with the MR. Although the actual glycan ligands were not identified, the data showed that binding was carbohydrate-mediated and dependent on interaction with the CRDs 4–7 of the MR. High levels of MR expression, induced by the Th2 cytokine IL-4, are found on alternatively activated macrophages that are recruited to mouse liver granuloma induced by schistosome eggs [43]. IL-4 plays a protective role during schistosomiasis by controlling the generation of reactive oxygen and nitrogen intermediates in the liver, which may suggest a role of alternatively activated macrophages and the MR in regulating the granulomatous pathology. IL-4 may also enhance MR levels on immature DC. Stimulation of DC from patients with house dust mite allergy by *Dermatophagoides pteronyssinus* Der p 1 allergen induced a Th2 cytokine profile. In addition, DC from patients with an allergy for house dust mites express more MR and more efficiently internalize Der p 1 than DC from healthy donors. The involvement of the MR in Der p 1 uptake was established by using mannan and blocking anti-MR mAb inhibitors, indicating a carbohydrate-mediated interaction of the MR with Der p 1 [44]. These data suggest that the MR could play a role in carbohydrate-mediated Der p 1 allergen uptake by DC and in the pathogenesis of allergic diseases in house dust mite-sensitive patients.

Host Protection Mechanisms May Include the Binding of Glycosylated Ags by Soluble Lectins in Schistosomiasis and Allergy

Soluble lectins, such as collectins and galectin-3 contribute to innate immunity. Collectins are soluble CLRs that contain a collagen-like domain, and assemble in large oligomeric complexes (fig. 3). Binding of collectins to glycosylated pathogens may facilitate clearance through aggregation, complement activation, opsonization and activation of phagocytosis. In addition, the collectins can modulate inflammatory and allergic responses, and modulate adaptive immune responses. Galectins constitute a family of soluble β-galactoside

binding lectins, that differ from CLRs in their binding to carbohydrate ligands, structure of the CRD, and their binding is not Ca^{2+} dependent.

Interaction of Helminth Glycan Ags and Allergens with Collectins

One of the best known collectins is the mannose-binding lectin (MBL) that interacts with both *S. mansoni* and *Trichinella spiralis*; however, the nature of the interacting glycans are unknown. Surfactant proteins A (SP-A) and D (SP-D) are collectins found in the alveolar lining of the lung. Both play an important role in innate immune responses against inhaled pathogens or allergens. SP-A and SP-D bind to extracts of the house dust mite *D. pteronyssinus* and the purified allergen Der p 1 in a carbohydrate-specific and Ca^{2+}-dependent manner. SP-A and SP-D showed inhibition of allergen-specific IgE binding to mite extracts, whereas recombinant human SP-D reduces allergic responses in mice sensitized to house dust mite allergens [45]. These data demonstrate that unidentified glycan Ags on inhaled allergens modulate innate immunity through interaction with SP-A and/or SP-D, which may play a role in resistance to allergy. Interestingly, SP-D also binds to the surface of *S. mansoni* schistosomula that transiently reside in the lung during infection [46]. In vitro analysis of the carbohydrate binding specificity of SP-D indicated that it binds to Fucα1,3GlcNAc and Fucα1,3GalNAc structural determinants. By contrast, SP-D showed a preferred binding to the F-LDN glycan antigen, whereas binding to Le^x and LDNF was weak. It will be of great interest to investigate whether SP-D also binds to the Fucα1,3GlcNAc determinant when it is present within the core of N-glycans to form the immunogenic core α_3-fucose antigen (fig. 1), a common plant and insect glycan allergen discussed above that also occurs on helminths.

Galectin-3 Recognizes LDN Glycan Ags within Schistosome SEA

Galectin-3 (also known as the MAC-2 antigen) is a well-known member of the galectin family and is composed of a C-terminal CRD, responsible for galactose and GalNAc recognition, and an N-terminal domain consisting of multiple PGAYPG repeats that mediates multimer formation. It is synthesized and secreted by many cell types including myeloid cells, e.g. macrophages and DC. Galectin-3 binding has been associated with several innate immune mechanisms, such as induction of an oxidative burst in neutrophils and monocytes. In addition, galectin-3 has been identified as an IgE binding protein that may trigger mast cell degranulation. Abs to galectin-3 inhibit IgE-mediated cytotoxicity of eosinophils to adult schistosomes.

Recently, we showed that in addition to β-galactosides, galectin-3 recognizes LDN glycan Ags containing a terminal β-GalNAc residue and interacts with LDN Ags within SEA [47]. High levels of galectin-3, and their

co-localization with LDN glycans on the parasite egg shells, have been observed in liver granulomas of *S. mansoni* infected hamsters. In addition, galectin-3 can mediate recognition and phagocytosis of LDN-coated particles by macrophages. Indeed, SEA has been detected in macrophages during *S. mansoni* infection. Using a mouse model based on hepatic implantation of Ag-coated beads, the glycans of schistosome egg glycoproteins, in particular LDN and LN that both bind galectin-3, have been identified as the primary driver of granuloma formation [Van de Vijver et al., pers. commun.]. These studies and the recent observation that granuloma formation during *S. mansoni* infection is significantly reduced in galectin-3-deficient mice [El-Cheikh et al., pers. commun.], provide evidence for a role of galectin-3 and LDN in granuloma formation during parasite infection in vivo. Our hypothesis is that multimeric galectin-3 facilitates the uptake and presentation of LDN-containing Ags by macrophages (and/or other Ag presenting cells), which may thereby trigger a more pronounced T cell response against the parasite and promote granuloma formation (fig. 5).

Does IL-10 Production Induced by Parasite Glycans Contribute to Protection Against Allergy?

An important consequence of the Th2 response during helminth infection is the production of a balanced array of cytokines that can attenuate or prevent pathology. Interestingly, heavily parasitized individuals are somehow protected against allergy. It is thought that a heavy burden of parasites stimulates iDC to secrete IL-10, leading to a regulatory T cell network that suppresses overly strong Th2 responses including allergic responses. There is a negative correlation between IL-10 levels and allergic symptoms, and it has been shown that IL-10 can inhibit mast cell degranulation, providing a mechanism for the observed protection [1]. Remarkably, schistosome egg glycolipids stimulate in vitro the production of IL-10, IL-6, and TNFα in human PBMCs derived from naïve donors, whereas worm glycolipids failed to do so. A strong cytokine production could also be observed with a neo-glycoprotein carrying the LDN-DF antigen that in vivo is present on egg glycolipids and schistosome SEA, but absent on worm glycolipids. The mechanism, however, of how the LDN-DF glycan Ags triggers innate immune responses is not yet known, and no receptor that recognizes this Ag has been identified thus far [13]. Schistosome SEA, as well as neoglycoconjugates carrying Le^x, can induce a proliferative response and IL-10 production from peripheral blood mononuclear cells (PBMCs) of schistosome-infected patients. Remarkably, the levels of IL-10 induced by the neoglycoconjugates containing Le^x were inversely correlated with the parasite burden,

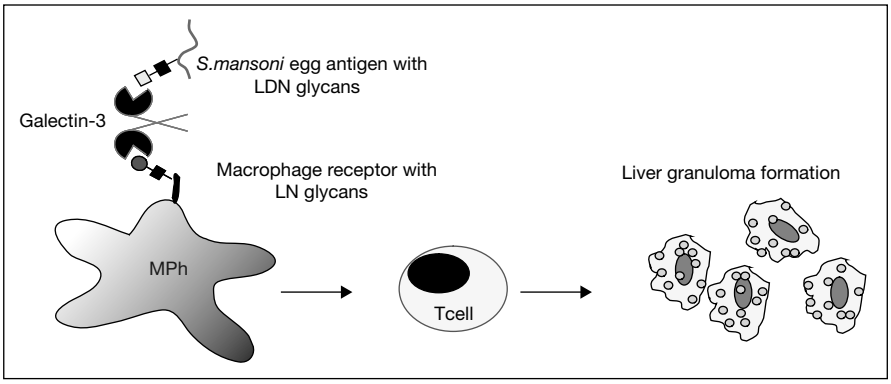

Fig. 5. Model of the role of galectin-3 in liver granuloma formation triggered by LDN glycan antigens from schistosome egg antigens (SEA). Dimeric galectin-3 may cross-link glycoconjugates carrying LDN glycan antigens and macrophage receptors carrying LN glycan antigens, resulting in induction of phagocytosis of the Ag and T cell responses.

suggesting that Le^x on SEA downregulates the immune response against this parasite Ag via the production of IL-10. Interestingly, it has been shown that the DC-SIGN-mediated binding of Le^x or other glycan ligands to human immature DC (iDC) can induce the production of IL-10. For example, mannosylated lipoarabinomannan (ManLAM), which is secreted by virulent *Mycobacterial* strains, is able to induce IL-10 production in iDC through interaction with DC-SIGN, in contrast to lipoarabinomannan that lack the mannose caps [32]. Elegant studies with *Helicobacter pylori* isogenic variants that only differed in expression of LPS-associated Le^x and Le^y, indicated that the Le^x/Le^y positive strain induced a DC-SIGN-dependent increased expression of IL-10 when cocultured with iDC, in contrast to the isogenic Le^x/Le^y negative strain [48]. These data clearly indicate that interaction of DC-SIGN with its glycan ligand can contribute to IL-10 production. However, this interaction may not be sufficient, since ligation of DC-SIGN by a blocking anti-DC-SIGN mAb could not trigger IL-10 production and no reports yet show that interaction of schistosome SEA to human iDC leads to IL-10 production, despite the strong binding of SEA to DC. It is likely that additional signals are needed to trigger the DC-SIGN-mediated IL-10 production in iDC, and it remains to be established whether that happens in vivo in schistosome or other helminth infections. In vivo, it is expected that many interactions occur between egg-derived components and host receptors and that concerted action of different DC-receptors will determine the final response, such as shown in other host-pathogen interactions.

Concluding Remarks

Schistosome- and allergen-type glycan Ags show profound immunomodulatory properties. Several of the glycan Ags interact with either soluble lectins or cell-surface lectins on DC and macrophages. These carbohydrate-binding proteins show a broad, but specific recognition profile and have overlapping carbohydrate binding profiles (table 1). For example, both DC-SIGN and hMGL interact with LDNF, and both galectin-3 and hMGL interact with LDN glycan Ags occurring within SEA glycoproteins. The glycan Ags, however, may be present in different abundance and spacing on protein- or lipid carriers that may influence their in vivo-binding and function; also, individual interactions may influence the recognition and function of other glycoconjugates. It is therefore important to identify the high-affinity ligands of the parasites recognized by the carbohydrate binding proteins in the innate immune system and study their immunomodulatory potential.

An important aspect of the interaction of the SEA-glycan Ags with CLRs may be the enhancement of humoral anti-glycan immune responses through efficient uptake and presentation of the captured glycoconjugates. In particular, high Ab levels are generated against immunogenic 'foreign glycan' Ags. A remarkable finding is that human DC recognize schistosome glycan Ags that are also found on human glycoconjugates, and thus can be regarded as 'self glycan' Ags. Evidence is accumulating that CLRs such as DC-SIGN function in normal homeostasis by the induction of regulatory T cells and tolerance to self-glycoproteins. However, during infection cross-talk between CLRs and other receptors such as TLRs determines the balance between induction of tolerance vs. immunity. In chronic schistosomiasis eggs trapped in the liver continuously secrete heavily glycosylated egg Ags (SEA) that trigger immature DC via interaction with different CLRs, and may prime them for the induction of regulatory T cells. The abundant expression within SEA of 'self-glycans' or glycan Ags that mimic 'self-glycans' due to their fit within the CRD of the host CLRs, may allow schistosomes to deceive the host immune system to their own benefit. By the induction of regulatory T cells, the host protects itself simultaneously against excessive damage by downregulating helminth-induced, Th2 cell-mediated immune responses. In such a strong anti-inflammatory environment, the host may also be protected against excessive Th2 cell-mediated allergic responses caused by invading allergens.

In conclusion, helminths produce glycans that interact in various ways with host lectins and modulate immune responses to promote their survival, and in doing so may simultaneously protect the host against allergy. Understanding the exact structures of the glycan Ags that induce immunomodulatory

responses, the immunological function of the host receptors, and possible cross-talk with other pattern-recognition receptors, may help to develop strategies to fight allergy, as well as infections with helminths and other 'sugar-coated' pathogens.

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Role of the Natural Killer T Lymphocytes in Th2 Responses during Allergic Asthma and Helminth Parasitic Diseases

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Abstract

The recent discovery that T cells recognize endogenous and foreign lipid and glycolipid molecules presented by CD1 proteins has brought a major contribution in the understanding of innate and adaptive immune response to certain harmless antigens and infectious pathogens. Among (glyco)lipid-reactive T cells, CD1d-restricted natural killer (NK) T cells represent a population of innate/memory lymphocytes that, upon stimulation, rapidly release important amounts of immunoregulatory cytokines that in turn can shape the acquired immune response in a Th1 or a Th2 direction. Here we review the general features of these cells as well as their diverse influence in various disease models. A particular emphasis will be placed on the role of NK T cells in the promotion of asthma, a typical Th2-related inflammatory disease. Moreover, recent studies suggest that NK T cells could also be important in the modulation of the host immune response during helminthic infections, generally associated with dominated Th2 responses. Our current understanding of the role and of the mode of NK T cell activation during the initial immunological events that lead to the promotion of Th2 responses will be discussed.

Traditionally, many immunologists regarded lipids as inactive molecules. However, it is becoming more and more evident that, by activating cells of the innate immune system, lipids play part in the promotion and the regulation of adaptive immune responses. The discovery of CD1-dependent antigen (Ag) presentation pathways has provided a mechanism by which T cells can recognize endogenous and foreign lipid Ags and in turn can activate the immune

system [1, 2]. CD1 molecules are MHC class I-like nonpolymorphic proteins that can be classified into two groups [3]: group 1, comprising CD1a, CD1b and CD1c, is present in humans (but absent in mice and rats) and group 2, the CD1d molecule, is present in most species examined so far. Presentation of lipid Ags has been principally described for the group 1 of CD1 molecules and their ligands include several structurally diverse endogenous as well as mycobacterial lipid Ags including fatty acids, glycolipids, phospholipids and lipopeptide Ags [3–5]. In contrast, the existence of self or foreign, naturally occurring CD1d-restricted lipid Ags is more limited and include glycosphingolipids and phospholipids [3]. In this review, we will focus on CD1d-restricted T cells.

Classification and General Features of NK T Cells

Studies of murine and human T cells have provided growing evidence for the existence of different types of T cells within the CD1d-restricted repertoire that are diverse with respect to TCR expression, structures of Ag recognized, and effector functions. Among them, CD1d-restricted natural killer (NK) T cells represent a heterogeneous population of unconventional T lymphocytes that express NK cell markers, such as NK1.1, and contain different categories of T cells [6]. The most abundant population expresses a semiconserved, canonical T cell receptor (TCR) consisting of an invariant Vα14-Jα18 (in mice) and Vα24-Jα18 (in humans) TCRα chain, combined with diverse TCRβ chains using a restricted number of Vβ region [6, 7]. In mice, this cell population, termed as classical, type-1 or invariant NK T cells (iNK T cells), are found at the highest frequency in the liver (10–40% of liver lymphocytes), but they are present at lower frequencies in thymus, bone marrow, spleen, lymph nodes, lungs, and blood [8]. iNK T cells recognize a limited number of synthetic or naturally occurring α- and, to a lesser extent, β-glycosylated sphingolipids bound to CD1d expressed on Ag presenting cells (APCs), such as dendritic cells (DCs) [8, 9]. Self CD1d-restricted Ags are believed to be generated during steady state conditions (for instance in the thymus) but also, in peripheral sites or systematically, during injury, infection and/or inflammation. That most CD1d-restricted T cells appear to be auto-reactive prompted several investigators to search for self glycolipids. Recently, Zhou et al. [10] elegantly demonstrated that isoglobotrihexosylceramide (iGb3), a lysosomal glycosphingolipid, represents one of the natural endogenous ligands of CD1d-restricted T cells. Moreover, growing evidence suggests that certain microorganisms, including mycobacteria, sphingomonas, *Leishmania*, and *Plasmodium* may also produce

CD1d-restricted ligands capable of activating some sub-populations of NK T cells [11–17].

Along with classical NK T cells, other populations of CD1d-restricted T cells with more diverse TCRs have been identified in mice and humans [6, 8]. However, these cells (designated as non-classical or type-2 NK T cells), have been less well studied (nature of the CD1d-restricted ligands, functions), mainly because of the absence of known specific markers for these cells. For the purpose of this review, the simple term NK T cells will represent solely classical (invariant) NK T cells.

NK T cells are part of the innate immune system and play a critical role in the control of the adaptive immune response. Most of the detailed understanding of the phenotype, development and modulation of immune responses by NK T cells has been derived from the use of the surrogate Ag α-galactosylceramide (α-GC) (an α-glycosylated sphingolipid initially isolated from a marine sponge), which is recognized by NK T cells in the context of CD1d [18]. Taking advantage of these features, several groups have produced tetramers of the CD1d molecule loaded with α-GC, which makes possible a complete and quantitative clonal description of the NK T cell population. In vivo and in vitro stimulation of NK T cells by α-GC promptly induces the production of large amounts of Th1-type (IFN-γ and TNF) as well as Th2-type (IL-4 and IL-13) cytokines that in turn activate and/or regulate the functions of several other cell types including DCs, macrophages, B cells, NK cells, and conventional T cells [9, 19, 20]. In addition, in certain circumstances, NK T cells can also release the immunoregulatory/immunosuppressive cytokine IL-10 [9]. Importantly, NK T cells are relatively flexible in their immediate cytokine response. Thus, according to the conditions of stimulation, they can either suppress or enhance the acquired immune response in a Th1 or a Th2 direction. In fact, many factors, including the doses of α-GC, the cytokines present in their microenvironment, the nature of the APC as well as the number and time periods between NK T cell activation, appear important in the orientation of the ensuing immune response [6–9]. Moreover, the nature of the CD1d-restricted ligand(s) determines the cytokine polarity of NK T cells and thus their ability to control or polarize the outcome of immune responses [9].

NK T Cells in Autoimmune, Neoplastic and Infectious Diseases

The spectrum of actions attributed to NK T cells is particularly broad. NK T cells have been shown to play a central role in some autoimmune diseases, inflammation and resistance to tumors. For instance, they potently promote

tumor rejection in murine models that use exogenously α-GC as a stimulus but they also contribute to the natural antitumor immune response in the absence of exogenous stimulation [21]. In terms of autoimmunity, the most extensive data concerning the role of NK T cells are in the prevention of Th1-mediated autoimmune diseases such as type 1 diabetes and experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis [22]. In these models, stimulation of NK T cells via administration of α-GC is effective in preventing the disease by shifting the balance from a pathogenic Th1 toward Th2 response to islet and central nervous system autoAgs, respectively. In both studies, inhibition of Th1 differentiation of autoreactive T cells was critically dependent on the ability of the NK T cells to produce IL-4 and/or IL-10. On the other hand, in certain experimental models, natural or intentional activation of NK T cells has been shown to enhance autoimmune disorders [23], thus underlining the complex and somewhat unpredictable nature of these cells [7, 9, 22]. Other examples showing the capacity of NK T cells to either induce or suppress the development of inflammation come from studies of murine models of intestinal inflammation, rheumatoid arthritis, dermatitis, hepatitis, atherosclerosis and contact sensitivity [9, 22, 24].

Along with their role in autoimmune/inflammatory diseases and cancer, CD1d-restricted T cells, including NK T cells, have been shown to naturally contribute to antimicrobial host response in some bacterial, protozoan parasitic, viral and fungal infections [12, 13]. However, the protective or deleterious role played by NK T cells depends on the infection model studied as well as the mouse genetic background [13]. Thus, they can control (or not) growth of microorganisms, influence antibody (Ab) production and adaptive immune responses against them, and contribute to immunopathologic tissue injury. Although NK T cells can produce IFN-γ in vivo during the course of some infections, the mechanisms by which they become activated are not fully elucidated. The current hypothesis is that the production of inflammatory cytokines in combination with CD1d-mediated presentation of endogenous ligands (probably iGb3) is involved in NK T cell activation during some infections [17, 25]. Although controversial in some cases, microbial-derived CD1d-restricted ligands may also directly activate NK T cells to produce immunostimulatory cytokines [11, 14–17]. In addition to their role during the natural course of infection, many studies have investigated the effects of pharmacological activation of murine NK T cells by α-GC and have demonstrated protection against viral hepatitis, cryptococcosis, *Mycobacteria* tuberculosis, malaria, trypanosomiasis and leishmaniasis [12, 13]. It is expected that the use of some CD1d-restricted Ags may have therapeutic potential in humans for the treatment of certain infectious diseases and/or for the improvement of vaccine efficacy.

NK T Cells in Th2 Inflammatory Response during Allergic Asthma

Consistent with their role in regulating Th1 response to self (autoimmune diseases) or foreign (infectious diseases) Ags, recent evidence also suggests that NK T cells are implicated in the initiation of some Th2-related inflammatory diseases, thus underlining the flexibility of this cell population in inflammatory pathologies. Two major examples illustrating the critical role of NK T cells in the development of Th2 immune/inflammatory responses will be discussed in this review.

Asthma is a manifold syndrome consisting of eosinophil-rich airway inflammation, bronchospasm and airway hyperreactivity (AHR), which has recently increased dramatically in prevalence in the industrialized world. In allergic asthma, recent advances have identified the complex interplay between Th2 effector cells, recruited inflammatory eosinophils, local immunoglobulin (Ig)E-activated mast cells, and released inflammatory cytokines and chemokines, as well as mediators of airway spasm and AHR [26]. It is generally admitted that, following the sensitization steps, rapid generation of IL-4 supports the crucial Ag-specific Th2 cell differentiation. The exact source of this required IL-4 is not known, but besides Th2 cells themselves, cells like mast cells, γ/δ T cells, and NK T cells might contribute to Th2 immune responses. Studies performed in both BALB/c and C57BL/6 mice suggest that NK T cells are indeed important in the promotion of Th2 immune responses in a model of asthma induced by systemic sensitization and airway challenges with ovalbumin (OVA) [27, 28]. Compared to wild-type (WT) counterparts, NK T cell-deficient ($J\alpha18^{-/-}$) and CD1d-deficient (CD1d−/−) mice displayed significantly reduced IL-4 and IL-5 in bronchoalveolar lavage fluid and decreased circulating anti-OVA IgE and IL-5, following airway Ag challenge. Furthermore, the development of eosinophilic airway inflammation and hyperreactivity were impaired in Jα18−/− and CD1d−/− mice. Importantly, adoptive transfer of NK T cells into Jα18−/− mice just before the first airway challenge restored asthma hallmarks [27, 28]. Finally, transfer of neutralizing anti-CD1d Abs in WT mice, before the sensitization step, prevented asthma development [28]. The implication of CD1d-restricted T cells, including NK T cells, in murine asthma has recently been confirmed using nonobese diabetic (NOD) mice [29] and using an experimental model with ragweed as an allergen [30]. It is noteworthy to mention that these studies are not in agreement with an initial work performed by Korsgren et al. [31]. In this study, no significant role for CD1d-restricted T cells was reported in a murine model of asthma. Among other possibilities, this discrepancy may be explained by differences in the mouse strains utilized (129/Sv × C57BL/6 mice in the later study [31]). Whilst

the influence of NK T cells in the development of experimental allergic asthma appears to be well established, whether or not they participate in asthma in humans is still an open question. One of the problems encountered in human studies is the low frequency as well as the great variability of NK T cell levels in the peripheral blood of human individuals. Also, NK T cell frequencies in peripheral blood do not necessarily mirror those in other tissues, including the lungs. Another limitation in human NK T cell research comes from the difficulty of studying NK T cells locally at the site of the immune reaction rather than in peripheral blood, which is frequently the only material available. Despite these limitations, future investigations aiming at determining NK T cell frequency and functions in the blood as well as in bronchoalveolar lavage samples from atopic vs. non-atopic individuals are warranted to confirm the potential role of NK T cells in human allergy.

Mechanisms of NK T Cell Activation and Mode of NK T Cell Actions during Allergic Asthma

The mechanisms by which NK T cells activate and influence the severity of murine allergic asthma are still under investigation. During asthma, pulmonary NK T cells (CD1d/α-GC tetramer+) are activated and produced both IL-4 and IL-13 [27, 28]. However, although endogenous production of CD1d-restricted glycolipids by pulmonary APCs probably play part in NK T cell activation in this model, the stimuli responsible for their generation are still unknown. After being activated, how do NK T cells enhance the severity of the allergic asthma? Adoptive transfer of NK T cells from double IL-4- and IL-13-deficient mice failed to restore asthma hallmarks in recipient Jα18−/− mice [27], suggesting the importance of these cytokines in this model. It might be hypothesized that NK T cells act either directly as an effector cell, at least through IL-4 and/or IL-13 production, or indirectly, either by licensing or regulating the functions of Th2 cells. They may also favor the recruitment of effector cells into the lungs through the production of some chemotactic factors. NK T cells may also act directly on other pulmonary cell populations to enhance the severity of the disease. For instance, the association between NK T cell activation and mucus overproduction by epithelial goblet cells, following Ag exposure, suggests a role for NK T cell-derived IL-13, a cytokine known to promote mucus production. Finally, as discussed in the last chapter, it is possible that NK T cells could inhibit the functions of some regulatory T cells implicated in the control of asthma. The different mechanisms by which NK T cells could impact asthma pathology are depicted in figure 1.

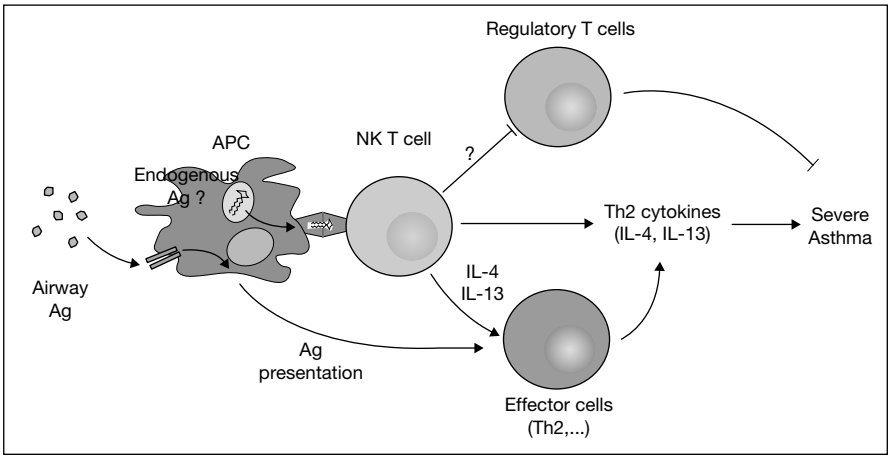

Fig. 1. Mechanisms by which NK T cells could impact asthma. During the sensitization steps, self CD1d-restricted ligands, through unknown mechanisms, are generated and activate resident or newly recruited NK T cells to produce IL-4 and IL-13. This early cytokine production might license the differentiation and/or impact the function of allergen-specific Th2 cells. NK T cell products could also promote the recruitment of effector cells into the lungs and/or act directly on lung resident cells (for instance epithelial goblet cells) to enhance the severity of the disease. Finally, NK T cells could also inhibit the functions of some regulatory T cells implicated in the control of asthma. Targeting NK T cells with synthetic α-GC analogues to counteract the negative influence they naturally exert in allergic asthma may be of therapeutic values, as recently suggested [Leite de Moraes, submitted].

NK T Cells during Helminth Infection

Albeit studied during viral, bacterial and protozoan parasite infection, the role of NK T cells has not been extensively investigated during metazoan parasite infection. Considering their involvement in both Th1 and Th2 responses, one may reasonably speculate that they may play part in the regulation of the immune response during helminthiasis.

Helminth parasites infect more than two billion people in the world today and induce marked morbidity and disability. Infection of humans with helminths, including nematodes (filarial parasites) and trematodes, has long been associated with the maintenance of a dominant Th2-type host immune response reflected by increases in IL-4- and IL-5-producing T cells, elevated IgE and IgG4 levels, and a pronounced eosinophilia. Surprisingly enough, very few studies have been devoted to investigate the role of NK T cells, and to a larger extent of CD1d-restricted T cells, during experimental helminth infections. Balmer and Devaney [32] initially reported the expansion of NK T-like

cells (CD3+/NK1.1+) in the spleen and popliteal draining LNs of C57BL/6 mice as early as 24 hours following infection with the nematode parasite *Brugia pahangi*. The number of IL-4-producing NK T cells was also increased at this time point suggesting that this early IL-4 production could polarize the immune response towards a Th2 profile during infection. On the other hand, Koyama et al. [33] observed that depletion of NK1.1-expressing cells, including some NK T cell subsets and NK cells, had no effect in the induction of Th2 responses and protection against the gastrointestinal nematode *Trichuris muris* infection. Thus, the role of the CD1d/NK T cell pathway during nematode infections is still controversial. On the other hand, several studies, including some from our laboratory, strongly suggest that NK T cells are important during murine schistosomiasis.

Schistosomes are trematodes that cause schistosomiasis, a chronic blood-vascular disease associated with a Th2-dominated response and, at chronic stages of infection, with regulatory mechanisms capable of controlling inflammation [34–36]. Among parasite molecules capable of inducing Th2 and regulatory responses, glycoconjugates including glycolipid Ags may be of particular importance, both acting on the innate and acquired immune systems [36, 37].

In the case of *Schistosoma mansoni*, infection is initiated by penetration of the skin by waterborne cercariae that migrate into the bloodstream and finally arrive in the hepatic portal system, where the sexually differentiated male and female mate. At approximately the fifth week postinfection, the mature female worms begin to produce large numbers of eggs, a substantial number of them being trapped in the liver and intestinal tissue where they induce a granulomatous response that results in the major pathologic manifestations of the disease [34]. The immune response to *S. mansoni* is reportedly initially a Th1 phenotype against the larval and adult worms, followed by a Th2 response to the parasite eggs 6–8 weeks postinfection [34]. Interestingly, as discussed in the next chapter, this sustained Th2-dominant immune response to *S. mansoni* egg Ags probably creates a cytokine environment that induces a Th1 to Th2 shift of the immune responses to unrelated Ags. The early mechanisms leading to the promotion of this Th2 dominated response, induced by parasite eggs, are still obscure. It has been clearly demonstrated that soluble egg Ags (SEA) are the main inducers of this Th2-dominated response [34]. More recently, we and others showed that the glycan moiety of SEA is essential for the induction of this Th2 response [38, 39]. Therefore, we recently hypothesized that egg glycolipids, through a CD1d-dependent mode of Ag presentation, may be important in the induction of Th2 responses during murine schistosomiasis. First, using a model of immunization based on the in vivo transfer of egg Ag-sensitized DCs, we found that DCs generated from CD1d−/− mice or treated with a neutralizing anti-CD1d Ab failed to mount a Th2 response in recipient mice [39]. This

prompted us to investigate the involvement of CD1d in the polarization of the immune response in *S. mansoni*-infected mice. We found that BALB/c CD1d−/− mice have a reduced Th2 response after egg laying and develop a less marked fibrotic pathology compared to WT mice [39]. Moreover, periovular granulomas from CD1d−/− animals displayed reduced numbers of eosinophils, a population that is normally driven by Th2 cytokines. Altogether, these results suggested the involvement of CD1d-restricted T cells in the early immunological events leading to the generation of the Th2 response during schistosomiasis. In agreement with this hypothesis, Zaccone et al. [40] recently reported that treatment with egg or adult worm Ags increased the number of NK T cells in autoimmune diabetes-prone NOD mice. Moreover, the Th2 response induced after immunization was able to prevent the onset of type 1 diabetes in NOD mice. These experimental evidences prompted us to investigate the possibility that NK T cells could become activated during the course of infection. Flow cytometry analysis revealed that splenic and hepatic NK T cells (CD1d/α-GC tetramer+) exhibit an activated phenotype as early as day 21 postinfection [Mallevaey et al., submitted], a time point that just precedes or coincides with full maturation of the worms and egg laying. In agreement with a recent report [40], this suggests that adult worms may be involved in the initial NK T cell activation. More importantly, using a synchronous model of egg deposition in the liver (intracecal injection), we found that NK T cells rapidly produce both IFN-γ and IL-4, but not IL-5 and IL-10 (as assessed by intracellular cytokine staining), in vivo. This suggested that schistosome eggs may activate NK T cells in vivo, probably by an indirect manner via DCs. Indeed, egg-sensitized WT, but not CD1d−/−, DCs activate liver NK T cells in vitro to produce both IFN-γ and IL-4, but not IL-5 and IL-10. Whether or not this early cytokine production also occurs during infection and whether it can influence the ensuing immune response are still open questions that necessitate further studies. Data from mice suggest that NK T cells are pre-programmed to transcribe the genes for both cytokines before activation and that their immediate cytokine response is difficult to polarize. Therefore, the fact that, after egg encounter, NK T cells immediately produce both IL-4 and IFN-γ is not reminiscent with their potential role in the promotion of Th2 responses during infection. Collectively, these data suggested that CD1d-restricted ligands may act as potent inducer of the innate response during schistosomiasis, via NK T cells [Mallevaey et al., submitted]. It should also be added that the use of Jα18−/− mice is necessary for us to determine whether it is the classical, invariant Vα14Jα18 fractions of CD1d-dependent cells involved during infection. Finally, whether parasite-derived or endogenously produced CD1d-restricted ligands (or both) are responsible for NK T cell activation during infection is still unknown and requires further investigation.

Mechanisms by which NK T Cells Become Activated during Infection

There are several, nonexclusive, hypotheses that may explain how NK T cells become activated during infection. First, an increasing body of evidence suggests that DC/pathogen interactions may lead to a DC activation/maturation process that generates CD1d-restricted self-lipids (as well as stimulatory factors such as IL-12) [17, 25]. Activation of innate receptors, including Toll-like receptors (TLRs) by pathogens is the main pathway by which DCs become activated during infection [41, 42]. Thus, it is possible that upon DC/pathogen interactions, some TLR members instigate in DCs intracellular pathways that change the metabolism of lipids, thus generating self or perhaps slightly modified self lipid Ags. In the case of *Schistosoma*, TLR2 and TLR3 appear to be selectively recruited by eggs in vitro to induce DC maturation [43, 44]. Whether or not TLR2 and/or TLR3 participate in NK T cell activation, by inducing in DCs the synthesis of self CD1d-restricted ligands, is an attractive hypothesis that is presently under investigation.

Another hypothesis proposes the existence of pathogen-derived ligands that may directly or indirectly bind to CD1d in DCs, and therefore activate NK T cells. Indeed, it is admitted that microbial pathogens produce many lipid and glycolipid molecules that are sufficiently different from mammalian molecules so that they could be recognized as foreign Ags by the mammalian immune system, via CD1d. Because *Schistosoma* expresses a wide panel of glycolipids, including unusual mono- and poly-glycosylceramides [45; Zanetta, pers. commun.], one may suppose that putative CD1d-restricted ligands may exist in this microorganism. Biochemical and functional studies are underway to elucidate this issue, for instance by testing the ability of different parasite lipid classes to activate NK T cells in vitro and in vivo.

Finally, in our experimental settings, the possibility that non-TCR-mediated signals (for instance via cytokine receptors) cooperate with signalling pathways induced by TCR engagement to activate NK T cells requires additional investigation. The different mechanisms by which schistosome egg/DC interactions may lead to NK T cell activation are depicted in figure 2.

NK T Cells in Regulatory Mechanisms during Th2-Related Inflammation

As stated before, NK T cells can exert diverse functions, according to the way they are activated (type of ligands and APCs, duration of activation, experimental models,...). This flexibility allows them to either promote Th1 or Th2

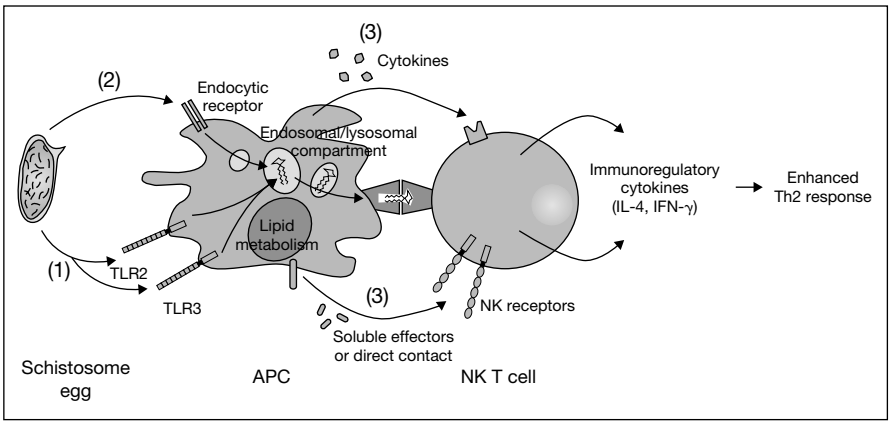

Fig. 2. Possible mechanisms by which NK T cells become activated during schistosomiasis. (1) Upon schistosome egg/DC contact, activation of innate receptors, including TLR2 and/or TLR3, may generate in DCs the synthesis of self CD1d-restricted glycolipids by modifying lipid metabolism. (2) Parasite Ag lipids may also be internalized by DCs, for instance through specific endocytic receptors, and selectively delivered to intracellular compartments (such as endosomes) to be associated with CD1d. (3) In parallel, CD1d-independent pathways may also be involved in NK T cell activation and functions. For instance, in response to eggs, DCs (through TLR engagement?), may produce soluble or membrane-bound factors (combined with self CD1d-restricted Ags) capable of modulating NK T cell functions via activation (or desactivation) of some cytokine and/or NK inhibitory/stimulatory receptors. Whatever the mechanisms, schistosome egg/DC interactions lead to the synthesis of soluble factors by NK T cells that in turn would shape the acquired immune response in a Th2 direction.

responses but also, in some cases, induce tolerance [7, 46]. One interesting feature of schistosomiasis, and in general of helminthiasis, is that infection appears to control both unrelated Th1 and Th2 pathologies [35, 47, 48]. For instance, *S. mansoni* infection and/or egg Ag exposure has been shown to suppress Th1 autoimmune diseases, including type 1 diabetes, experimental autoimmune encephalomyelitis, colitis, arthritis, and Graves' disease [40, 49]. In these settings, it is suspected that some regulatory cell populations, including CD4+CD25+ regulatory T cells, may be involved [50]. The potential regulatory role of NK T cells during murine schistosomiasis therefore needs to be examined in future studies. For instance, they may play part in the resolution phase of the pathology that characterizes the chronic phase of infection by acting directly on inflammatory/effector cells or indirectly by promoting the expansion and functions of other regulatory populations.

In the case of allergic diseases, current concepts postulate that some regulatory cell populations could regulate the effector responses of the disease, and

thus the pathology [26]. Regulatory T cells recognized to influence asthma outcome are the CD4+CD25+ regulatory T cells, the CD4+CD45RB^{low} and the newly described populations, Th1-like and Th2-like T regulatory cells [26, 51]. In such a complex system, could NK T cells impact the expansion and function of regulatory/suppressor T cell populations implicated in the control of the asthma symptoms? Studies are in progress to solve this issue.

Conclusions

NK T cells have diverse and sometimes antagonistic functions on the host system. The identification and characterization of the various molecules/signals that lead to the constitutive or exogenous activation of NK T cells is obviously an important goal for future research. This may lead to design news strategies to harness in vivo the activity of NK T cells and drive them in the desired direction (Th1, Th2 or regulatory responses). Concerning atopic diseases and helminthiasis, both of them being characterized by Th2 and regulatory responses, NK T cells appear to constitutively play a critical role in the initiation of the Th2 response. However, the mechanisms involved in their initial activation are still elusive as are their effects, if any, on subsequent regulatory mechanisms. Also, it will be important to determine whether NK T cells are also involved in Th2-related diseases in the human system. For instance, it will be particularly interesting to determine the frequencies and functions of NK T cells in nonatopic vs. atopic patients, in individuals chronically infected with *Schistosoma* but also in asthmatic individuals living in an area of polyhelminthic endemicity. Finally, it will be crucial to understand how NK T cells are pathogenic in asthma. It may help to develop new therapeutic strategies to neutralize their deleterious role in asthma, for instance by using α-GC analogues that favor the secretion of type 1 cytokines by pulmonary NK T cells. In the case of helminthiasis, it is expected that studies on NK T cells will ameliorate our understanding on the role of lipid/lipid-reactive T cell pathway on the regulation of immune/inflammatory responses. This may also lead to the discovery of new immunostimulatory and/or immunoregulatory molecules applicable in humans.

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The Mast Cell and Gut Nematodes: Damage and Defence

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Abstract

Gut nematode infection induces a dominant type 2 immune response, crypt hyperplasia and mucosal mastocytosis. Despite their strong association with nematode infection, the role of mast cells in the mechanism of worm expulsion is yet to be fully defined. Recent work suggests that they contribute to resistance, aiding the effector mechanisms which ultimately result in worm expulsion. Although it is widely accepted that both connective and mucosal mast cells arise from a common progenitor, it is clear that mucosal mastocytosis is dependent on the presence of type 2 cytokines such as interleukin 4 (IL-4), IL-9, IL-10 and IL-13. Importantly, it is now evident that mucosal mast cells can amplify this protective response, as well as contributing to intestinal pathology. Here we discuss current areas of interest in this field, including the potentially conflicting role that mast cells play in intestinal inflammation. We also highlight the significance of these responses to current ideas relating to parasite infection and allergy.

Response to intestinal nematode, or geohelminth infection has been widely studied and is consistently associated with elevated levels of IgE, histamine and increased blood eosinophilia. Elevated Th2 cytokines are a hallmark of human intestinal helminth infection, but it is the use of animal models that has allowed the mucosal immune response to be studied in detail. Although their precise role in the mechanism effecting worm expulsion has not been completely defined, mucosal mast cells are key immune mediators, and are associated generically with a Th2 mucosal immune response. It has been known for many years that *Trichinella spiralis*, a natural parasite of mice, is absolutely dependent on mast cells for the resolution of infection; mast cell-deficient mice are susceptible [1, 2]. Similarly, the administration of IL-3 to *Strongyloides ratti*-infected mice increases the number of mucosal mast cells and accelerates

worm expulsion [3]. Mast cells play a dominant role in the immune response against *Heligomosomoides polygyrus*; a resistant phenotype during primary or trickle infection correlates with a large mastocytosis [4]. Both *Trichuris muris* and *Nippostrongylus braziliensis* generate a large mastocytosis in response to the parasite, but in both cases the mast cell response is not crucial for worm expulsion [5, 6].

The Th2 response generated during geohelminth infection has been reviewed extensively elsewhere, as has the mast cell and its involvement in allergy and paradoxically, Th1-mediated disorders such as autoimmunity, irritable bowel disease (IBD) and cancer. These specific aspects of mast cell biology will not be repeated here. This review will centre on recent discoveries regarding the mucosal mast cell and its specific role in the immune response to intestinal helminth infection.

Regulation of Mucosal Mast Cells

Mast cell regulation has been studied in many systems. In the context of gut nematode infection, the differentiation and proliferation of mast cells becomes of prime importance when considering expulsion and effector mechanisms in the gut. In the context of allergy, mast cells and their mediators clearly play a significant role in the pathology and etiology of this disease.

Mast cells can be broadly classified as either connective or mucosal mast cells depending on granule content and tissue location. It is widely accepted that both classes derive from the same progenitor in the bone marrow and that it is tissue environment which confers phenotype on the resident immature cell. Significantly, connective and mucosal mast cells produce different proteases, probably in response to the cytokine and tissue environment. Observations at mucosal and tissue sites suggest that mucosal mast cells are generated in response to infection/injury, whereas tissue mast cells are present in naïve/uninfected hosts. Another crucial difference is the clear dependence of mucosal mastocytosis on type 2 cytokines, and therefore on the CD4+ adaptive T cell response.

Stem cell factor (SCF) is an essential growth factor for mast cells, and is particularly important in the generation of a mucosal mastocytosis. Early work with SCF loss-of-function mutant mice (SI/SI^d) and SCF receptor (c-kit)-deficient mice (W/W^v) demonstrated that mastocytosis absolutely relies on signalling through the SCF receptor (c-kit) [1]. This was reinforced by the use of SCF in vivo to increase mast cell number and maturation in the small intestine [7]. Subsequently, the use of a neutralising monoclonal antibody to SCF or *c-kit* in vivo showed definitively that mast cells are involved in the expulsion of *T. spiralis* [8].

More recently, Fukao et al. [9] have generated PI3 kinase-deficient mice, specifically lacking the p85α subunit of the class I PI3K pathway. This subunit transmits a portion of the signalling downstream of SCF:c-kit ligation. The mice are phenotypically similar to the WW^v, but with fewer haematopoietic defects. Although IL-3 signalling is unaffected in the p85α knockouts, these mice selectively lack gastrointestinal and peritoneal mast cells and are highly susceptible to *Strongyloides venezuelensis*. Gab-2-deficient mice, lacking another key player in the PI3 kinase signalling pathway, also suffer selective loss of mast cells [10]. These data support the hypothesis that SCF is particularly important in the generation of a mucosal mast cell response. This pathway is either redundant or non-essential in connective tissue or dermal mastocytosis.

The specific association of Th2 cytokines with mucosal mastocytosis has been widely shown. IL-4 and IL-13 play a significant role in protective immunity to geohelminths [11, 12]. IL-4-deficient mice make a reduced mastocytosis to *T. spiralis*, *Strongyloides ratti*, *H. polygyrus* and *N. brasiliensis*. IL-9 transgenic mice generate a massive gut mastocytosis and subsequently expel *T. spiralis* in a matter of days and IL-9-deficient mice are unable to mount a mast cell response to *N. brasiliensis* [13]. Similarly, although mast cells have been shown to be non-essential in *T. muris* infection, abrogating IL-9 delays worm expulsion and prevents blood eosinophilia [14]. IL-10 is also important in the generation of mastocytosis in response to *T. spiralis* [15]. Lorentz et al. [16] have shown in vitro that although IL-4 alone cannot support the proliferation or differentiation of mast cells, when used in conjunction with SCF it promotes both proliferation and more importantly, the expression of Th2 cytokines by the mast cells. Interestingly, in the absence of IL-4 the mast cells default to produce pro-inflammatory cytokines including TNFα and IL-18. Although it would be convenient to assume that a polarising source of IL-4 must be T cell derived, Urban et al. [17] have elegantly shown using STAT6 knockouts that this is not the case for all helminth infections. It appears that during *N. brasiliensis* infection, IL-4 is required for mastocytosis but does not need to be secreted by lymphocytes; a strong Th2 response is made in the absence of IL-4 responsive T cells. Recently they have pinpointed an additional source of IL-4 to be basophils [18].

Traditionally IL-3 has been used to culture mast cells for study in vitro and for a long time its role was established as an essential cytokine in this context. However in 1998, Lantz et al. [19] showed that although IL-3 deficient mice were susceptible to *S. venezuelensis*, they could mount a gut mastocytosis, albeit reduced. This suggested that in fact IL-3 amplified the mast cell response, rather than specifically controlling it. Work in our laboratory [Pennock, Tybulewitcz and Grencis, unpubl.] has corroborated this observation, but highlighted the possibility of a threshold mast cell number required in order

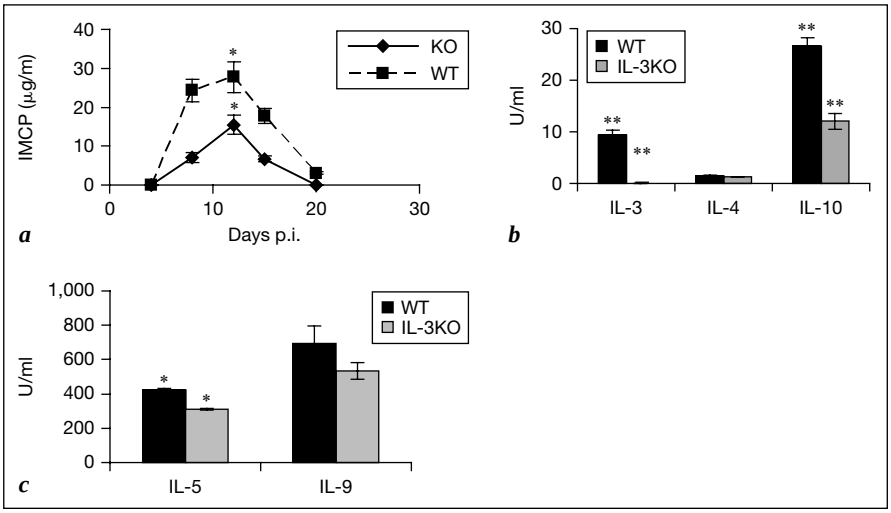

Fig. 1. *T. spiralis* infection of IL-3-deficient mice. ***a*** IL-3KO had reduced intestinal mast cell protease (IMCP) in serum compared to wild-type controls. ***b***, ***c*** In the absence of IL-3, animals made reduced IL-10 and IL-5 in response to antigen restimulation of mesenteric lymph node cells. Error bars represent SE. * $p < 0.05$, ** $p < 0.001$.

to achieve *T. spiralis* expulsion. Although IL-3 deficient mice are able to expel *Trichinella* as rapidly as wild-type controls, mast cell number, and consequently intestinal mast cell protease, is significantly reduced (fig. 1a). Interestingly, antigen restimulation of lymph node cells demonstrated significantly reduced IL-5 and IL-10 production (fig. 1b, c). This supports recent work defining a paracrine role for mast cells in the perpetuation of the Th2 response (see 'Effector Mechanisms' below), and in the chemoattraction of eosinophils to the site of inflammation.

Adhesion and Homing of Mast Cells to the Gut

For many years, the study of mast cells in vitro relied on 2- to 3-week bone marrow cultures using primarily IL-3 and SCF. The physiological relevance of these IL-3-dependent mast cells to mucosal biology remained in question until Wright et al. [20] demonstrated that supplementary factors IL-9 and TGFβ could induce a mucosal phenotype in BMMC, as measured by mucosal mast cell protease-1 (mMCP1) expression. This study has led to the observation that TGFβ can upregulate α_E integrin expression on mast cells in vitro. In mice

lacking the potential to activate TGFβ in the mucosa, intraepithelial mucosal mast cells are absent, despite no difference in number in the lamina propria [21]. Thus TGFβ can potentially control mast cell migration into the epithelial site of the mucosa.

This is a significant finding when considering the effector function of mast cells at the gut/lumen interface (see below), but also bearing in mind how and why mast cells arrive at the gut mucosa in response to helminths. We and others have shown that homing to the small intestine specifically requires β7 integrin [6, 22, 23]. Establishment of the gut-associated lymphoid tissue (GALT) is dependent on β7 expression. In fact this adhesion molecule is critical for small but not large intestinal leukocyte immigration; β7 is required for mastocytosis in response to *T. spiralis*, but β7-deficient mice demonstrate unimpaired leukocyte homing to the large intestine upon infection with *T. muris* [24]. It appears that mast cells require β7 both for maturation (coupled with αE) and for specific homing to the small intestine (coupled with α4). We have shown that in fact mast cell progenitors are generated on demand in the bone marrow of *T. spiralis*-infected mice, and exit into the periphery expressing α4β7 integrin, primed for small intestine entry [22]. Furthermore, these progenitors exit the bone marrow in response to a stem cell factor gradient, supporting a chemotactic role for this essential growth factor. This data corroborated previous studies with *N. brasiliensis* in rats, which demonstrated the presence of mast cell progenitors in the blood during infection using ex vivo colony-forming units [25].

Additional chemoattractants have been demonstrated for mast cells, including TNFα, IL-8 and IL-4, so the reason for an absolute requirement by mucosal mast cells for IL-4 is becoming clear. This essential cytokine is able to induce differentiation, proliferation and migration of mast cells at mucosal sites.

Effector Mechanisms

What precise effector role do mast cells play in the resolution of intestinal helminth infection? Since the actual number of mast cells affects expulsion kinetics, this implies a role for the whole cell in the effector mechanism, either via a soluble or membrane-bound factor. Mucosal mast cells produce various mediators which have the potential to cause pathology, but which also form part of their role in defence against nematodes. *T. spiralis* has provided an excellent infection model to examine their role more closely. Although mucosal mast cells express various tryptases and chymases, the specific role of these proteases has been unknown until recently, despite observations that expression corresponds with cell position within the villi. Mice deficient in mMCP-1, a

chymase found in the cytoplasmic granules of mast cells, were unable to expel *T. spiralis*, despite mounting a sufficient mastocytosis in the small intestine [26]. Furthermore, the mast cells of the mMCP-1-deficient animals appeared to accumulate in the submucosa of the small intestine, suggesting a role for mMCP-1 in the localisation of these cells. Interestingly, *Trichuris suis* has evolved a trypsin/chymotrypsin inhibitor which may be able to modulate mast cell activity in vivo [28]. TsCEI is an effective inhibitor of mMCP-1 and other host trypsin activity in vitro, suggesting that some nematodes may be able to modulate the generic anti-helminth response mounted by the host.

Subsequent work has proposed a mechanism which would explain why mucosal mast cell location is of prime importance. It has been demonstrated that nematode infection causes enteric leakiness of the gut, which is increased by IL-4 and IL-13 [29]. During infection, mast cells migrate from the submucosa, through the lamina propria into the intestinal epithelium, where they begin to express mMCP-1. In fact, mast cells can specifically degrade the tight junction protein occludin during *T. spiralis* infection; both mast cell and mMCP-1-deficient mice did not demonstrate increased paracellular permeability [30]. This suggests that the specific role of mMCP-1 is to disrupt epithelial barrier function and allow the influx of solutes and water into the lumen, potentially contributing to parasite expulsion. Other mast cell secretagogues such as histamine and prostaglandins have been shown to have similar effects in the presence of IL-4 [29].

So mast cells are generated in a Th2 environment, where IL-4 can promote both migration and proliferation of progenitors. But do mast cells contribute to the type 2 cytokine environment once up and running? The primary mode of activation and degranulation has long been understood to be under the control of IgE. An early paper [31] suggested that both dermal and gastrointestinal mast cells express IL-4 constitutively, and that rapid release can be stimulated by IgE. It has since been shown that IL-13, IL-9 and IL-10 can be released from mast cells through FcIRI ligation, although the presence of FcIRI is not essential for mucosal mast cell secretion of mMCP-1. FcγR-deficient mice infected with *T. spiralis* exhibit elevated mMCP-1 secretion and effective worm expulsion [32]. It is known that switching to IgE is under the regulation of Th2 cytokines, so this type 2 milieu can clearly be amplified by the mast cell. However it is becoming apparent that mast cell cytokine secretion and degranulation is significantly enhanced in the presence of type 2 cytokines [33]. Work by Wong et al. [34] has recently shown that mast cells are able to amplify a type 2 response by stimulating the production of IL-13 from T cells. This is achieved by a membrane-bound tryptase (TMT) on the surface of the mast cell which interacts directly with the T cell. This has added significantly to our understanding of why mast cells appear to be so ubiquitous in Th2 responses.

It is understood that mast cells can contribute significantly to the pathology of allergic conditions. Drugs used to control allergic inflammation traditionally involve anti-histamines or mast cell stabilizers. Mast cells are associated with fatal asthma, and have been implicated in the tissue remodelling associated with chronic asthma [35, 36]. Given the pathological potential of mast cell mediators, can their role in helminth infection be viewed as a balance between damage and defence? Do mast cells contribute to the intestinal enteropathy which ultimately provides an unfriendly living space for the parasite, or can resistance be achieved without the villus crypt hyperplasia associated with geohelminth infection? Work by Lawrence et al. [37] has suggested that during *T. spiralis* infection, intestinal pathology is under the control of IL-4; IL-4-deficient mice exhibit delayed worm expulsion, and a suppression of crypt hyperplasia and histological inflammation. Intestinal pathology can also be ameliorated in the absence of TNFα or iNOS [37, 38]. Furthermore, treatment of mice with anti-ICOS reduced TNFα levels, and subsequently pathology, whilst maintaining both mast cell numbers and resistant phenotype [39]. In fact, levels of mMCP-1 were elevated in the treated group. This suggests primarily that the mast cell is able to retain its effector role in the absence of extreme pathology, but also that the expulsion effector mechanism can operate in the absence of crypt hyperplasia, at least in resistant animals.

It is likely that different nematodes elicit different dominant effector mechanisms despite the immune response having evolved generically to expel gut invaders. For example, although *N. brasiliensis* infection induces a typical mastocytosis in the gut, mMCP-1 deficient mice demonstrate expulsion kinetics similar to wild types [40]. Furthermore, SCID mice treated with anti-*c-kit* antibody, subsequently T, B and mast cell deficient, are still able to expel the parasite when treated with IL-4 [41]. It is clear that type 2 cytokines can affect non-immune cells such as smooth muscle, goblet cells and intestinal epithelium, and it has been postulated that these tissues play a primary role in inducing the expulsion of *Nippostrongylus* through peristalsis, enteric leakiness and mucus production. However administration of anti-β7 antibodies to *N. brasiliensis*-infected rats resulted in delayed worm expulsion from the small intestine [6], suggesting that β7+ cells do play a role in the immune response. The observation that basophils express IL-4 during *N. brasiliensis* infection [18] and the knowledge that human basophils express β7, suggests that these cells play an essential role in this helminth infection. However it is possible, and indeed likely, that a large part of the function of mast cells and basophils overlap and that both contribute subtly to the decline of a friendly milieu for the parasite. An early paper by Newlands et al. [7] suggested that mast cells can affect the fecundity of *N. brasiliensis.* rSCF or anti-SCF treatment increased or decreased faecal egg output, respectively. Therefore, the implication that

mast cells are redundant in such a model is perhaps to underestimate their potential.

It is evident that alongside mucosal mast cell location, the actual magnitude of mastocytosis can determine the speed of worm expulsion. Although it has been known for many years that the bone marrow plays a significant role in determining the kinetics of mastocytosis, the molecular factors affecting the real-time accumulation and number of mast cells in the gut during infection are only just being studied. Early work using chimeras strongly suggested that genetic variation could influence expulsion kinetics. This has lead to the hypothesis that in mice at least, strain specific differences in mast cell precursor frequency could influence the immune response. Whether these precursors reside at a mucosal or haematopoietic site remains controversial; limiting dilution assays have demonstrated mast cell progenitors in several tissues including the small intestine, bone marrow and spleen. Early adoptive transfer experiments demonstrated that the mast cells generated during helminth infection were from haematopoietic origin [2]. It is likely that the response to a massive insult such as a helminth infection would involve mobilisation and differentiation in both locations. As mentioned, we have recently shown that mast cell progenitors leave the bone marrow destined for the small intestine during *T. spiralis* infection [22]. Furthermore, limiting dilution assays have demonstrated that in the bone marrow precursor frequency between strains varies widely [42], supporting the strain-dependent observations. However, these studies are based on in vitro culture techniques. Our unpublished work suggests that it is the potential of the precursors to respond to growth factors rather than their frequency which is different between strains.

An important recent development in the allergy and mast cell biology field is the discovery that mast cells express toll-like receptors (TLRs). Given their prime location in the epithelium, this could be an important aspect of their role in gut immunology. Even though it has not been formally shown in situ that gut mucosal mast cells express TLRs, cultured bone marrow-derived and cord blood-derived mast cells do. Work has centred on TLR2 and TLR4 and their role in the immune response against bacterial infection. A natural TLR2 ligand, peptidoglycan, can stimulate degranulation and the release of IL-4, IL-5, IL-6 and IL-13 from bone marrow-derived mast cells (BMMC) [43]. Similarly, stimulation with LPS can induce secretion of TNFα, IL-13, IL-6 and IL-1β but not IL-4 or IL-5. Varadaradjalou et al. [44] have shown that in fact cultured human mast cells require priming with IL-4 to respond to LPS at all. The TLR field has been reviewed extensively [45, 46] and will not be dealt with in detail here. However this area is expanding all the time. Despite a propensity for TLR ligation to be associated with a type 1 response, throughout the recent literature there are examples of TLR2 signalling inducing Th2 responses. In addition,

TLR responses can be regulated by helminth antigens [47, 48] and there is some evidence that TLR4 binding can prevent mast cell apoptosis [49].

Given the prime location of mucosal mast cells in the epithelium of the gut, the role of TLRs in anti-parasitic immunity is beginning to come under investigation. Interestingly, treatment of *T. muris*-infected IL-10/IL-4-deficient animals with antibiotics can abrogate the severe pathology that normally results in 100% mortality [50]. An earlier study showed that whipworm infection actually promotes bacterial colonisation and growth, suggesting that in fact toll receptor ligation may still be promoting a concurrent Th1 response in the chronically infected gut [51]. This has been supported elsewhere [33]. In susceptible animals, the persistence of a significant mastocytosis in a Th2 environment could allow otherwise innocuous infections to sustain pathology through the activation of inflammatory pathways. Additional studies have shown that in fact ligation of TLRs by LPS or peptidoglycan on BMMCs can induce the secretion of TNFα without inducing degranulation [52]. This supports work discussed above relating to the role of TNFα in intestinal enteropathy [38, 39]. The importance of TLRs in the immune response and pathology in the gut remains to be seen, but it is likely that as this field progresses it will shed more light on the role of the mast cell in allergy and resistance to nematode infection.

Current Ideas

It is clear that mast cells play a fundamental role in the pathophysiology of Th2-induced allergic conditions such as asthma, and it has been suggested that the decline of nematode infection in the west has contributed to the increased incidence of asthma and related disease [53]. This field is still controversial; recently *Ascaris lumbricoides* infection has been associated with an *increased* incidence of asthma [54], but other studies have found no such association. Rather, an increased resistance to worm infection, i.e. heightened Th2 response, has been suggested as one evolutionary contributory factor to the asthma prevalence and inflammatory bowel conditions we see in the west today. A recent study by Cooper et al. [55] has shown that in an area where *A. lumbricoides* infection is endemic (rural Ecuador), it is the genetic predisposition to atopic disease which protects against helminth infection, rather than the other way around. In other words, a resistant phenotype may have provided an evolutionary advantage in the past, but has subsequently promoted atopy in the absence of any geohelminth infection. An alternative hypothesis is that early exposure to intestinal helminth infection can tolerize the host against cross-reactive allergens, thereby dampening down the allergic response and providing protection.

Mast cells are also being implicated in the etiology of distinctly Th1-driven disorders, such as Crohns, ulcerative colitis and autoimmunity. Although the localisation and phenotype of the mast cells are different when comparing Crohns and a helminth infection [56], the co-existence of both conditions clinically may prove the undoing of inflammatory bowel disorders. Significantly it seems that localisation of a parasite (*Trichuris suis*) in the intestine can switch an inflammatory Th1 site to a protective Th2 environment and give temporary relief from IBD [57 and references therein]. The mechanism inducing this dramatic remission (86%) has not been studied closely, but it is clear that both IBD and *Trichuris* infection are associated with a strong mastocytosis. It will be interesting to see how this field develops. Is this a pertinent example of mast cell collusion in the immune response, conspiring to elicit damage or defence depending on the environment or pathogenic trigger? Perhaps simply the presence of a dominant antigen is sufficient to switch the immune response from pathology to protection. Or maybe the parasite itself is inhibiting the pathological inflammation which is so damaging in IBD.

Equally interestingly, the same research group has shown in mice that an anti-nematode response in the duodenum can positively modulate a damaging inflammatory response in the colon [58]. The colitis induced in IL-10 deficient mice was reversed by infection with *H. polygyrus* after the colitis had been established. Furthermore, this effect was adoptively transferred using MLN cells from *H. polygyrus*-infected colitic mice. Mast cells were not studied in this investigation, but IL-13 appeared to be the primary Th2 cytokine induced; there was no difference in IL-4 production between treated/non-treated groups. Since mastocytosis correlates clearly with a resistant phenotype in *H. polygyrus* infection [4], and given the recent discovery of TMT (see above), it would be pertinent to repeat the same experiments in a mast cell-free system.

In conclusion, recent literature has reinforced the integral role of the mucosal mast cell in Th2-driven responses. This milieu can both drive and maintain the mast cell response to gastrointestinal nematode infection. Furthermore, the role of mast cell mediators in the effector mechanism associated with resistance to geohelminth infection is gradually being uncovered. Importantly, it seems that mast cells may in fact play a pivotal role in the resolution of inflammatory disorders that are increasingly familiar clinical problems challenging medicine today.

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Basophils, Basophilia and Helminth Infections

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Abstract

A growing body of evidence suggests basophils are important components of the human immune response to helminth infections. Basophil numbers are increased in several animal models of helminth infection, and basophils have been shown to release both histamine and IL-4 in response to helminths. Helminth infections typically provoke type 2 immune responses characterized by eosinophilia, elevated levels of Ag-specific and polyclonal IgE, and T cell production of type 2 cytokines such as IL-4, IL-5, and IL-13. IL-4 plays a central role in this type 2 response. As basophils are the only peripheral blood mononuclear cells with the ability to release IL-4 rapidly in response to appropriate stimuli, releasing large quantities of preformed IL-4 within minutes of surface IgE cross-linking, it appears likely that basophils play an important role in amplifying ongoing type 2 immune responses to helminth infections once Ag-specific IgE is present. Basophils may also function to initiate type 2 responses upon first exposure to helminths and to potentially re-establish these responses upon re-exposure. This article reviews basic basophil biology and physiology, evaluates the evidence for the presence of basophilia in helminth infections, and then focuses on the possible roles basophils serve in the immune response to helminth infections.

Basophils are leukocytes with large cytoplasmic granules that originate from CD34+ progenitor cells. Although present at tissue sites of inflammation in allergic diseases and helminth infections, basophils reside predominantly in the circulation. Basophils typically degranulate through an IgE-dependent pathway thereby releasing multiple inflammatory mediators, with histamine being the prototypical effector molecule. While basophils are typically considered part of the innate immune system, they also modify the adaptive immune system through the release of cytokines and chemokines. In addition, histamine, itself, has multiple immunomodulatory effects. Finally, because basophils are

clearly involved in the immune response to helminth infections, this monograph will focus on the role these cells play in the immune response to helminth infection.

Basophil Biology

Basophils are the least common white blood cell, making up 0.5–1% of the circulating peripheral blood cell population and accounting for 0.3% of the nucleated cells in the marrow. They have lobulated nuclei of various forms and contain multiple prominent amorphous granules that stain purple to red with blue aniline dyes. This staining pattern is due to the presence within the granules of highly sulfated proteoglycans that are often complexed to proteases, histamine, and cytokines.

Lineage

Basophils originate from pluripotential CD34+ progenitor cells found in cord blood, peripheral blood, and bone marrow. They are believed to derive from a subset of CD34+ cells that are also IL-3R+ and IL-5R+. Existence of a common progenitor cell for eosinophils and basophils is supported by the finding of granulocytes with hybrid eosinophil/basophil phenotype in vivo in patients with chronic myelogenous leukemia and in vitro in cultures of peripheral blood and bone marrow from normal human donors. There is also evidence that basophils share a common progenitor with mast cells.

Traditionally, basophils have been thought to arise from a separate cell lineage than mast cells, whose progenitor cells are CD34+/CD38+ or CD34+/c-kit+/CD13+. Recently, however, a new monoclonal antibody (97A6 – which targets ectonucleotide pyrophosphatase/phosphodiesterase 3 (CD203c)) has been described that is specific for mast cells, basophils, and their precursors [1], suggesting that mast cells and basophils arise from a similar lineage.

Growth and Development

The principal cytokine required for basophil growth and differentiation is IL-3 [2]; up to 50% of cord blood and bone marrow cells differentiated in the presence of IL-3 become basophils, with the remaining cells consisting of eosinophils, neutrophils, and macrophages. Studies with IL-3 knockout (KO) mice have demonstrated that IL-3 is not required for development of baseline basophil numbers in the bone marrow and circulation, but IL-3 is necessary for the enhanced basophil production seen in mouse models of helminth infection [3].

Cytokines other than IL-3 also contribute to basophil development. Differentiation of the human basophil-like cell line KU 812 can be promoted by IL-6 and TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and possibly IL-5 can cause basophil differentiation and increased production from the peripheral blood.

Basophils typically remain in the circulation after maturation in the bone marrow but can infiltrate tissues at sites of active inflammation in parasitic diseases, asthma, and allergy. The typical basophil has a relatively short lifespan, ranging from a few days to a few weeks. IL-3, IL-5, and NGF all improve the basophil lifespan in culture, with IL-3 improving viability the most [4]. Basophils usually die through apoptosis, which can be induced by glucocorticoids and inhibited by IL-3 (although not by IL-5 or GM-CSF). The type 1 cytokines IL-12 and IL-18 induce Fas-dependent apoptosis of circulating immature basophils, likely through NK cell-mediated cytotoxicity [5].

Trafficking

Trafficking of basophils from the peripheral circulation to sites of inflammation occurs through a multistep process, with leukocytes rolling on endothelial cells through binding of selectins, firmly adhering to endothelium through integrins, transmigrating through the endothelial cell layer by diapedesis, and finally proceeding to sites of inflammation through the effects of chemotactic agents.

Expression of basophil adhesion molecules is modulated by several factors. Pretreatment with IL-1 and TNF-α, as well as FcεR1 aggregation, enhances the ability of basophils to bind to endothelial cells. IL-3 increases basophil adhesiveness to endothelial cells, possibly by increasing CD11b – a molecule that can bind to ICAM-1, fibrinogen, and C3bi – on basophils. In addition to CD11b, basophil activation also increases surface expression of CD11c and CD11d, two other integrins [6].

Multiple factors – including cytokines, chemokines, complement components, and lipid products – serve as basophil chemoattractants. Of the CC chemokines, RANTES and MCP-3 have the greatest ability to attract basophils, followed by MCP-1 and MIP-1α [7]. In terms of the effects of the CC chemokines on basophils, RANTES acts mostly as a chemoattractant, MCP-1 as an inducer of basophil degranulation, and MCP-3 as a combination, serving as both a strong chemoattractant and inducer of basophil degranulation. MCP-2, another CC chemokine, is also chemoattractive for basophils. Stromal cell-derived factor 1 (SDF-1), a CXC chemokine that is the only known ligand for CXCR4, has been shown to recruit basophils with even greater potency than RANTES and MCP-1 [8]. Of the cytokines, IL-3 and GM-CSF are strong basophil chemoattractants and IL-5 and IL-8 are weak ones [9].

Eotaxin, eotaxin-2, and eotaxin-3, usually considered primarily eosinophil chemoattractive proteins, are also potent basophil chemoattractants. Other basophil chemoattractants include the complement components C3a and C5a, leukotriene B_4, and platelet-activating factor (PAF). Recently, prostaglandin D_2 has been shown to induce migration of basophils through the CRTH2 receptor [10] and insulin-like growth factors 1 and 2 have been shown to selectively promote basophil chemotaxis [11].

Basophil Function

Basophil Activation

Due to their ability to bind IgE, individual basophils can specifically recognize and be activated by many different antigens. Activation of basophils causes release of pre-formed and newly synthesized inflammatory mediators that play important roles in vascular reaction, exudation, leukocyte accumulation, and wound healing. In addition, basophils have the ability to produce and release cytokines and to phagocytose particles.

IgE-Mediated Activation

The most important molecules for basophil function are their high-affinity IgE receptors (FcεR1). These receptors are highly specific for IgE and bind them in a 1:1 ratio, with the CH3 domain of the Fc portion of the IgE antibody serving as the principal binding site for FcεR1. When a multivalent antigen binds to several IgE molecules, the receptors aggregate and initiate cell activation, stimulating mediator generation and release. Basophil degranulation occurs very quickly, with cross-linking of IgE causing half-maximum histamine release from basophils in 7 min [12].

While basophil sensitivity varies between different individuals, the average number of IgE cross-links necessary to trigger basophil activation is approximately 2,000 [13]. Because basophils can express several hundred thousand FcεR1 per cell, a single basophil can hold enough different IgE molecules to enable that basophil to specifically recognize and be activated by well over a thousand antigens.

FcεR1 upregulation on basophils is mediated by the interaction of IgE with FcεR1. Because of this, the number of FcεR1s expressed on basophils is proportional to the amount of IgE in the serum [14].

Human FcεR1 can be expressed as a tetrameric ($\alpha\beta\gamma_2$) or trimeric structure ($\alpha\beta_2$). Because the β subunit of FcεR1 amplifies IgE-triggered signaling

[15] and increases surface FcεR1 expression [16] the finding of different FcεR1β:FcεR1α ratios suggests that basophil responsiveness is modulated in part by changes in this ratio.

In addition to FcεR1, human basophils also express FcγRIIb, a low-affinity IgG receptor. In vitro studies using human basophils have shown that co-aggregation of FcγRIIb and FcεR1 molecules decreases IgE-dependent activation [17].

Non-IgE Activation

Basophils can be activated by a number of substances through IgE-independent mechanisms. These factors, known as histamine-releasing factors, include complement proteins, human histamine-releasing factor (HRF), chemokines, cytokines, substance P, PAF, contrast media, ionophores, opiates, the bacterial peptide f-Met-Leu-Phe (fMLP), and HIV glycoprotein 120.

The complement proteins C3a, C4a, and C5 cause basophil as well as mast cell degranulation. They may have a role in anaphylactoid reactions and are therefore known as anaphylatoxins. It has been suggested that anaphylatoxins may be activated in immune complex-mediated diseases, reactions to iodinated contrast media, and reactions to dialysis tubing.

HRF (p23) is made by lymphocytes of atopic children and causes histamine and IL-4 release from a subset of basophils in patients with allergy. Initially believed to act through IgE, it has recently been shown to activate basophils in an IgE-independent manner, although its basophil receptor has yet to be identified [18].

Of the CC chemokines, MCP-1 and MCP-3 are the most potent histamine-releasing factors described [7]. MCP-1 has been shown to be equivalent to IgE and C5a in its ability to cause basophils to degranulate, and it induces histamine release from basophils within 30 s. Other CC chemokines that induce basophils to release histamine include MCP-2, RANTES, and MIP-1α. Of interest, while it is a weak inducer of basophil degranulation, MCP-2 also causes basophils not pre-treated with IL-3 to lose their ability to be degranulated by MCP-1 and MCP-3, suggesting that MCP-2 may act as a functional inhibitor of MCP-1 and MCP-3. SDF-1, a CXC chemokine, induces histamine release from basophils equally as well as does MCP-1 [8].

Other less potent basophil activators include fibroblast-induced cytokine, MIP-1α, connective tissue-activating peptide III (CTAPIII), secretory IgA, neutrophil activating peptide-2 (NAP-2), and stem cell factor. IL-18, a proinflammatory cytokine that activates NK cells and Th1 responses, induces basophils to release histamine, IL-4, and IL-13 when co-cultured with IL-3 [19]. When IL-18 is given with IL-12, however, it inhibits IgE-triggered histamine activation [19].

Degranulation

Basophils release granule contents in two different ways. Anaphylactic degranulation (AND), the typical fashion in which basophils degranulate after IgE-mediated activation, occurs through granule-to-plasma-membrane fusion and allows for very rapid release of large amounts of granule materials. Piecemeal degranulation (PMD), on the other hand, occurs through trafficking of small vesicles from granules to the plasma membrane and results in a slow, piecemeal loss of granule contents. The most effective trigger for AND is anti-IgE (then MCP-1, then rHRF), whereas rank orders for piecemeal degranulation and granule-vesicle attachments are MCP-1 > anti-IgE > rHRF [20]. While morphologically they appear very different, AND and PMD actually represent anatomic extremes of a continuum of basophil degranulation, because at very high rates of PMD, vesicles form into tubules by vesicle-to-vesicle fusions, allowing for direct granule-to-plasma-membrane fusion and leading to the morphologic release pattern termed AND.

Activation Markers

CD63 has been the most commonly used marker of basophil activation. IgE-dependent activation of basophils is associated with upregulation of CD63, and the kinetics of this upregulation parallel those of histamine release from basophils [21]. Increased expression of CD63 on basophils as detected by flow cytometry after in vitro allergen challenge correlates strongly with in vitro assays of histamine and leukotriene C4 release and has been shown to correlate with a history of allergy to both pollen and hymenoptera venom. In addition to CD63, activated basophils also upregulate several β_2 integrins.

Because CD63 and integrins are not specific only for basophils, studies for other markers of activation are being actively pursued. One possible candidate is E-NPP3 (CD203c), a type II transmembrane protein exclusively expressed on basophils, mast cells, and their CD34+ precursor cells [1]. CD203c has recently been shown to be upregulated on peripheral blood basophils from sensitized individuals after exposure to antigen [22] and prostaglandin D2 [23].

Effector Molecules

Basophils release both preformed and newly synthesized inflammatory mediators. Preformed inflammatory mediators include histamine, chondroitin

sulfates, neutral protease, elastase, β-glucoronidase, major basic protein, cathepsin G-like enzyme, Charcot-Leyden crystal protein, tryptase, chymase, and carboxypeptidase. Basophils also store basogranulin, the function of which is unknown, and IL-4. Factors that basophils synthesize upon activation include leukotriene C4, PAI-1, IL-4, IL-13, and MIP-1α.

Histamine

Basophils are the predominant source of histamine in human peripheral blood. Histamine binds to the carboxyl groups of proteins and proteoglycans in secretory granules through ionic forces. During degranulation, histamine dissociates from the proteoglycan-protein complex by cation exchange with extracellular sodium at neutral pH. Histamine likely acts close to its site of release, as it is degraded into either methylhistamine or imidazole acetic acid within minutes of release. Histamine affects cells through its interactions with cell-specific H1, H2, H3, and H4 receptors [24]. H1 receptors induce vascular permeability, dilate arterioles, and stimulate intestinal and bronchial smooth muscle contraction. H2 receptors stimulate gastric acid production by parietal cells, increase mucus secretion, cause endothelial cells to release prostacyclin and modulate the immune response by inhibiting secretion from cytotoxic lymphocytes as well as neutrophils and basophils. H3 receptors modulate neuroconduction by affecting neurotransmitter release in the central and peripheral nervous systems. H4 receptors were first cloned in 2000 and their physiologic role is still being investigated.

Other Effector Molecules

The effects of leukotriene C4 overlap significantly with those of histamine, as it causes smooth muscle contraction, increased vascular permeability, and mucus secretion. Because it is not preformed, LTC4 is released several hours after basophil activation and, thus, is a contributor to the late-phase response seen in allergic reactions.

MIP-1α (macrophage inflammatory protein-1) is a potent inflammatory mediator that stimulates further histamine release, causing a positive feedback look of basophil degranulation.

C5a stimulation of the basophil cell line KU 812 results in production of plasminogen activator inhibitor-1, suggesting that basophils may play a role in the modulation of fibrinolysis.

Basogranulin is a recently identified basic protein that appears to be unique to basophils, is located in secretory granules, and is secreted. Its physiologic role is as yet undetermined.

Immunomodulatory Capabilities

While basophils are typically thought of as cells of the innate immune system, they also serve to modify the adaptive immune system by releasing cytokines. Additionally, histamine released by basophils has immunomodulatory effects.

Cytokine Release

Basophils release both IL-4 and IL-13. IL-4 induces CD4+ T helper (Th)0 cells to become Th2 cells and both cytokines can, in combination with ligation of CD40, cause B cells to switch to IgE isotype. Of all PBMC, basophils are the only cells with the ability to provide IL-4 early in response to a stimulus, releasing preformed IL-4 within 5–10 min of IgE stimulation. After activation, basophils also synthesize IL-4 and IL-13 de novo, with time-course experiments showing a second peak of IL-4 release after 4 h and a first peak of IL-13 release at 24 h [25].

There are likely some differences in the regulation of IL-4 and IL-13 production by basophils, as only IL-13 is secreted from basophils after stimulation with IL-3 alone and IL-4 production is inhibited by the immunosuppressant FK506, whereas IL-13 production is not [26]. Also, 20 h after activation, the amount of IL-4 released from basophils after stimulation with IL-3 and anti-IgE correlates strongly with the percent of histamine secreted, while the amount of IL-13 released does not [26].

Eotaxin also modulates basophil cytokine production, augmenting antigen-dependent IL-4 production and release from basophils by 2- to 4-fold and lowering the threshold for basophil activation and IL-4 production by 40-fold [27]. Other factors that enhance basophil IL-4 production include the chemokines MCP-2, MCP-3, MCP-4, RANTES, and eotaxin-2, all of which act on basophils through CCR-3 [27], and the recombinant HRF (rp21) [28].

Because they can express CD40 ligand as well as release IL-4 and IL-13, basophils have the ability to induce B cells to switch to IgE isotype, potentially creating a positive feedback loop whereby IgE-dependent activation of basophils results in the production of more IgE by B cells.

Immunomodulatory Properties of Histamine

Basophils can modulate the immune response through secreted histamine. Histamine modulates the expression and action of several cytokines, having been shown to increase production of IL-2, IL-5, IL-6, IL-8, IL-10, IL-11, IL-16, GM-CSF, and RANTES and to downregulate production of IL-2, IL-4, IL-12, IFN-γ, and TNF-α [29].

Signaling through the different histamine receptors mediates different effects on the Th response. Triggering of the H1R on CD4+ T cells enhances Th1-type responses, causing increased IFN-γ production and subsequent downregulation of Th2-type responses, while triggering through the H2R downregulates both Th1 and Th2 responses [24]. Because the Th2-type response is integral to the production of IgE through the actions of IL-4 and IL-13, the downregulation of the Th2 arm of the cellular immune system through H1 and H2 receptors may serve as a negative feedback on the response that initially accounted for the production of IgE that led to the basophil degranulation. Clinical evidence that histamine has the ability to act as an immunosuppressant comes from studies showing that histamine suppresses contact dermatitis in mice [30] and decreases anti-tumor cytokine expression, with subsequent increases in tumor growth, in colorectal cancer implants [31].

Histamine exerts its immunomodulatory effects by acting on a variety of cell types and modulating the Th1/Th2 balance at several different points [24]. Histamine affects monocytes by decreasing their production of IL-12 and by inhibiting ICAM-1 expression induced by IL-18. Through interaction with the H2 receptor, histamine causes increased IL-10 and decreased IL-12 production by dendritic cells. Histamine also increases the release of IL-10 from unstimulated and LPS-stimulated human alveolar macrophages by PGE_2 and NO production. One study suggests that many of the effects of histamine may be due to its upregulation of IL-18 production, as addition of anti-IL-18 abolished all cytokine changes caused by histamine in in vitro studies of PBMC [32]. Finally, histamine has been reported to negatively feedback on basophils by decreasing basophil activation as measured by Alcian blue staining and CD63 expression [33].

Basophils and the Immune Response to Helminth Infections

The immune response to helminths is markedly different than that to most viral, bacterial, and fungal infections. Helminths initially induce a type 2 immune response characterized by eosinophilia, elevated serum levels of Ag-specific and polyclonal IgE, and increases in T cell production of IL-4, IL-5, and IL-13. Eosinophilia is likely due to the production of IL-5 by CD4+ T cells, whereas the IgE production is due to IL-4 and IL-13, both of which, in conjunction with CD40-CD40 ligand interaction, induce IgE switch recombination in B cells. The initial signal that induces production of type 2 CD4+ T cells in helminth infections, however, remains unknown. IL-4, which can induce T cells to differentiate toward a type 2 phenotype, may be the initial trigger of

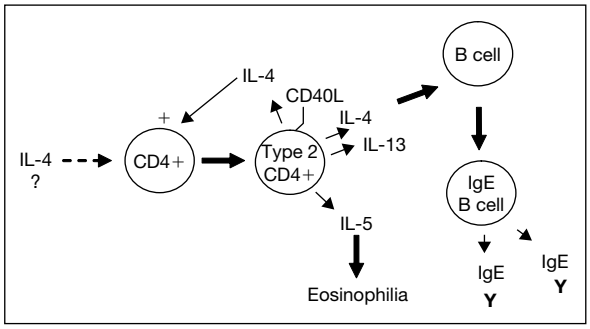

Fig. 1. Possible mechanism for increased IgE levels and eosinophilia in human helminth infections.

this response, or may serve simply to amplify an ongoing type 2 response. A schematic of this immune response is diagrammed in figure 1.

Basophilia

Several factors suggest basophils play an important role in the immune response to helminth infections. While there are no good data that basophilia occurs in helminth infections of humans it clearly occurs in animal models of parasitic infection (references in [34]). One of the earliest reports of this phenomenon came from Chan in the 1960s, who showed that bone marrow and circulating basophil counts increase within 48 h following subcutaneous injection of Ascaris body and ova fluid into guinea pigs. Since then, several other investigators have reported similar findings in several different animal models of parasitic infection. *Trichostrongylus colubriformis* infection has been shown to increase basophil numbers in the bone marrow, small intestine, and peripheral circulation of guinea pigs. Infection of rats and gerbils with *Nippostrongylus brasiliensis* resulted in up to a 50-fold increase in peripheral basophil counts at 2 weeks. *Trichinella spiralis* infection in rats and guinea pigs also results in a marked basophilia that precedes the onset of eosinophilia by approximately 1 week. Fasciola infection of guinea pigs is associated with a chronic peripheral basophilia that is detectable up to 4 months after infection, and Strongyloides infection of *Erythrocebus patas* monkeys causes peripheral basophilia that is occasionally detectable for more than a month after infection.

The presence of peripheral basophilia in human parasitic infections has not been as clear. While basophilia is mentioned as occurring in human parasitic infections in immunology and parasitology book chapters and review articles, the evidence to support this phenomenon is scant. Indeed, in a retrospective study of >600 patients with confirmed parasitic infection only 4 had an

elevated peripheral basophil count, and there were no statistically significant differences in either absolute or relative basophil counts between individuals with and without parasites [34].

While this finding clearly demonstrates that basophilia is not a useful clinical marker of parasitic disease, it does not exclude the possibility that basophils play an important role in helminth infections of humans. As the subsequent sections illustrate, there is a growing body of evidence that suggests basophils are important components of the human immune response to helminth infections.

Basophil Activation in Helminth Infections

It is clear that basophils are activated by helminth infections, as basophils from patients infected with *Toxocara, Ascaris, Onchocerca, Wuchereria, Loa loa, Strongyloides,* and *Schistosoma* have been shown to release histamine in response to parasite Ag (references in [35]). Basophil activation in helminth infections is likely due in part, if not entirely, to the presence of Ag-specific IgE, the levels of which are proportional to the amount of histamine released by basophils after stimulation with helminth antigens [36].

In addition to histamine, basophils have also been shown to release IL-4 in response to helminth infections. Flow cytometry studies have demonstrated that the absolute numbers of basophils and CD4+ T cells in filaria-infected patients that release IL-4 in response to filarial antigen are equivalent [35]. Moreover, in filaria-infected individuals, basophils release more IL-4 on a per cell basis than do CD4+ T cells and do so after stimulation with 100-fold lower concentrations of antigen than that required for Ag-specific CD4+ T cell activation [35].

The finding of basophils as a major contributor to the IL-4 pool in helminth infection has been corroborated by other studies. A study in Papua New Guinea showed human basophils capable of releasing IL-4 in response to larval and adult filarial Ag [37]. In a murine study using the IL-4 promoter to drive green fluorescent protein expression, basophils in the liver and lungs of mice have been demonstrated to be the principal source of IL-4 production in vivo after infection with *N. brasiliensis* [38]. Similarly, a model of antigen-specific induction of Th2 memory CD4+ T cells [39] has also shown basophils to be the major initiator of IL-4 activity.

Helminth Antigens as 'Super Allergens'

It has been demonstrated that a glycoprotein from *Schistosoma mansoni* eggs and *Echinococcus multilocularis* extracts can cause basophils to degranulate and release IL-4 by binding and cross-linking non-antigen-specific IgE [40, 41], raising the possibility that some helminth antigens may act as a type of 'super allergen' by being able to bind directly to the non-variable portion of

IgE. This suggests that, in some cases, basophils may serve as the initial source of IL-4, providing the stimulus for T cells to differentiate toward a Th2 phenotype. This concept of 'super allergenity' of certain helminth antigens has found support in the identification of secreted proteases of the hookworm *Necator americanus* that have been shown to induce both IL-4 and IL-13 release from basophils in a non-IgE specific manner [42]. Most interestingly, basophils from mice that lack B cells, and thus have no IgE, have been convincingly shown to release IL-4 in response to *Nippostrongylus* infection [38]. Theoretically, at least, some of the IgE binding parasite-encoded lectins also have the potential to activate basophils in a non-classical manner through binding to the heavily glycosylated IgE molecules bound to the basophil surface through FcεR1 [43].

Similarly, homologs of mammalian translationally controlled tumor protein, a calcium-binding protein that directly stimulates histamine release from basophils, from *Schistosoma mansoni* [44] and the filarial parasites *Wuchereria bancrofti* and *Brugia malayi* [45] have been identified and characterized at a molecular level. Indeed, this is one of the major transcripts found in both Brugia and Wuchereria microfilariae based on abundance in expressed sequence tag (EST) studies [Ndi et al., unpubl.]. These studies all suggest that basophils are functionally important in the immune response to helminth infections. The exact role they play, however, remains to be characterized.

Potential Roles of Basophils in Helminth Infections

Some of the potential roles basophils may play in the immune response to helminth infections are diagrammed in figure 2. If helminth antigens truly act as 'super-allergens' in vitro and non-specifically activate basophils, then basophils may act as the initial source of innately derived IL-4, initiating the type 2 response observed in helminth infections (fig. 2 pathway A).

T cell receptor engagement in the presence of IL-4 causes naïve T cells to differentiate to a type 2 phenotype. As basophils release large quantities of IL-4 after IgE-mediated activation, it stands to reason that stimulation of basophils in close proximity to the presentation of an antigen by an APC to a T cell could result in that T cell differentiating to a type 2 phenotype. While not yet demonstrated by either in vivo or in vitro studies, this potential immunomodulatory effect of basophils has been postulated by several researchers. If true, it would suggest that basophils could serve to differentiate naïve Ag-specific CD4+ T cells to type 2 CD4+ T cells in helminth infections once Ag-specific IgE is present (fig. 2 pathway B).

In vitro studies have already demonstrated that basophils, cells which express CD40 ligand as well as release IL-4, have the ability to induce B cells to switch to IgE isotype [46, 47] (fig. 2 pathway C). If either pathway B or C occurs in vivo, then basophils would function to amplify ongoing Ag-specific

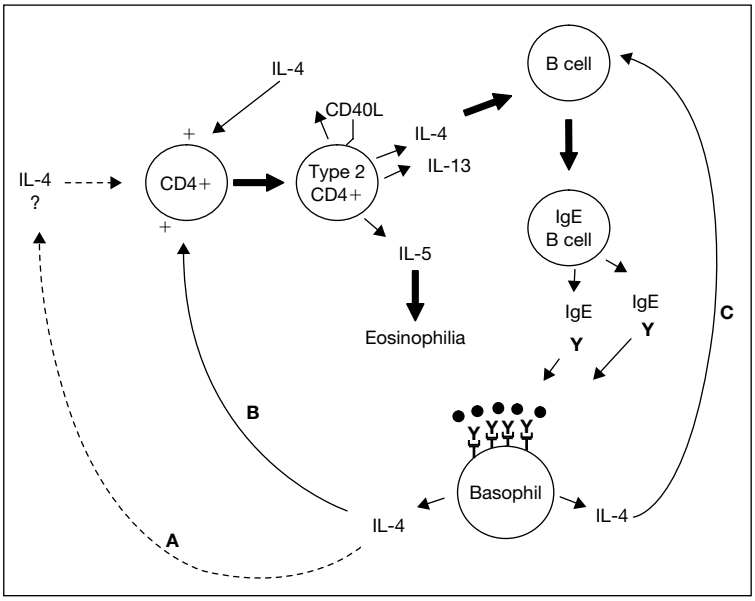

Fig. 2. Possible roles of basophils in the immune response to systemic helminth infections. Pathway **A** suggests that basophils may be the initial source of IL-4 in helminth infections. Pathways **B** and **C** suggest basophils serve to amplify an ongoing type 2 immune response by causing non-differentiated Ag-specific CD4+ T cells to convert to type 2 T cells and by causing B cells to undergo isotype switching to IgE production. Pathway **A** requires a nonspecific means of basophil activation by helminth antigens. Pathways **B** and **C** could occur in the presence of nonspecific or Ag-specific IgE-mediated basophil activation. ● = Helminth Ag; **Y** = helminth Ag-specific IgE; ⍊ = FcεR1.

IgE and type 2 immune responses in helminth infections. Finally, basophils, in conjunction with persistent, long-term Ag-specific IgE production by B-cells, may serve to quickly re-establish a type 2 immune response by either pathway B or C in individuals who have previously been infected with helminth infections.

In addition to improving our understanding of the human immune response to helminth infections, further study of the role basophils play in helminth infections may provide insights into the mechanisms behind and potential therapeutics for allergic and autoimmune diseases. For example, if basophils can be shown to drive CD4+ T cells toward a type 2 phenotype (fig. 2 pathway B), then an entirely new field of practical immunomodulation may be opened. If one wanted to initiate a type 2 response against a certain antigen, for example, one could hypothetically vaccinate a person with the antigen of interest concurrently with Ag-specific IgE. Since IL-4 downregulates production of Th1 cells this type of strategy could also possibly be used to attenuate

unwanted type 1 responses, which are seen in autoimmune diseases such as diabetes and multiple sclerosis. Unlike therapies involving systemic administration of cytokine, such an approach would be antigen-specific, with basophils being activated and releasing IL-4 only in areas of the body containing the Ag for which the injected IgE antibody was specific. Suggestive evidence that IL-4 may be efficacious in the treatment of autoimmune diseases driven by type 1 responses comes from animals studies; in particular murine models of arthritis and experimental autoimmune encephalomyelitis in which injection of IL-4-expressing cells and recombinant IL-4 have ameliorated autoimmune disease [48, 49]. More recently in both murine systems and human trials, IL-4 inducing parasite material has been shown to ameliorate inflammatory bowel disease although the source of the IL-4 (basophil vs. T cell) was not addressed [50, 51].

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Innate, Adaptive and Regulatory Responses in Schistosomiasis: Relationship to Allergy

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Abstract

Helminth infections have profound effects on the immune system. Here, recent insights in the molecular interactions between schistosomes and the host are described with respect to adaptive but also with respect to innate immune responses. Furthermore, the different mechanisms of immune hyporesponsiveness are depicted with emphasis on regulatory T cells. Finally, the relationship between downregulatory responses and allergy is discussed.

Worldwide more than 200 million people are infected with schistosomes, sometimes causing severe morbidity. Infectious cercariae penetrate the human skin, transform into schistosomula, and migrate via the bloodstream through the lungs to the portal vein, where they mate. Four to 5 weeks after infection, the mature paired worms migrate from the portal vein to the plexus of the bladder (*Schistosoma haematobium*) or the mesenteric veins of the intestine (*Schistosoma mansoni* and *Schistosoma japonicum*), where the female worms lay hundreds of eggs per day. About 50% of the eggs penetrate the tissues and are excreted via the urine or faeces. The rest of the eggs remain in the body, where they get trapped in the intestinal or bladder wall. Some of these eggs will be transported via the blood circulation to the liver or other organs. The trapped eggs get encapsulated in large granulomas, which evokes granulomatous inflammation, responsible for the main pathology of schistosomiasis. In a subset of the infected individuals, the granulomas may cause liver fibrosis and

portal hypertension in case of intestinal schistosomiasis and bladder cancer or renal failure in case of urinary schistosomiasis. Schistosome infection is accompanied by the induction of a prominent T helper (Th) 2 response, characterized by the production of interleukin-4 (IL-4), IL-5, IL-13, and high serum levels of immunoglobulin E (IgE). Recent molecular studies have revealed that this is the result of direct interaction of schistosome products with specific receptors, such as members of the Toll-like receptor family, on immune cells. This interaction leads to the activation of specific intracellular pathways, ultimately resulting in skewing of the immune response. Individuals chronically infected with schistosomes additionally develop lymphocyte hyporesponsiveness to parasite antigens. This feature of chronic infection might both protect the host from excessive inflammation as well as enhance parasite survival by decreasing immune reactivity to the parasite. As a result of bystander suppression, this hyporesponsiveness can also extend to third party antigens.

In this chapter, we will mainly focus on the innate and adaptive immune responses in populations residing in areas where schistosomiasis is endemic, but we will also address other helminths when relevant. We will describe what is known about the mechanisms employed by the parasite that are involved in the downmodulation of the immune response, with emphasis on the innate immune response and regulatory T cells (Treg cells). An immunological framework will be put forward that can explain the lower prevalence of inflammatory diseases in helminth-infected populations.

Innate Immune Responses in Schistosomiasis

The development of an appropriate immune response to a pathogen requires recognition of signature molecules of the pathogen by specific receptors of the host. This recognition initiates an immediate response from innate immune cells and sets the stage for the ensuing adaptive response. Pathogen-expressed molecules that stimulate innate immune responses are called pathogen-associated molecular patterns (PAMPs). PAMPs are recognized by means of pattern recognition receptors (PRRs), including the families of Toll-like receptors (TLRs) and C-type lectins, expressed by innate immune cells as well as resident tissue cells. PRR activation on innate effector cells such as macrophages and neutrophils results in activation of microbicidal effector pathways and inflammation. In addition, dendritic cells (DC), also expressing high levels of PRRs, play an important role in both the initiation of innate responses and in the translation of innate pathogen recognition into the induction of an adaptive immune response by instructing the appropriate subset(s) of T helper cells: Th1, Th2 or Treg cells.

The TLR family is the best-characterized class of PRRs in mammalian species. TLRs recognize a wide variety of PAMPs, from proteins to lipids and nucleic acids. Engagement of the TLRs results in activation of intracellular signaling pathways leading to the expression of multiple genes encoding inflammatory mediators and co-stimulatory molecules. Individual TLRs are differentially distributed within the cell. TLR1, TLR2, TLR4, TLR5 and TLR6 are expressed at the cell surface, whereas TLR3, TLR7, TLR8 and TLR9 localize in intracellular compartments such as endosomes. The TLRs at the cell surface are mostly involved in the recognition of bacterial products. In contrast, the intracellular TLRs specialize in viral detection and recognition of nucleic acids [1].

Recent studies have identified several schistosome structures that induce innate immune responses. A soluble extract of *S. mansoni* eggs (SEA) contains a mixture of carbohydrates, some of which are unique to the schistosome. Glycoconjugates from both schistosome eggs and worms contain the LacdiNAc (LDN) motif (GalNacβ1–4GlcNAc-R) and fucosylated LacdiNAc derivatives such as LDNF (GalNacβ1–4(Fucα1–3)GlcNAc) and LDN-DF (GalNacβ1–4 (Fucα1–2Fucα1–3)GlcNAc), as well as the LewisX epitope (Galβ1–4(Fucα1–3) GlcNAc). Stimulation of peripheral blood mononuclear cells (PBMC) from non-exposed individuals with synthetic neoglycoconjugates containing these sugar epitopes resulted in the production of IL-10, IL-6 and TNF-α, LDN-DF being the most potent stimulus [2]. This showed that schistosome carbohydrates could induce both pro- and anti-inflammatory cytokine responses in nonexposed individuals. It is not clear yet how schistosome glycans trigger innate immune responses and which cellular receptors are involved, although the C-type lectin dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) was shown to recognize LewisX and possibly LDNF [3]. Interestingly, injection of *S. mansoni* eggs into mice led to the production of IL-12 by lymph node cells, followed by the production of IL-6 and IFN-γ. IL-6, together with IL-12, was subsequently involved in the upregulation of the production of IL-10. Next, IL-10 and IL-6 caused a reduction in IL-12 and IFN-γ, revealing a role for IL-6 in the downregulaton of the Th1 response [4]. Stimulation of PBMC from *S. mansoni*-infected individuals with the schistosome glycan lacto-*N*-fucopentaose III (LNFPIII) containing LewisX, conjugated to human serum albumin, induced the production of IL-10, whereas no response could be measured in non-infected individuals [5]. Interestingly, there was hardly any production of IL-10 by PBMC of individuals with high egg counts (>400 eggs per gram feces) in this particular study, suggesting that IL-10 production may be an early event after egg laying. However, only 4–11 individuals per group were studied, and the production of other cytokines was not investigated. Another study did not find a correlation between infection intensity of *S. mansoni* (0–8,100 eggs

per gram feces) and the level of IL-10 production by PBMC after stimulation with schistosome egg antigens (SEA) [6], which could possibly be explained by a difference in duration and intensity of infection.

Carbohydrate moieties in schistosomes do not only occur on proteins, but can also be lipid-bound. Indeed, glycolipids isolated from eggs of *S. mansoni* stimulate the production of IL-6, TNF-α and IL-10 in monocytes from uninfected individuals [2].

Very recent work by Aksoy et al. [7] revealed that live schistosome eggs contain double-stranded (ds) RNA that can trigger mouse dendritic cells via TLR3. Recognition of parasite ds RNA results in phosphorylation of signaling transducer activator of transcription (STAT) 1 and the production of several interferon-induced inflammatory gene products, as well as in elevated levels of TNF-α and IL-12, suggesting that this leads to increased type 1 inflammation [7]. It is difficult to imagine how ds RNA of live eggs would get access to the DC, since RNA would normally be present inside the egg. It would be interesting to know whether ds RNA is released from the eggs, or whether DC and macrophages phagocytose dying eggs, followed by degradation inside endosomal compartments that leads to release of ds RNA, which would be in agreement with the intracellular localization of TLR3.

The accessibility of schistosome molecules will define which structures will interact with the immune system. Therefore, in vitro stimulations with a specific schistosome molecule might not reflect the responses in vivo when these structures are components of the whole egg or worm. Although for some of the schistosome structures the receptor that transduces the activation signal into the cell is known, for many schistosome components the receptors involved have not been identified yet. The simultaneous binding of schistosome components to different receptors on the surface of immune cells, for example to a TLR and a C-type lectin, will trigger different intracellular signaling pathways. The integration of all these signals will determine the final outcome of the immune response.

Adaptive Immune Responses in Schistosomiasis

In schistosomiasis, there is a dynamic series of changes in the adaptive immune response mounted to the parasite. The first stage of infection is characterized by an immune response that is primarily Th1 in nature, and can cause acute schistosomiasis with toxemic symptoms, especially in patients from non-endemic areas. PBMC from individuals with acute schistosomiasis produce higher IFN-γ and lower IL-5 than PBMC from chronically infected individuals, reflecting a Th1 dominated immune response [8]. This Th1 response switches

into a Th2 polarized response as soon as the adult worms release the eggs. The schistosome egg antigens stimulate granuloma formation, partitioning the eggs and its antigens from the tissues. Animal models of schistosomiasis have shown that Th2 cytokines play an important role in the formation and maintenance of these granulomatous lesions. Although the Th2 response can cause severe pathogenesis in the liver, bladder and intestine, it might also prevent an even more dangerous Th1 inflammatory response that could be fatal to the host. Accordingly, the immune response present in schistosome-infected individuals is characterized by high levels of Th2 cytokines upon stimulation of immune cells with parasite antigens, and high levels of serum IgE.

Recent studies have identified several schistosome structures that are involved in the strong Th2 skewing characteristic for schistosome infection. α_3-Fucose- and unusual β_2-xylose-containing sugar modifications of egg molecules appear to play an important role in Th2 polarization [9]. For example, the carbohydrate LNFPIII induces signaling via TLR4 on murine dendritic cells, which is dependent on the fucose moiety. This results in activation of dendritic cells that preferentially induce T cells with a Th2 cytokine profile [10]. In vivo, LNFPIII acts as a strong Th2 inducer if it is coupled to non-schistosome proteins. Interestingly, LNFPIII stimulation of DC leads to preferential activation of extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinases (MAPK) [10]. LPS (lipopolysaccharide) activates all three species of MAPKs, demonstrating a differential activation of intracellular signaling pathways, even though both LPS and LFNPIII signal via TLR4.

A better understanding of the adapter molecules that bind to the intracellular domains of the TLRs may explain how stimulation of the same TLR leads to such diverse responses. For example, the adapter MyD88 seems to be dispensable for Th2 induction by SEA, whereas it is required for Th1 polarization. In addition, simultaneous signaling via other receptors may modify the responses generated via TLRs. For instance, the LewisX moiety present in LNFPIII can bind to the DC-specific ICAM-3-grabbing nonintegrin [11], thereby possibly influencing the signaling via TLR4. LewisX on LPS from a phase variant of the bacterium *Helicobacter pylori* skews the immune responses towards Th2 via binding to DC-SIGN on DC [12]. In addition to stimulating innate immune responses, lipids also play a role in influencing the adaptive response, as seen for the lipid fraction containing phosphatidylserine from *S. mansoni* eggs and worms, that induces Th2 skewing in human DC [13].

Similarly, molecules from other helminths induce strong Th2 responses. Excretory-secretory glycoproteins from the rodent nematode *Nippostrongylus brasiliensis* (NES) have Th2-promoting activity on DC and a Th2-inducing adjuvant function on other antigens. The exact nature of molecules involved in

NES are unknown, however, the activity is heat labile and protease sensitive, suggesting that the active component is protein in nature [14]. The Th2-driving capacity of extracts from the adult filarial nematode *Brugia malaya* critically depends on the presence of intact glycans, as this activity is abolished by periodate treatment [15]. The ES-62 glycoprotein from the rodent filarial nematode *Acanthocheilonema viteae* has immunomodulatory effects on a variety of cells of the immune system, including B and T cells as well as antigen-presenting cells. Many of these activities have been attributed to the phosphorylcholine (PC) moieties attached via N-glycans to the ES-62 protein. The immunomodulation of ES-62 on macrophages and dendritic cells seems to be dependent on TLR4, although the involvement of other receptors cannot be excluded. Like LNFPIII and NES, ES-62 has the capacity to induce DC to acquire a phenotype that supports the differentiation of Th2 cells. ES-62 induces phosphorylation of the MAPK ERK and suppresses activation of p38, leading to a decrease in the production of IL-12 [16]. Altogether it appears that helminth glycans have a major contribution to the induction of Th2 development. The receptors that bind these glycans and transduce the signal into the cells of the immune system are beginning to be discovered and so far include members of the TLR family and C-type lectins.

Downregulation of the Immune Response during Chronic Schistosome Infection

Modulation of Innate Responses

The downregulation of immune responses seen in individuals chronically infected with helminths, including schistosomes, is instrumental for parasite survival, but might also benefit the host by preventing excessive inflammation. Although this downregulation of immune responses in infected individuals has been recognized for a long time, the mechanisms that underlie this hyporesponsiveness are less clear. There are many studies on the modulation of adaptive responses, however, much less is known about the modulation of innate immune responses during the chronic phase of schistosome infection. Regulatory mechanisms of both innate and adaptive immune responses that are induced upon schistosome infection are shown in figure 1.

Recently, our group has studied the innate immune responses of schoolchildren living in an area in Gabon endemic for *Schistosoma haematobium*. The schistosome lipid fraction phosphatidylserine of the worm stimulated the production of IL-8, IL-10, IL-6 and TNF-α in PBMC from all children. However, the responses were lower in the infected children, in particular for IL-8 and TNF-α production [17]. The responses to another TLR stimulus derived from

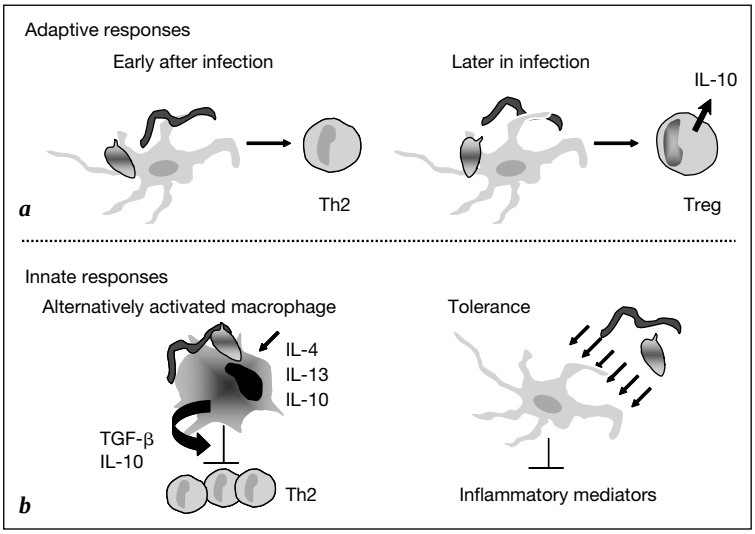

Fig. 1. Regulatory mechanisms induced by schistosome infection. ***a*** Adaptive immune response. Early in the infection with schistosomes, the interaction of schistosome molecules from eggs and worms with dendritic cells (DC) will induce skewing towards a strong Th2 response, characterized by high levels of IL-4, IL-5, IL-13 and IgE. Later in the infection, schistosome components, such as lyso-phosphatidylserine, will modulate DC, resulting in the induction of regulatory T cells. IL-10 is one of the important products from these Treg cells that affect not only the proliferation of effector T cells, but also will affect cells from the innate immune system, such as macrophages and DC. ***b*** Innate immune responses. Exposure to schistosome molecules, or to a combination of the cytokines IL-4, IL-13 and IL-10, can change the activation status of the macrophage, resulting in alternatively activated MΦ (aaMΦ). AaMΦ produce high levels of IL-10 and TGFβ, instrumental in inhibiting the proliferation of other T cells, such as Th2 cells. Repeated stimulation of DC with schistosome components will induce tolerance, leading to a reduction in the production of inflammatory mediators by the DC.

bacteria, LPS, followed a similar pattern, showing that the responses to other TLR stimuli are also modified during chronic schistosome infection. This reduced innate immune response in individuals infected with schistosomes could be the result of continuous exposure of the cells to parasite molecules. It has been shown for bacterial PAMPs that upon repeated stimulation, the cells of the innate immune system become unresponsive to the same or other TLR ligands, a phenomenon termed tolerance or cross-tolerance, respectively. Thus, it is possible that repeated exposure to high levels of parasite antigens, engaging the TLRs, results in a more generalized state of unresponsiveness to TLR

ligands. Alternatively, the schistosome-infected children live in an environment with higher exposure to bacterial pathogens, explaining the additional tolerance to LPS. More recently, several molecular events that might explain the mechanism of tolerance have been discovered. Repeated stimulation of monocytes can lead to altered surface expression of TLR2 and TLR4, and can also affect their intracellular signaling pathways. For example, it has been reported that there is a decrease in the association of interleukin-1 receptor-associated kinase (IRAK) with MyD88 or TLR4, and a reduction in both MAP kinase activity as well as nuclear factor kappa B (NF-κB)-induced gene transcription. Furthermore, negative regulators of TLR signaling, such as Toll-interacting protein (Tollip) and IRAK-M, are induced in vitro after repeated stimulation with LPS [18].

Interestingly, recent work has shown that exposure of mouse dendritic cells to SEA can downmodulate TLR-mediated inflammatory responses. SEA inhibited the LPS-induced induction of IL-12 and suppressed the upregulation of co-stimulatory molecule expression. Simultaneous exposure of DC to SEA and LPS changed the expression of more than 100 LPS-regulated genes, of which many were proinflammatory [19]. This indicates that helminth-derived molecules can modulate the innate immune response by repressing inflammatory responses from DC.

The presence of alternatively activated macrophages (aaMΦ) is another feature of helminth infection. Early studies already showed that adherent phagocytic mononuclear cells from patients with schistosomiasis mediated suppression of lymphocyte proliferation in vitro [20]. Now we know that there are several subsets of macrophages that develop under different environmental conditions. For example, the classically activated macrophages, induced in a Th1 cytokine environment, are essential in protection against intracellular bacteria. Recently, it became clear that a different macrophage type arises in a Th2 environment, when IL-10 is present. Since these cells exhibit a different activation program, these are termed aaMΦ to distinguish them from the deactivated macrophages formed in an exclusive IL-10 environment [21]. They are found in healthy individuals in the placenta, lungs and sites of immune privilege, as well as during chronic inflammatory diseases. Injection of the egg-derived glycoconjugate LNFPIII from *S. mansoni* in mice induced the presence of a macrophage population that lacked secretion of NO and produced high levels of IL-10 and TGF-β. These macrophages efficiently suppressed the proliferation of T cells in vitro [22]. Gene expression analysis of aaMΦ isolated from animals infected with the filarial worm *Brugia* revealed an abundant expression of the Ym1 and Fizz1 genes. At this moment it is still unclear whether Fizz1 and Ym1 are beneficial or exacerbate disease during helminth infection [23].

Modulation of Adaptive Responses

Downmodulation of immune responses is not restricted to the innate responses, but is also found in the adaptive compartment. During the later course of schistosome infection, a downmodulated or modified Th2 response develops, leading to compromised antigen-specific T cell responses. This hyporesponsiveness persists for the remainder of the infection and is reflected in vitro by the greatly diminished lymphocyte proliferation to parasite antigens [20, 24]. However, responses are not completely absent, since T cells from schistosome-infected patients continue to produce IL-4, but show suppressed production of IL-5 and IFN-γ [25]. Treatment of schistosomiasis leads to an increase in Th2 cytokines such as IL-5 and IL-13, in response to worm antigens [26], although another study detects an increase in IL-4, but not in IL-5 to worm or egg antigen after treatment [27]. Chronic infections with schistosomes can also modulate in vivo immune responses to other antigens, as reflected in the lower response to tetanus toxoid (TT) after immunization of schistosome-infected patients. In this study, the interferon-γ response to TT was inversely correlated with the intensity of infection, however, the response did not switch to Th2 [28].

Suppression of the immune system seems to depend on the continuous presence of the parasites. A study of Ethiopian immigrants to Israel showed that T cells from individuals with heavy helminth infections (including *S. mansoni* and *Trichuris trichuria*) were defective in several signaling pathways, shown by attenuated phosphorylation of cellular kinases, lower secretion of the chemokines RANTES and MIP-1α, and reduced proliferation to the recall antigen tuberculin purified protein derivative (PPD). All these responses were restored gradually following anti-helminthic treatment, indicating that chronic helminth infection causes hyporesponsiveness and anergy at the T cell level and that the eradication of helminths reverses this hyporesponsiveness [29].

Immunoepidemiological studies in Gabon showed that peripheral blood cells from schistosome-infected children produced higher amounts of IL-10 upon stimulation with parasite antigens [30]. Additionally, the presence of antibodies to IL-10 or TGF-β could reverse the suppression of in vitro proliferation of cells isolated from schistosome-infected individuals [23, 24]. Moreover, helminth-infected individuals have higher concentrations of TGF-β in their plasma than non-infected subjects [29]. All together, IL-10 and/or TGF-β seem to play an important role in the immunosuppression induced by helminth infection, which points towards a potential role for regulatory T cell populations.

Modulation via Regulatory T Cells

Recently, regulatory T cell populations have attracted considerable scientific interest, since these cells are able to suppress both Th1 and Th2-mediated

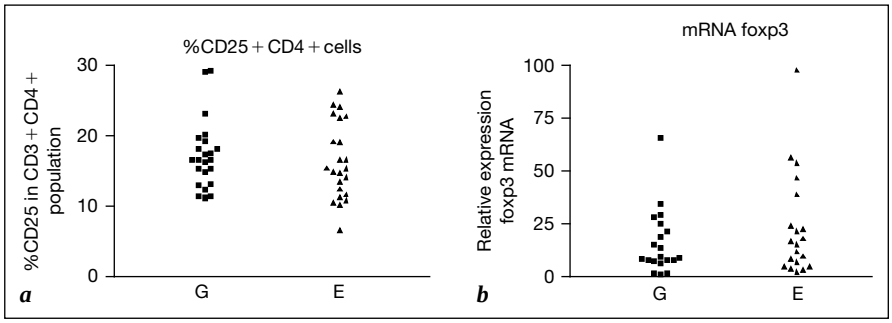

Fig. 2. The number of natural Treg cells in infected and non-infected populations. ***a*** PBMC were isolated from a Gabonese population infected with schistosomes (G, n = 23) and non-infected Europeans (E, n = 23), and analyzed by FACS (fluorescence activated cell sorter) for the surface expression of CD3, CD4 and CD25. The percentage of CD4+CD25+ T cells was determined in the CD3+CD4+ T cell gate. By using the Mann-Whitney test, no significant difference was found between the two populations. ***b*** Total RNA was isolated from PBMC of the Gabonese (G) and European (E) populations, and cDNA prepared. The relative mRNA expression levels of foxp3 were detected by real-time quantitative PCR (Taqman), using 18S rRNA to normalize for the amount of input RNA. The sample with the lowest expression of foxp3 was set to a value of 1. No significant difference was found between the two populations (Mann–Whitney test).

immune responses and have been shown to be crucial for the prevention of both autoimmune and allergic diseases in many animal models [31]. Most of the animal work on Treg cells has concentrated on the CD4+CD25+ population of Treg cells, also called natural(ly occurring) Treg cells, which are selected on self-antigen in the thymus and play a major role in the control of pathogenic autoimmunity. Contact-dependent signals play an important role in the suppression of effector cells by natural Treg cells, although they also produce IL-10 and TGF-β, which may be functionally involved in the suppression. Furthermore, Treg cells were shown to expand in a large variety of parasite infection models with helminths and with protozoa, where they prolong pathogen survival by inhibiting potentially harmful immune responses. For example, mice infected with *S. mansoni* have elevated numbers of IL-10 producing natural CD4+ CD25+ Treg cells, which suppress Th1 responses to schistosome eggs and ensure Th2 polarization [32]. These cells are essential to protect the animals from fatal inflammatory responses to eggs in the liver [33].

Data on the role of Treg cells in human schistosomiasis, or in any other helminth infection are scarce. We have analyzed the percentage of CD25+ T cells within the CD4+ T cell population in the peripheral blood of individuals from an area in Gabon endemic for *Schistosoma haematobium* (fig. 2). So

far, we could not detect any differences when comparing these percentages to European individuals not exposed to schistosome infection. This observation was confirmed by similar mRNA expression levels of foxp3, a transcription factor specific for natural Treg cells, in the peripheral blood of both populations (fig. 2). Larger populations need to be tested to confirm this finding, and functional analysis might detect a qualitative difference between the Treg cells of either population, as seen in studies on allergy.

Importantly, next to the natural Treg cell population, there are also other Treg cell populations with a different phenotype that are induced peripherally upon encounter with pathogens or self-antigens not present in the thymus. These Treg cells are called either 'adaptive' or 'induced' Treg cells. To date, the interrelation of these adaptive Treg cells with the natural Treg cells is unclear. According to their cytokine profile, adaptive Treg cells can be divided into several subsets. Although the subsets seem to be heterogeneous in nature, two main subsets have been identified; Tr1 cells, producing high levels of IL-10, and Th3 cells, which primarily secrete TGF-β. Due to the lack of specific markers for these Treg cells, very little is known about their role in helminth infection. One study showed that antigen-specific T cells could be cloned from onchocerciasis patients. These cells secreted elevated levels of IL-10 and/or TGF-β and could inhibit effector T cell proliferation [34]. This argues for the fact that helminth infection in vivo induces Treg cell subsets that mediate hyporesponsiveness. Interestingly, the schistosome lyso-phosphatidylserine (lyso-PS) can modulate dendritic cells in vitro to drive polarization of naïve T cells to cells that produce IL-10 and have suppressive activity. Lyso-PS modulates DC via TLR2, whereas other TLR2 ligands such as lipotechoic acid from *Staphylococcus aureus* or heat-killed *Listeria monocytogenesis* do not induce DC to activate IL-10-producing T cells [13]. Lyso-PS contains acyl chains that differ in length and possibly the position of the double bond from mammalian lyso-PS [van der Kleij, unpubl. data], indicating that schistosomes contain specific molecules that can induce Treg cells in vitro. At present, its capacity to regulate inflammatory responses in vivo is being investigated in a mouse model of asthma. The lack of specific markers for Treg cells has so far hampered clear insights into the role of Treg cell subsets in human helminth infection.

Relationship between Schistosomiasis and Allergy

Prevention of Atopy

There are abundant studies in human showing a negative association between heavy infection with helminths and allergic reactivity to common allergens. Many of these studies describe a protective effect of intestinal

helminths, such as ascaris, trichuris and hookworm, towards the prevalence of atopy. These observations have been confirmed for schistosome infection, demonstrating lower skin reactivity to allergen in infected individuals [30, 35]. Since infection with schistosomes and allergic diseases are both associated with strong Th2 responses, their negative association cannot simply be clarified by the balance between Th1 and Th2 responses. Chronic infection with a high burden of helminths is thought to induce a regulatory network that can alter immune responses to both parasite and harmless antigens. PBMC of individuals infected with schistosomes or filarial worms express high levels of the regulatory cytokine IL-10, whereas, in contrast, asthmatic patients in general secrete lower levels of IL-10, both in the bronchoalveolar lavage (BAL) and by PBMC [36].

A recent study of *S. mansoni* infection in an experimental model of systemic fatal anaphylaxis, discovered a role for IL-10 in the prevention of allergic hypersensitivity. This effect was most strikingly observed in mice infected with male cerceriae (infection with worms only), rather than infection with both male and female cercariae (infection with worms and eggs), and was dependent on the presence of B cells. However, it was unclear whether B cells producing IL-10 played a direct role in the modulation of the immune response [37].

Role of Regulatory T Cells

Notably, both allergic and nonallergic individuals seem to respond to harmless allergens with a Th2 profile, whereas only nonallergic individuals have CD4+CD25+ Treg cells that are capable of suppressing allergen-specific proliferation of CD4+CD25− cells in vitro [38]. However, other studies found no major functional differences between the CD4+CD25+ T cells derived from either healthy or atopic donors [39, 40]. The fact that CD25 is not exclusively expressed by Treg cells, but also on activated effector T cells, could explain the discrepancy between the studies. Akdis et al. [41] analyzed the adaptive Tr1 like cells that produce high amounts of IL-10 and found an increased ratio of IL-10-producing Tr1 cells to Th2 cells in nonatopic individuals, indicating that atopic individuals have a lower proportion of Tr1 like cells. These studies indicate that allergic disease might partly be caused by a defect in the function or induction of Treg cell populations. In addition, successful immunotherapy was found to be associated with the induction of Treg cells that secrete IL-10 and TGF-β [41, 42], suggesting again that protection from allergy is mediated by the induction of Treg cells. Active and chronic helminth infection might also induce strong Treg cell responses that play a role in the protection from inflammatory responses. The induction and expansion of Treg cells appear to require antigen-specific activation, but they seem to function through antigen-independent mechanisms**.** Therefore, Treg cells induced by schistosome

infection might inhibit allergen-specific Th2 cells, either directly or by secreting high amounts of IL-10 and/or TGF-β. High levels of these regulatory cytokines create an environment that favors the induction of allergen-specific Treg cells, possibly via modulation of antigen-presenting cells. It has been shown that Treg cells can modulate dendritic cells in vitro, resulting in a more regulatory DC that can promote the generation of Treg cells [43]**.** A summary of potential regulatory actions of Treg cells induced by chronic helminth infection in the control of inflammatory allergic responses is depicted in figure 3.

Although regulatory T cells could be the source of IL-10 production, IL-10 might also be produced by cells of the innate immune system upon contact with helminth molecules. A recent study of a mouse model of schistosomiasis used IL-10 and T cell-deficient mice to show that the IL-10 that controls severe pathogenesis is not only derived from Treg cells, but also from cells in the non-T cell compartment [33].

IgG4 Antibodies and IL-13 Decoy Receptor

Two additional mechanisms of immune suppression that are induced in helminth infection and could play a role in the prevention of allergic disease are the induction of high levels of IgG4 antibodies, and the production of the IL-13 decoy receptor.

Next to IgE, high levels of IgG4 are produced during helminth infection. Interestingly, IL-10 induces B cells to switch from IgE to IgG4 antibody production [44]. High IgG4 antibody titers are associated with decreased atopy and have been found at increasing levels upon allergen immunotherapy. Since the IgG4 isotype is not associated with any effector response, this might be a mechanism that suits both parasite and host. IgG4 protects from IgE-mediated allergic responses by competing for allergen binding with IgE, and reduces excessive pathology in the host as a result of parasite infection. Recent studies in Ecuador showed that children with IgG4 to ascaris have a lower frequency of atopy than those who were IgG4-negative [45].

Animal studies have shown that the Th2 cytokine IL-13 is a major stimulus for the development of egg-induced liver fibrosis, possibly by directly stimulating collagen deposition by fibroblasts [46]. Interestingly, humans infected with *S. mansoni* have increased levels of the soluble decoy receptor for IL-13 in their serum [46]. This decoy receptor consists of a dimer of the IL-4Rα chain and the high affinity IL-13Rα2 chain, which lacks an obvious signaling motif or Janus kinase/STAT binding sequence in its cytoplasmic region. Thus, this decoy receptor might decrease the levels of circulating IL-13 and thereby control the progression of schistosomiasis. Indeed, an animal model of schistosomiasis showed that the presence of the decoy receptor was critical for host survival [46]. Interestingly, the IL-13Rα2 seems to be upregulated by IL-13 itself as well

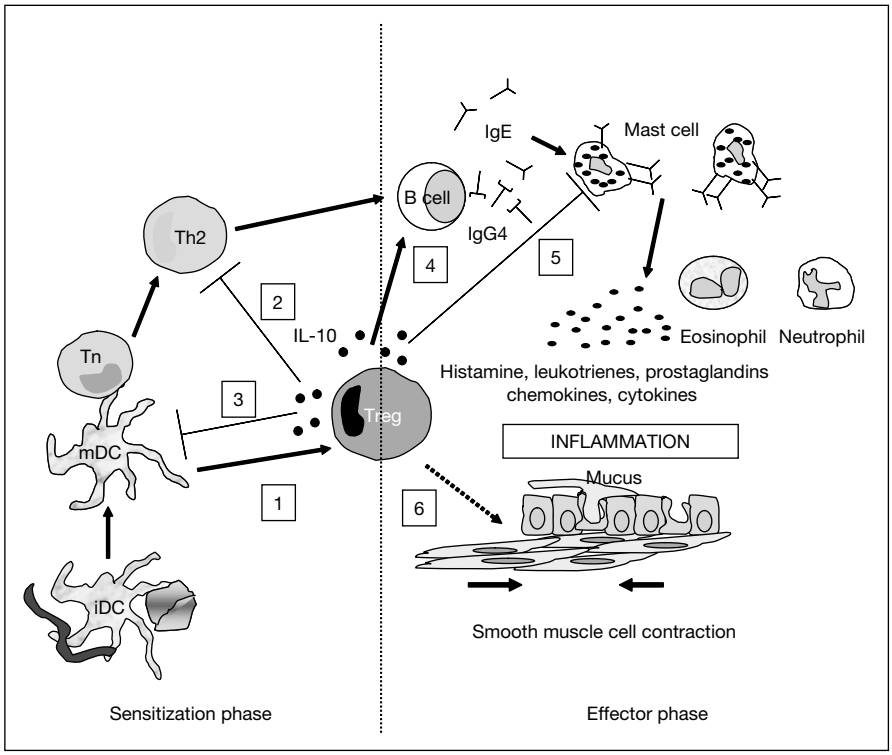

Fig. 3. The role of Treg cells in the development of allergic inflammation. The immune response leading to allergic inflammation can be divided into a sensitization phase and an effector phase. In the sensitization phase, mature dendritic cells (mDC) present allergens to naïve T cells, which results in the development of Th2 cells producing cytokines such as IL-4, IL-5 and IL-13. IL-4 and IL-13 help B cells to switch to IgE antibody production, whereas IL-5 facilitates eosinophil accumulation. In the effector phase, IgE binds to FcεRI receptors on mast cells and basophiles, triggering the release of inflammatory mediators such as histamine, leukotrienes, prostaglandins, and chemokines. This will leads to an influx of more eosinophils and neutrophils, resulting in a strong inflammatory reaction, involving mucus secretion and smooth muscle cell contraction. During infection with schistosomes, the contact of immature DC (iDC) in the periphery with molecules derived from the worms and/or eggs leads to maturation of the DC (mDC) and migration to lymph nodes, where the DC can drive the development of Treg cells (1). Treg cells can either directly inhibit allergen-specific Th2 cells (2), or, via the interaction with DC, lead to the activation of Treg cells rather than Th2 cells (3). IL-10 produced by Treg cells induces B cells to switch from IgE to IgG4 production (4). IgG4 can compete with IgE for allergen binding, and since it does not induce effector functions, IgG4 is able to block the IgE-mediated degranulation of mast cells. IL-10 has also been shown to inhibit mast cell degranulation, preventing the release of inflammatory mediators (5). Finally, Treg cells might have a direct influence on the activation status of the peripheral tissues, although there is no clear evidence for such an effect so far (6).

as by IL-10. The feedback mechanism of the IL-13Rα2 decoy receptor apparently prevents over pathology from helminth infection. Future studies will have to tell whether this feedback mechanism induced by helminth infection can also lead to the suppression of inflammatory responses that are mediated by IL-13, as in allergic asthma.

The Window of Immune Suppression

An important question is how early the downmodulatory effect of helminth infection on the immune response in children occurs and at which stage of allergic inflammation it is operational: is it at the initiation or at the effector phase of allergic disease? Early exposure to microbial compounds, including those from helminths, will help to form a balanced development of both effector and regulatory T cell populations, which might protect from subsequent pathologies in the long term. At this moment, not much is known about the longevity of Treg cells, and the existence of a memory pool of Treg cells. Since exposure early in life to a farm environment seems to have a long-term effect to protect children from asthma, this would argue for the existence of a long-lasting mechanism that may be mediated by such a memory Treg cell subset. Children in endemic areas might have been exposed to helminths since early childhood, educating the immune system at an age where allergy develops.

Several studies have shown that eradication of helminths in older children causes a rise in mite reactivity, suggesting that continuous presence of helminths is needed to control allergic inflammation [47–49]. However, the rise in allergic reactivity was modest, which could be a result of a strong imprinting event earlier in life. These studies might reflect an additional effect of active helminth infection, where peripheral induction of a stronger regulatory network prevents the inflammation in the effector stage of allergic disease. The persistence of sensitization to house dust mite in the helminth-infected children from Gabon that had no skin reactivity against mite [47], suggests also that regulation takes place at the level of the effector phase. IL-10 has been shown to inhibit mast cell degranulation [50], providing an explanation for the effect of a downmodulatory immune response on the effector phase of inflammation. However, in a Brazilian population with chronic and heavy *S. mansoni* infections, uninfected individuals had greater allergen-specific IgE response, indicating that both sensitization and reactivity were compromised in infection [35]. There is some evidence that IgE to mite in helminth-infected individuals is of low affinity [van Ree, pers. commun.]. This could play a role in the difference seen between sensitization and atopy in helminth-infected children.

In summary, the type of helminth, the chronicity and intensity of infection together will determine the effect of helminth infection on the immune responses that are ongoing. Furthermore, the development of allergy is also dependent on the degree of allergen exposure and on the genetic background of an individual. These factors will all contribute to the susceptibility of the host to develop allergy and to the capability to induce mechanisms of effective immunoregulation.

Concluding Remarks

Allergic disease might result from inappropriate activation of the immunological networks and effector pathways that were initially aimed at helminths. Conversely, chronic infection with helminths induces a regulatory network that can prevent excessive inflammation resulting from both helminth infection and allergic disease. The identification of helminth structures that downregulate immune responses will be particularly helpful in order to dampen inflammation. A potential drawback could be that although immune suppression can reduce inflammatory reactions, this might also render the host more susceptible to secondary infections. A better insight into the molecular pathways that cause downregulation of immune responses might lead to more specific targets for therapeutic intervention. In addition, the use of Treg cells that induce immunoregulation could be a major step forward in the treatment of inflammatory diseases. However, in order to avoid generalized immunosuppression the use of antigen-specific pathways would be preferential. This remains a challenge for the future. Finally, not only modulation of adaptive responses but also of innate immune responses may be important to achieve effective downmodulation of unwarranted immune responses.

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Regulatory T Cells Induced by Parasites and the Modulation of Allergic Responses

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Abstract

The inverse relationship between helminth infection and overt allergic reactivity has intrigued medical scientists for three decades. In the past 5 years, detailed epidemiological studies coupled with new experimental model approaches, have substantiated the negative effects of infection on allergic disease manifestation, and begun to provide mechanistic explanations for this fascinating interaction. Several key conclusions can now be drawn. First, the modulation of allergies, such as Th2-dependent pathologies, is not primarily through immune deviation (e.g. switching responsiveness to Th1), as helminth infections themselves drive strong Th2 responses. Second, helminth-infected hosts show similar levels of immune sensitisation to allergens as do uninfected counterparts, but the expression of overt allergic reactivity is suppressed. Third, the down-modulation of allergy in both human and experimental infections is consistent with the activity of T regulatory (Treg) cell populations, which suppress effector mechanisms of both Th1 and Th2 type.

The epidemiology of human allergic disease displays two remarkable features. First, the prevalence of asthma, eczema and allergic rhinitis has risen sharply in the western world over recent decades, and, secondly, incidence of these Th2-mediated disorders remains low in developing countries with high endemicities of Th2-inducing helminth parasites. These observations have stimulated new, ground-breaking research with implications for the immunology of both allergies and of parasitic infections, which is the focus for this review.

The suggestion that parasitic helminths negatively associate with allergic reactivity dates back almost 30 years [1]. Detailed field-based epidemiological studies verify these early observations, while comparisons before and after

anthelminthic treatment, have documented that helminth infections suppress overt allergic reactivity despite high levels of underlying allergen sensitivity [2, 3]. Laboratory-based animal studies have defined potential mechanisms underlying the inverse relationship between infections and allergies, focussing on immunoregulatory pathways in particular. In this review, we first outline the essential characteristics of the allergic reaction and the potential for regulatory lymphocyte control of allergy. We then relate this knowledge to the immunological sequelae of helminth infection, with a spotlight on immunoregulatory networks and regulatory T cells (Tregs). While based primarily on data from animal models, the proposition that reduced allergic reactivity operates via a regulatory T cell route is borne out by recent studies identifying deficient regulatory networks in allergy-prone human individuals. Thus, a regulatory network centered around Treg cells can in key respects account for the negative association between helminth infections and allergic reactivity.

The Cellular Basis of Allergic Diseases

A florid allergic reaction is the result of a muticomponent immune response encompassing initiating cell types (dendritic cells and Th2 cells), inflammatory chemokines and cytokines (such as eotaxin, interleukin (IL)-5 and IL-13), effector molecules (such as immunoglobulin (Ig)E, leukotrienes, and anaphylatoxins) and effector cells (such as eosinophils). Allergic reactivity to harmless food proteins, such as those from milk, peanut, shellfish and soybeans, and to innocuous airborne allergens such as plant pollens, mould spores and dust mite particles, afflicts an increasing number of sufferers. The nature of the allergen and site of contact determine the development and progression of allergic disease, ranging from upper and lower airway complications such as allergic rhinitis and asthma, to intestinal discomfort, skin rashes and eczema from food and skin-contact allergens. This diversity of allergens and symptomatic manifestations belie similar early and late-stage cellular inflammatory reactions amongst the allergic diseases. Hence, our focus in this review on airway allergy and the development of asthma should serve to illustrate the common pathways of cellular activation and mediator release culminating in allergic immunopathology.

Airway allergic reactivity is thought to be initiated by dendritic cells (DCs) which line the sub-epithelial layers and intercellular spaces of the alveolar walls, monitoring the local mucosal environment. Upon allergen encounter, accompanied by appropriate pathogen-associated molecular pattern (PAMP) stimulation, DCs mature and migrate to the draining lymph nodes. Within the local lymph nodes, the encounter between allergen-loaded DC and

peptide-specific T cells generates allergen-reactive cells of the Th2 phenotype, although the exact determinants of this commitment have yet to be defined. Th2 cells then egress from lymph nodes back to the site of initial allergen encounter, following chemokine gradients of RANTES (regulated on activation, normal T cell expressed and secreted), TARC (thymus-associated and regulated chemokine) and MDC (macrophage-derived chemokine). A chemotactic gradient of prostaglandin D_2 (PGD_2) also attracts inflammatory Th2 populations.

Allergen re-encounter by DCs and presentation to antigen-specific Th2 cells, results in a secreted barrage of the Th2 cell-derived cytokines IL-4, IL-5, IL-9 and IL-13. These mediators are present at almost all intermediate stages of airway inflammation and pathophysiological asthma. Animal models have shown that depletion of CD4+ Th2 cells or of IL-4 sufficiency severely attenuates allergen-induced airway inflammation, and similar effects result from disruption of the Th2 signalling molecules GATA-3 or STAT-6. Thus, CD4+ Th2 cells and IL-4, orchestrate the allergic inflammatory response and appear to be essential in the aetiology of allergic asthma.

In addition to IL-4, the cytokine IL-13 is intimately involved in allergic reactivity [4]. Both IL-4 and IL-13 share many properties, and cell surface receptors for the two cytokines share a common signalling chain (IL-4Rα). While IL-4 is an essential Th2-inducing mediator, IL-13 is the primary driver of effector responses such as mucus hypersecretion and goblet cell hyperplasia. IL-13 also promotes chemokine secretion, enhancing cellular recruitment, acting as a critical factor in the development of airway hyperresponsiveness (AHR) [4].

In terms of cellular inflammation, the infiltration of eosinophils is considered to be the hallmark of allergic responses. In allergic asthma, IL-5 works in close collaboration with the predominant eosinophil chemoattractant eotaxin to mobilise and recruit eosinophils. Th2 cell-derived IL-4 and IL-13, along with TNF-α released from macrophages, also stimulate eotaxin release from non-immune populations including lung fibroblasts, epithelial cells, and smooth muscle cells. In the local milieu, eotaxin recruits eosinophils, stimulates their respiratory burst activity, and up-regulates VCAM-1-mediated adhesion to endothelial cells. IL-5 further promotes eosinophil differentiation and T-cell dependent airway mucus production, AHR and tissue damage. In the periphery, eotaxin and IL-5 synergistically mobilise further mature eosinophils within bone marrow. Allergen-induced degranulation of eosinophils, releasing major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO), exacerbates localised tissue damage observed in allergic asthma.

The production of allergen-specific IgE is directly related to the Th2 bias of the allergic response, as IL-4 promotes immunoglobulin switching to the Cε isotype. Because allergen-specific IgE represents a very small fraction of total

serum immunoglobulin, its function depends on the high-affinity receptor, FcεR1, found on mast cells, basophils and human eosinophils. Allergen cross-linking of FcεR1-bound IgE on mast cells or basophils triggers the rapid release of pre-formed mediators, including histamine, proteases, carbohydrate-cleaving enzymes, cytokines and chemokines, prostanoids and leukotrienes, each of which contribute to the allergic inflammatory cascade.

Allergic asthma is thus a complex disease resulting from considerable cellular influx into the airways, accompanied by tissue damage and excessive secretion of mediators, cytokines, chemokines and mucus, which ultimately leads to airway remodelling. Thickening of the airway wall smooth muscle is the major concern in a syndrome typified by reduced baseline airway calibre and hyperresponsiveness to allergen provocation.

Regulatory T Cell (Treg) Biology

The field of immunology, having been dominated by the paradigm of Th1/Th2 antagonism for nearly 20 years, has now been overturned by the re-emergence of the concept of suppressor, now regulatory, T cells [5]. As yet, definitions of Treg population(s) are primarily functional, and controversy continues over issues of surface marker expression and modes of suppressive activity. Nevertheless, the field is slowly piecing together the basic biology of Treg cells, with a greater appreciation of their influence in the immune system as a whole.

Treg cells reduce the magnitude of immune responses by suppressing either, or both, Th1 and Th2 cell function as well as that of other immune cells. Different Treg cell types have been described based on origin, generation, and mechanism of action, with 3 consensus phenotypes: 'natural' CD4+CD25+ Tregs which develop in the thymus and regulate self-reactive T cells in the periphery; Tr1 cells, which arise in the periphery and secrete IL-10 in the absence of IL-4; and Th3 cells, which are activated in the mucosal surface of the gut and secrete IL-10 and TGF-β.

'Natural' Treg cells acquire their phenotype before leaving the thymus and regulate self-reactive components of the peripheral immune response. In the absence of 'natural' Tregs, spontaneous organ-specific autoimmune diseases ensue [5]. 'Natural' Treg cells express CD4 together with high levels of the alpha chain of IL-2R, CD25, and hence are referred to as CD4+CD25+. They also show elevated expression of the forkhead box P3 (FoxP3) transcription factor. FoxP3 may be a critical component of Treg cell differentiation, as its mutation or genetic dysfunction results in several autoimmune disorders in humans (IpEX/XLAAD syndrome) and mice (Scurfy). Whether natural Treg cells are

expanded or stimulated by encounter with exogenous antigen in the periphery (as, for example, during infection) is as yet unknown. CD4+CD25+ cells can regulate both Th1- and Th2-associated responses.

Other Treg populations can arise from non-regulatory peripheral T cell populations, indicative of reactivity to exogenous antigens. Among these are Tr1 cells, generated during activation in the presence of IL-10 [6], which typically secrete high levels of IL-10 with low levels of IL-2 and no IL-4, and have the capacity to suppress both Th1 and Th2 responses. OVA-specific Tr1 cells can be induced in vitro with IL-10-treated BM DCs in the presence of OVA peptide. The importance of DCs in Treg induction is explored below.

Th3 regulatory cells have been defined from murine gut-associated lymphoid tissues (GALT) and human peripheral blood [7]. Typically, Th3 cells secrete high levels of TGF-β and either IL-4 and/or IL-10. Th3 cells were initially described in oral tolerance models, maintaining tolerance to self-antigens. Indeed, mice genetically deficient in TGF-β die of inflammatory bowel disease within approximately 3 weeks of birth. Thus, the primary function of TGF-β-secreting Th3 cells may lie within the intestinal mucosa, maintaining tolerance to commensal bacteria, food antigens and self antigens.

The relationship between 'natural' CD4+CD25+ Treg cells and Th3 cells remains uncertain. The definitive high secretion of TGF-β from Th3 cells and the expression of surface-bound TGF-β by suppressive CD4+CD25+ cells [8] suggests a common function between these Treg populations. Significantly, CD4+CD25+ cells can regulate intestinal and airway inflammatory responses, suggesting that Th3 and CD4+CD25+ cells can both regulate responses to exogenous antigens. Th3 and Tr1 populations also overlap significantly. For example, IL-10-secreting Tr1 cells, isolated from respiratory-associated mucosal sites, have been shown to regulate both airway inflammation [9] and intestinal colitis [6]. The functional cross-over of these three Treg subtypes, Tr1, Th3 and CD4+CD25+, with regard to mucosal-associated tissues illustrates the difficulty of classifying them as independent cell phenotypes.

Further complexity is added by evidence that naïve, peripheral T cells may be converted into a regulatory phenotype. For example, forced expression of FoxP3 in naive T cells induces them to adopt regulatory functions, capable of inhibiting several autoimmune diseases [10]. Moreover, CD4+CD25– cells can be converted into CD4+CD25+FoxP3+ regulatory cells through stimulation by TGF-β [11], implying that CD4+CD25+ Treg cells can be generated de novo outside the thymus. Similarly, Tr1 cells can be generated from naïve CD25– peripheral blood CD4+ cells, by immature allogeneic DCs.

CD4+CD25+ Tregs can, moreover, recruit a secondary population of Tr1-like IL-10-secreting CD4+CD25– cells with their own regulatory capacity, analogous to infectious tolerance. Thus, natural CD4+CD25+ cells may

conscript antigen-specific peripheral T cells into acting as professional regulators. The concept of primary and secondary Treg populations may account for the discrepancies and overlaps with Treg heterogeneity.

Increasing interest is centering around the role of DCs in the induction of Tregs, particularly in the context of the peripheral immune system. Antigen presentation of either self or foreign antigens by immature DCs may preferentially induce Treg populations. Such DCs, with a $CD11c^{low}CD45RB^{high}$ phenotype, are significantly enriched in IL-10 over-expressing animals. Akbari et al. [9] isolated Tr1-inducing DCs, secreting IL-10, from the lung following respiratory exposure to antigen, and Th3-inducing DCs, secreting TGF-β, from the gut following oral exposure to antigen. The undoubtedly defining role of DCs in commanding a regulatory outcome raises the question of how DCs are themselves regulated: for example, could the infectious tolerance referred to above be mediated by altering DC phenotype prior to encounter with secondary T cell populations?

Regulatory T Cells in Allergic Diseases

The first suggestions that allergies could be controlled by regulatory T cells arose early in the current decade [12] in the light of both human and model system studies. Within mouse models, it had been demonstrated that forced expression of either IL-10 or TGF-β, the two canonical regulatory cytokines, prevented allergic airway inflammation in animal models [13, 14]. For example, T cell lines expressing latent TGF-β were able, on adoptive transfer into OVA-sensitised SCID or BALB/c recipients, to abolish airway inflammation and AHR. Protected animals carried increased concentrations of active TGF-β in the bronchoalveolar lavage (BAL) fluid, and protection from airway inflammation was reversed by neutralisation of TGF-β [13]. T cell lines expressing IL-10 can also protect OVA-sensitive recipient mice from AHR and inflammation [14]. Subsequently, the ability of Tregs to control allergic airway inflammation has been formally demonstrated, and the mechanisms involved shown to include T cell expression of CTLA-4. Thus, monoclonal antibodies against CTLA-4 exacerbate allergen-induced inflammation, suggesting that co-stimulatory interactions as well as modulating cytokines are at play in the regulation of allergy.

In parallel to mouse models, studies on human patients document the effects of Treg populations on allergies. The first evidence for downregulation occurring in the clinical setting derived from allergen-specific immunotherapy (SIT). Successful SIT for insect venom allergy was shown to be accompanied by enhanced IL-10 and TGF-β production [15]. IL-10 has a key influence,

promoting blocking antibody IgG4 responses while inhibiting specific IgE production, comparable to a 'modified Th2' response described with natural exposure to high levels of cat or dog allergen [16]. In the case of SIT with high doses of the house dust mite (HDM) allergen Der p 1, the IL-10 and TGF-β was shown to be derived from CD4+CD25+ T cells, and involved concomitant suppression of the pro-allergic cytokines IL-5 and IL-13, as well as of IFN-γ [17]. These CD4+CD25+ Tregs showed classical suppression of allergen-responsive effector T cells in vitro [17].

These first indications of Treg control of allergies were extended by more direct comparisons of Treg activity in allergic and non-allergic patients. Although there is no distinction in the overall levels or functions of CD4+CD25+ T cells from atopics and non-atopics, subtle differences exist in the ability of Tregs from allergic individuals to suppress only allergen specific Th1 responses and not Th2-derived IL-4/IL-5 [18]. Moroever, suppression of Th2 cytokines in atopics wanes during the pollen season [19], indicating the failure to control responses in the face of renewed allergen challenge. Thus, in allergic patients a skewed Th2 response and greater proportion of allergen-reactive Th2 may be due to an inability of Treg cells to control Th2 cytokines.

This notion is supported from a recent study from Switzerland, comparing Th1, Th2 and Tr1 cellular compartments in birch pollen- and HDM-allergic patients with non-atopic individuals. Tr1 cells were the dominant allergen-specific population in non-atopic individuals, whereas atopic individuals had a high frequency of allergen-specific IL-4-secreting Th2 cells [20]. Responses from non-atopic individuals could be amplified by neutralising IL-10, TGF-β or CTLA-4, suggesting multiple mechanisms of Tr1-mediated suppression. HDM-allergic donors, as expected, showed a strong response to Der p 1, which was suppressed by IL-10-secreting Der p 1-specific cells. This study provides the strongest evidence to date of a deficient or sub-optimal proportion of Treg cells in allergic individuals, as allergen-reactive Th2 cells from allergic patients are still suppressible with sufficient numbers of Tr1 cells.

Finally, Ling et al. [21] found similar patterns of deficient regulatory activity within the CD4+CD25+ compartment of allergic individuals. CD4+CD25+ T cells from hay fever patients, during the pollen season, had the weakest suppressive effect on allergen-induced CD4+CD25− T cell proliferation and IL-5 secretion. CD4+CD25+ T cells from atopic individuals, with no present symptoms, had significantly greater suppressive function, with CD4+CD25+ T cells from non-atopic individuals displaying the greatest suppressive capacity.

The malfunction of Treg cells from allergic contact dermatitis patients, sensitive to nickel, has also been reported, with CD4+CD25+ cells presenting a limited or absent capacity to suppress metal-specific CD4+ and CD8+ cells

[22]. Thus, Treg cells may operate to control allergic reactivity at dermal as well as mucosal sites. As a result, a consensus has emerged from many authorities in the field that some form of Treg population is at the centre of an anti-inflammatory network governing potential allergic responses of all types.

Because Treg cells can suppress both Th1 and Th2 effector responses, they can modulate not only allergies, as above, but also autoimmune pathologies. Consequently, the activity of Tregs in infectious diseases may be instrumental, irrespective of whether the infection is primarily controlled by Th1 or Th2 type responses. Indeed, a series of recent papers have documented the role of Tregs in infectious diseases, including viral, bacterial, fungal, protozoal and helminth infections (reviewed in [23]). Interestingly, most report that removal of, or inhibition of, Treg cells lead to effective clearance of infection and restoration of antigen-specific activity, providing direct evidence of dampened effector responses by Tregs during infection.

Helminth-Mediated Immune Regulation and Treg Cells

One of the striking features of helminth infections is the preponderance of asymptomatic infections; in which either parasite immune suppression or host feedback inhibition regulate the degree of inflammatory immunopathology, which results from infection [23]. Helminth infections are associated with specific T cell hyporesponsiveness manifest by depression of specific cytokine responses such as IL-5, IL-8 and TNF-α, and a more generalised switch away from inflammatory Th1 reactivity. While the Th2 response measured by IL-4 production is not diminished, Th2 effector mechanisms are blunted in a clear parallel to the 'modified Th2 response' observed following allergen desensitisation. Despite many pathological conditions observed in schistosomiasis and filariasis, most chronic infections remain asymptomatic [23]. Compared to symptomatic patients with high IgE, asymptomatic filariasis patients mount a strong IgG4 response with lower levels of IgE, reminiscent of the 'modified Th2' response discussed above.

Evidence of regulatory T cells during helminth infection is steadily accumulating. T cell hyporesponsiveness in filarial and schistosome-infected patients can be reversed in vitro with antibodies to IL-10 and/or TGF-β, suggesting a Treg component in these infections (reviewed in [23]). In the mouse model for schistosomiasis, IL-10 has proved to be critical to restrain immunopathology and ensure host survival, in particular exerting a critical restraint on granuloma formation and size. Crucially, IL-10 is produced not only by CD4+CD25+ Treg cells but also by innate cell types [24]. McKee et al. [25] further identified that both Treg cells and Th2 cells generated during

Schistosoma mansoni infections regulate Th1 development and immunopathology. Most recently, in our own laboratory, we have demonstrated that Treg populations expand during filarial infection of mice, using the model system of *Litomosoides sigmodontis.* In this system, clearance of adult worms from chronically infected mice could be induced by antibody neutralisation of Treg activity, specifically by administering anti-CD25 together with anti-GITR [26].

Among the most chronic of helminth infections are those which establish in the gastrointestinal (GI) tract. Although the intestinal locale is known to be favourable to Th3 induction, few reports conclusively identify T cells with regulatory function in GI helminth infections. Wohlleben et al. [27] have described a population of CD4+CD25+IL10+ T cells in the BAL fluid of mice infected with *Nippostrongylus brasiliensis*. However, these cells also secrete IL-4, suggesting that they may be, or at least contain, activated Th2 cells. Schopf et al. [28] identified the critical role of IL-10 in Th2 development and resistance, with suppression of IFN-γ and IL-12 induced Th1 development, and the maintenance of colon barrier function during *Trichuris muris* infection. Furthermore, mice deficient in IL-10 production show increased fatalities following *T. muris* or schistosome infections.

Most recently, the generation of Treg cells, with elevated expression of FoxP3, during chronic intestinal nematode infection with *Heligmosomoides polygyrus* has been observed [29]. As will be described in greater detail below, this infection induces a functionally active regulatory T cell population capable of down-modulating the allergic response to an unrelated antigen.

Helminth Infection and Allergic Disease

In the light of immune depression in helminth infection, it is not surprising that allergic inflammation, appears also to be modulated during infection. Indeed, epidemiological data demonstrating low levels of asthma in rural Gambians were first published 30 years ago [1]. A more recent study in Ethiopia likewise found that asthma correlated negatively with infection with *Ascaris* and/or *Necator* [30]. Understanding immunoregulatory mechanisms, in particular their induction by helminths, may lead to new therapeutic avenues for immune disorders and also bring some clarification to why inflammatory reactions occur in autoimmunity and allergy.

Recent reviews [12, 31] have discussed the inverse association between chronic helminth infection and allergen-induced inflammation in exposed individuals. A consensus we highlight is that allergen sensitisation occurs normally, including significant induction of IgE, in helminth-infected subjects [30].

However, there is clear evidence for suppression of effector phase mechanisms and patent allergen reactivity associated with pathology.

The negative interaction between Th2-biasing helminth infections and Th2-dependent allergic diseases has forced a reconsideration of the original 'hygiene hypothesis'. In its simplest form, this hypothesis suggested that reduced levels of Th1-inducing infections result in exaggerated Th2 responses and allergic immunopathology. However, this postulation is not compatible with reduced allergies in helminth-infected persons, nor with the independent epidemiological data recording increased Th1-associated autoimmune diseases in Western populations.

Indeed, the Th1/Th2 dichotomy cannot readily be applied to the allergic setting. Although Th1-polarising pathogens such as *Chlamydia* can be used experimentally to deviate responses away from Th2 allergy [32], it is also true that allergen-specific Th1 cells can themselves cause allergic airway inflammation [33]. Together with the studies discussed below, evidence now available has led to a new 'hygiene hypothesis' built around the role of regulatory T cells and networks in dampening both Th1 and Th2 effector responses.

Among the most influential of the new series of studies into the helminth-allergy interaction have been those of van den Biggelaar et al. [2]. In a study comparing 520 Gabonese schoolchildren (from 5–14 years), skin-prick test (SPT) responses to HDM were significantly lower in those infected with *Schistosoma haematobium* than in uninfected classmates, despite both groups having similar allergen-specific IgE levels. Furthermore, a negative correlation between SPT scores and parasite-specific IL-10 response was observed in infected children. Further studies in the same population discovered that older age-groups who had higher levels of schistosome and filarial infections, as well as increased mite sensitisation, were in fact lowest in terms of skin test reactivity [34], suggesting an immunomodulatory mechanism, possibly mediated via IL-10, aimed at the effector stages of an allergic reaction.

In one of the largest epidemiological studies documenting the interaction between helminth infection and allergic reactivity (693 adults in rural and urban areas of The Gambia), Nyan et al. [35] reported an inverse association between skin-test reactivity and the presence of intestinal helminth (*Ascaris* or hookworm) infection. Importantly, the authors pointed out that this relationship could in fact reflect a higher level of anti-parasite immunity among atopics. However, higher infection intensities were found to generate higher Der p 1 sensitisation rates among non-atopics, suggesting that only effector phases are down-modulated by infection.

Likewise, Araujo et al. [36] studying Brazilian patients with chronic, heavy *S. mansoni* infections (>200 eggs/g of faeces), observed a higher prevalence (24%) of HDM reactivity in uninfected controls compared to chronically

infected individuals (5%). In this study, however, uninfected individuals mounted a greater allergen-specific IgE response, indicating that both sensitisation (allergen-specific IgE production), and reactivity can be reduced during infection.

In an original key study, Lynch et al. [37] discovered that treatment of helminth infections increased allergen-specific IgE and SPT responses in subjects living in high parasite-transmission areas. Thus, not only did infection repress atopy, but removal of infection resulted in the reappearance or introduction of allergic reactivity. Similarly, in *S. mansoni*-infected patients, Araujo et al. [3] reported ablated allergen reactivity with reduced secretion of Der p 1-specific IL-4 and IL-5 and elevated Der p 1-specific IL-10 in. Following treatment and eradication of worm burdens, Der p 1-specific IL-10 levels declined whilst Der p 1-specific IL-5 responses increased, suggesting that an active *S. mansoni* infection may promote an allergen-specific IL-10 response.

However, not all studies of helminth-allergy interactions have concluded with an inverse association. In one study, anthelmintic treatment for a year, improved clinical asthma [38]. Thus, in some cases helminth infection may actually exacerbate allergic symptoms. Migratory phases of invading larvae, travelling via the pulmonary system may potentiate reactivity to allergens in a polarised Th2 environment. Additionally, cross-reactivity between parasite-derived antigens and allergens, or acute florid responses to short-lived infections may be more pro-allergic, while frequent or chronic infection may build up sufficiently to generate a regulatory unit.

From the majority of human studies a scenario in which helminth infections prevent allergen reactivity can be clearly seen. Furthermore, it appears that an active infection is required to prevent allergen reactivity. Harnessing the active elements of a highly evolved helminth without the pathogenic consequences of an infection must be the priority to obtain new therapeutic agents for the treatment of hyperactive immune response. To distinguish cause from consequence and to dissect potential mechanisms, experimental models must be tested.

Model Systems for Infection and Allergy

Model systems offer great opportunities to dissect the interplay between pathogens and allergic disease. The suppressive influence of several bacteria has been well established in animal models of Th2-type allergic lung inflammation, and more recently studies on helminth models of infection have been described.

Using the human parasite *Strongyloides stercoralis*, Wang et al. [39] provided some of the first evidence for mitigation of allergic outcomes by helminth

infections. Mice infected with two doses of *S. stercoralis,* producing a short-lived tissue infection, were sensitised to ovalbumin (OVA) shortly after the second infection. Wang et al. [39] showed greatly reduced allergen-specific IgE production and eotaxin secretion, but no overall change in pulmonary eosinophilia or lymphocyte recruitment to the BAL fluid. Because *S. stercoralis* introduces an abortive and highly immunogenic infective episode and migrates via the lungs, its effects may be less far-reaching than a long-lived chronic infection; nevertheless these data show significant inhibition on components of both the sensitisation (IgE) and effector (eotaxin) phases. A more overt suppression of airway allergy, including airway eosinophilia, eotaxin levels and OVA-specific IgE and IgG1 in BALF, was achieved with longer-term exposure to *N. brasiliensis* infection [27]. IL-10-deficient animals showed no signs of protection with infection. This study is suggestive of a regulatory mechanism generated during helminth infection, with the capacity to dampen airway allergy.

Allergic responses to food allergens are also pliant to helminth infection. Bashir et al. [40] demonstrated that prior infection with the gastrointestinal nematode *Heligmosomoides polygyrus* significantly reduced allergen sensitisation, measured by allergen-specific IgE, and diminished production of the effector cytokine, IL-13. The effect observed with *H. polygyrus* could be reversed with anti-IL-10, suggesting a suppressive mechanism involving an immunoregulatory pathway.

Importantly, as with human studies, not all experimental helminth infections modulate allergic responses. Under conditions of existing elevated IL-4 and IL-5 and consequent IgE and localised eosinophilia, an incoming wave of helminth larvae migrating through the respiratory system may exacerbate airway hyperresponsiveness [41]. A key distinction may lie between acute and chronic helminth infections, and the extent to which the allergic state pre-exists at the time of parasite invasion.

In our laboratory, we have studied the interaction between *H. polygyrus*, a natural intestinal parasite of mice, and airway allergy. We selected this combination, as *H. polygyrus* follows an exclusively enteric life cycle, does not enter the tissues or the lungs during its development, and readily establishes as a chronic infection in immunocompetent mice. Thus, airway inflammation reflects the allergen-specific response without physical interference from migrating parasites. Using a standard sensitization-challenge system, we found that infected mice develop significantly lower allergic responses. Both C57BL/6 and BALB/c mice, harbouring a chronic *H. polygyrus* infection, display significantly reduced cellular airway inflammation following OVA or Der p 1 airway challenge, respectively. Of greatest importance, airway eosinophilia is almost completely inhibited by the presence of a chronic infection, mirrored

by reduced BAL IL-5 and eotaxin levels. Pathologically, infected mice have less peri-bronchial inflammation and goblet cell accumulation accompanied by reduced mast cell activity, measured by β-hexosaminidase activity. In our model, with two allergens in two strains of mice, the Th1/Th2 balance is not shifted, with comparable levels of IL-4 and reduced IFN-γ in the BAL fluid of both infected and uninfected mice, following airway challenge. Thus, a Th2-inducing infection can modulate a Th2 allergic inflammatory response in an unrelated organ. These findings do not support 'immune deviation' away from the pro-allergic Th2 response, as a mature Th2 response was created by the infection. Rather, an additional regulatory response was induced in infected mice, including the cytokines IL-10 and TGF-β.

To address the role of a regulatory response generated during infection and its ability to suppress allergen-induced airway inflammation, we neutralised IL-10 signalling, using a monoclonal anti-IL-10R Ab, or removed CD25+ cells, using anti-CD25 (PC61), prior to airway challenge of sensitised, infected mice. The protective effect of a chronic *H. polygyrus* infection was lost on depletion of CD25+ cells, but was undiminished following anti-IL-10R Ab treatment, indicating that a CD25+ regulatory population was active through a non-IL-10-dependent mechanism [Wilson et al., submitted].

Using an adoptive transfer model, transferring cellular populations from the mesenteric lymph nodes (MLN) of chronically infected mice into uninfected allergen-sensitised recipients, we were able to identify cellular populations within the MLN of infected donors that could transfer the protective effect observed in infected mice. CD4+CD25+ MLN cells from chronically infected mice were found to suppress allergen-induced airway inflammation in uninfected but allergen-sensitised mice [Wilson et al., submitted] providing additional evidence to support the hypothesis that helminth infection down-regulates allergic reactivity, through the action of a regulatory population rather than by altering the Th1/Th2 balance. In our experiments, CD4+CD25+ cells are introduced 7 days before airway challenge of sensitised recipients. Thus, donor Tregs inhibit the reactivation of differentiated Th2 cell effector cells and the subsequent mobilisation of eosinophils.

The suppressive MLN cell population show elevated expression of Treg-associated markers, including CTLA-4, GITR, CD103, surface bound TGF-β and intracellular IL-10. Interestingly, the transfer of Treg cells from infected donors does not require exogenous parasite antigen, raising questions of antigen specificity. Thus, it is not yet known whether the donor cells directly suppress the allergen response, or perhaps induce allergen-specific regulatory cells by virtue of surface ligands or secreted mediators present in the donor population.

The transfer experiments also help in resolving the issue of IgE interference. The stimulation of polyclonal IgE during infection has been proposed as a

protective mechanism against allergy, through the dilution of allergen-specific IgE and saturation of FcεR on mast cells or basophils. In our experiments, polyclonal IgE is indeed elevated in infected mice, however, allergen-specific IgE is of equal quantity in the presence or absence of infection prior to allergen challenge, and is not altered by the transfer of MLNC capable of suppressing the allergic response.

It is interesting to note how our observations illustrate the functional crossover of the three Treg subtypes. Functional, suppressive CD4+CD25+ cells, frequently defined as 'naturally occurring Treg' cells, were isolated from the 'Th3 location' (MLN of chronically infected mice) and show functionality in the 'Tr1 location' (in the airway tissues).

Alternative Models

Several contending hypotheses are available to explain how infections in general, or helminth infections in particular, could downregulate allergies and inflammatory disorders. The simplest is a homeostatic model which supposes that if the immune system has a predetermined capacity for responsiveness, then infection would distract from a proto-allergic (or autoimmune) pathway, restraining it below the threshold of pathology. Mechanistically, this could operate at the level of competition between two simultaneous responses (one to pathogen, one to allergen) for cytokines and growth factors, or for suitable physical niches within lymphoid tissue of antigen-specific peripheral T cell populations.

A second generic model for downregulation in infection arises from the sustained high antigen levels associated with chronic parasitism. With repeated transmission of infective stages and long-lived adult worm populations, humans in endemic areas suffer continuous antigen exposure. The repeated generation of pro-inflammatory signals in more reactive patients may lead to chronic pathology such as elephantiasis or hepatosplenic schistosomiasis. Conversely, in asymptomatic individuals, continuous stimulation may create counter-regulatory conditions such as receptor downmodulation or signalling cross-talk, preventing inflammatory responses to pathogen stimuli.

Finally, a more explicit model invoking regulatory T cells has been formulated. This postulates that the increasing prevalence of allergic disease is linked to diminished downregulatory effects within the immune system, such as those mediated by regulatory T cells [12, 42]. This model, which is essentially a derivation of the 'hygiene hypothesis' postulates that the protective effect observed with chronic helminth infections may be attributed to induction or expansion of immune self-regulation. Similar regulatory populations may be

induced in other chronic infections, such as those caused by viral, bacterial and fungal pathogens. The 'regulatory' hypothesis also takes into account the parallel increase in autoimmune diseases, as a common consequence of an ill-balanced immune system and deficient regulatory population.

Molecular Mechanisms at the Parasite-Allergy Interface

A mechanistic explanation for the suppression of allergy should go beyond the description of regulatory T cells, and establish at the molecular level how changes in key mediators, cytokines and cell surface receptor ligation may block the allergic reaction. A very wide range of additional cytokines, chemokines, receptors and antagonists are intimately involved in the generation and regulation of allergies. We discuss here some of the factors known at this stage to be involved in the interactions between infection and allergy.

IL-10 is one of the primary down-regulatory cytokines which has received much attention in the allergic setting. IL-10-deficient mice succumb to a heightened inflammatory response in an OVA-induced airway model, while administration of IL-10 reduces the antigen-presenting capacity of DCs as well as exerting direct effects on innate effector populations such as mast cells and eosinophils. In humans, reduced IL-10 secretion in BAL fluid of asthmatic individuals, compared to non-asthmatics [43], is often found. Because IL-10 is not only prominent in most helminth infections, but is actually essential to prevent lethal pathological reactions to parasite infection, it is highly likely to influence concurrent responses to bystander antigens such as allergens.

TGF-β, like IL-10, is a major feature of chronic helminth infections, and as part of the anti-inflammatory network may be a significant player in helminth infections and allergy [12]. In addition to the above-mentioned study of TGF-β-mediated suppression of airway allergy [13], evidence exists that high-dose allergen challenge induces a TGF-β-dependent suppression of airway eosinophilia [44]. TGF-β can directly reduce proliferation of antigen-specific CD4+ T cells, and, as discussed above, induce the differentiation of FoxP3+CD4+CD25+ Treg cells [11]. Finally, it is important to note the synergistic effect of both IL-10 and TGF-β in the induction of T cell hyporesponsiveness.

In the context of allergy, IL-13 is a remarkably important cytokine. As well as essential functions in Th2 induction, fibrogenesis, airway hyperreactivity and goblet cell accumulation, IL-13 is a necessary component in the development of acute and chronic allergic asthma [45]. A soluble form of the receptor, IL-13Rα2, acts to remove active IL-13 and mitigate immunopathology in helminth infections. Release of this decoy receptor is stimulated by IL-10 as well as by

IL-13 itself [46], revealing an elegant feedback mechanism to prevent a run-away pathogenic reaction to infections and allergens alike.

Where the role of co-stimulation in regulating pro-allergic responses has been explored, intriguing results have been obtained. Ligation by CTLA-4 to B7 molecules on DCs triggers the activation of indoleamine 2,3-dioxygenase (IDO) [47], a rate-limiting tryptophan-catabolising enzyme that can inhibit T cell proliferation through depletion of local tryptophan levels. In helminth infections, enhanced CTLA-4 expression in particular has been recorded [26]. Thus, enhanced CTLA-4 interactions with DCs and increasing IDO activity provides a potential mechanism whereby Treg cells responding to infection could modulate allergen-specific T cell responses.

Infectious Tolerance and the Specificity of Treg Cells

We have so far considered the potential for direct suppression by parasite-induced Treg cells on the bystander response to allergens. This supposes that the Treg cells are either parasite-specific, or else are self-reactive 'natural' Tregs which are expanded in a non-TCR-specific fashion during infection. However, it is possible that allergen-specific regulatory T cells emerge in the presence of a chronic infection, if parasite-induced Tregs are able to convert naive or effector T cells into the regulatory cells phenotype. Newly converted Treg cells could then block the activation of additional T cells, in a regulatory cascade [48]. One mechanism for this process could be through the known action of TGF-β, which converts normal T cells into suppressive, TGF-β secreting FoxP3+ Tregs [11]. In the HDM-induced airway inflammation model, Chen and colleagues [11] have demonstrated that secondarily recruited Treg cells can suppress HDM-induced airway inflammation.

This model of infectious tolerance accommodates many of the results we have observed in the *H. polygyrus* system of inhibition of airway allergy. Here, TGF-β expression is elevated in MLNC of infected mice, and in the cell population able to confer protection on recipient, uninfected mice. The transfer of a relatively small number of CD4+CD25+ cells can block allergic reactivity, even though relatively few donor cells are observed to localise to the airways. It is enticing to suggest that a small number of donor Treg cells propagate the expansion of host, allergen-specific, Treg populations. Infectious tolerance may be self-sufficient with a healthy supply of naïve CD4+ cells to be converted by donor Tregs. A role for IL-10 may also be considered; although donor IL-10 is not required [Wilson et al., submitted], TGF-β from donor cells may induce host IL-10 secretion [49] while, IL-10 can mediate the up-regulation of the TGF-βR2 on activated T cells, rendering them more susceptible to the suppressive

effects of TGF-β. Thus, a synergistic role for these regulatory cytokines is entirely possible.

Conclusions

The present rise in allergic and autoimmune diseases are associated with diminished levels of immune regulation. Experimentally enhancing T cell regulation can reduce these diseases, as shown in the *H. polygyrus* system. Extrapolating to the human situation, the inverse association between helminth infections and allergic reactivity may thus be explained by helminth-induced regulatory networks. The evidence for this is threefold.

First, human allergic responses correlate inversely with levels of allergen-specific regulatory activity. High-dose allergen-specific immunotherapy used to treat allergic patients, generates significant populations of allergen-specific regulatory T cells, producing IL-10 and/or TGF-β. This therapy also induces the secretion of non-inflammatory IgG4 antibodies and is promising to be a successful step in treating IgE mediated allergic disease.

Second, helminth infections stimulate regulatory T cell populations which prolong survival of parasites in the immunocompetent host. Thus, helminths are associated with expansion of a cell type known to be capable of downregulating immunopathologies.

Finally, we show in our own experiments that helminth-induced Tregs are capable of transferring the suppression of allergy into uninfected but allergen-sensitized animals. Thus, we have provided proof-of-principle that the regulatory pathway can effectively be mobilised in the parasite-allergy interaction.

Evolutionarily, allergies may be a consequence of our helminth-infected evolutionary history, when helminth infections were considerably more abundant. A STAT6 polymorphism originally linked to susceptibility to allergic asthma has more recently been discovered to confer resistance to *Ascaris* infection in human populations [50]. Thus, in evolutionary time, alleles conferring stronger immune responsiveness to a helminth parasite were originally advantageous; however, in a helminth-free environment, the same alleles now render individuals over-reactive to environmental allergens and confer a risk of pathological disadvantage. Perhaps, by unravelling the intricate relationship between infections and allergies we will achieve a deeper understanding of both threats to human health, and discover new pathways to alleviate disease and pathology for the future.

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Author Index

Araujo, M.I. 29

Capron, M. 113
Cummings, R.D. 91

Dalton, J.P. 45
de Carvalho, E.M. 29
Donnelly, S. 45

Faveeuw, C. 113

Grencis, R.K. 128

Hartgers, F.C. 157

Jankovic, D. 65

Kane, C.M. 82
Kullberg, M.C. 65

Leite-de-Moraes, M. 113
Loukas, A. 45

Maizels, R.M. 176
Mallevaey, T. 113
Mitre, E. 141

Nagler-Anderson, C. 1
Negrão-Corrêa, D. 14
Nutman, T.B. 141

Pearce, E.J. 82
Pennock, J.L. 128

Sher, A. 65
Smits, H.H. 157
Steinfelder, S. 65
Sun, J. 82

Teixeira, M.M. 14
Trottein, F. 113

van der Kleij, D. 157
van Die, I. 91

Wilson, M.S. 176

Yazdanbakhsh, M. 157

Subject Index

Asthma
  airway allergy pathophysiology 177–179
  demographic trends 1, 2, 36, 176
  diagnosis 35
  hygiene hypothesis 2
  Mycobacterium tuberculosis exposure, effects 2
  natural killer cell Th2 inflammatory response 117, 118, 122–124
  schistosomiasis, protection 36–42
  Th2 cytokines 35, 36

Basophil
  activation
    degranulation 146
    immunoglobulin-E-independent activation 145
    immunoglobulin-E-mediated activation 144, 145
    markers 146
  cytokine release 148
  effectors 146, 147
  growth and development 142, 143
  helminth infection response
    activation 151
    basophilia 150, 151
    superallergens 151, 152
    Th2 response initiation 152–154
  histamine immunomodulatory properties 148, 149
  lineage 142
  protease allergen modulation of function 57
  trafficking 143, 144

CD1 molecules
  classes 114
  T cell recognition 113
CD4+CD25+ regulatory T cells
  allergy control 181–183
  classification of regulatory T cells 179, 180
  dendritic cells in induction 181
  differentiation 180
  helminth-mediated protection against allergy 9, 183, 184
  infectious tolerance and specificity of cells 191, 192
  intestinal inflammation protection 8
  Leishmania major survival role 8, 9
  markers 179, 180
  schistosomiasis infection
    atopy and asthma protection 168, 169
    hygiene hypothesis 189, 190
    immune response downregulation 165–167
  Th3 cell relationship 180
Collectins, schistosome egg glycan antigen and allergen binding 105
Complement, basophil activation 145
Crohn's disease, schistosomiasis, protection 34, 35

CTLA-4, allergy protection role in
helminth infection 191

Dendritic cell (DC)
airway allergy initiation 177, 178
protease allergen modulation of function 50
regulatory T cell induction 181
schistosome egg glycan antigen interactions
DC-SIGN 102, 103, 107
L-SIGN 103
Th cell activation role 83, 84
Th2 polarization role in schistosomiasis
downregulation in negative pathway 71–73
evidence against default pathway 69–71
prospects for study 77
selective activation in positive pathway 73, 74
soluble egg antigen interactions 70, 71, 74–77, 85–87
Toll-like receptors 72–74
Der p 1
allergen activity 48
collectin binding 105
mannose receptor interactions 104

Egg antigens, *see* Glycan antigens, schistosome eggs
Eosinophil, airway allergy role 178

Food allergy, *see Heligmosomoides polygyrus*, allergy protection

Galectin-3, schistosome egg glycan antigen and allergen binding 105, 106
GATA-3, Th2 cell differentiation induction 77
Glycan antigens, schistosome eggs
antigen-presenting cell interactions
DC-SIGN 102, 103
L-SIGN 103
macrophage galactose-type lectin 103
mannose receptor 104
cross-reactive immunoglobulin E 98, 99
dendritic cell interactions 70, 71, 74–77, 85–87, 102, 103
humoral immunogenicity 95, 96
interleukin-10 induction and allergy protection 106, 107
lectin interactions
host protection in schistosomiasis and allergy
collectins 105
galectin-3 105, 106
overview 100, 101
prospects for study 108, 109
soluble egg antigen preparation 96, 159
structures and types 92–95, 159
Th2 polarization induction 97, 98, 159
Toll-like receptor interactions 101, 102

Heligmosomoides polygyrus, allergy protection
airway allergy studies 187, 188
dietary adjuvant response studies 3–5
life cycle 187
mechanism 8, 9, 11
mesenteric lymph node transfer studies 188, 189
overview 2, 3, 187
peanut antigen challenge studies 5, 7, 8
regulatory T cell role 188
Helminth Th2-polarizing immune response
allergy protection
Heligmosomoides polygyrus model, *see Heligmosomoides polygyrus*, allergy protection
overview 2, 3, 16, 53, 177
Schistosoma mansoni model, *see Schistosoma mansoni*
Strongyloides venezuelensis model, *see Strongyloides venezuelensis*
allergy similarities 92
autoimmune disease incidence 2
basophil role, *see* Basophil
evolutionary implications 192
glycan antigen roles, *see* Glycan antigens, schistosome eggs
mast cell role, *see* Mast cell
natural killer cell role, *see* Natural killer cell

overview 53, 54, 82, 83, 149, 150
polarization pathways in schistosomiasis
dendritic cell role
downregulation in negative pathway 71–73
evidence against default pathway 69–71
prospects for study 77
selective activation in positive pathway 73, 74
soluble egg antigen interactions 70, 71, 74–77, 85–87
Toll-like receptors 72–74, 87–89
interleukin-4 role 67–69, 74–77
protease allergens 54, 58–61
superallergens 151, 152
Hepatitis C virus (HCV), schistosomiasis and susceptibility 32
Histamine
basophil release 147
immunomodulatory properties 148, 149
receptors 147
Histamine-releasing factor (HRF), basophil activation 145
Human T cell lymphocytotrophic virus (HTLV-1), schistosomiasis and susceptibility 32–34
Hygiene hypothesis
helminth protection against allergy, *see* Helminth Th2-polarizing immune response; *Strongyloides venezuelensis*
overview 2, 15

Immunoglobulin E (IgE)
airway allergy role 178, 179
basophil activation 144, 145
protease allergen sensitization via cross-reactive immunoglobulin E 54–56
schistosome egg glycan antigen cross-reactivity with allergens 98, 99
Immunoglobulin G4 (IgG4), schistosomiasis prevention of atopy and allergy 169
Interleukin-3 (IL-3)
basophil regulation 142
mast cell regulation 130, 131
Interleukin-4 (IL-4)
airway allergy role 178
basophil production in helminth infection 152–154
mast cell regulation 130, 133
sources 68
Th2 polarization role in schistosomiasis 67–69, 74–77
Interleukin-5 (IL-5), airway allergy role 178
Interleukin-10 (IL-10)
helminth Th2-polarizing immune response and allergy protection 9, 22, 25, 190, 191
schistosome egg glycan antigen induction and allergy protection 106, 107
schistosomiasis and allergy protection 31, 39–42
sources 40, 41
Interleukin-13 (IL-13)
airway allergy role 178
allergy protection in helminth infection 190, 191
schistosomiasis prevention of atopy and allergy with soluble decoy receptors 169, 171

Lectins, schistosome egg glycan interactions
host protection in schistosomiasis and allergy
collectins 105
galectin-3 105, 106
overview 100, 101
Leishmania major, regulatory T cells in survival 8, 9

Macrophage, schistosomiasis response 164
Mast cell
adhesion and homing in gut 131
allergy role 134
classification 129
helminth infection
effector mechanisms 132–136
prospects for study 136, 137
regulators in gut nematode infection 129–131

Mast cell (continued)
response 119–121
Th2 environment 133
protease allergen activation 50, 51
Toll-like receptor expression 135, 136
Mold allergy, proteases as allergens 49
Monocyte chemoattractant protein (MCP), basophil activation 145
Mycobacterium tuberculosis, exposure effects on asthma incidence 2

Natural killer (NK) cell
activation mechanisms in infection 122
CD1d-restricted ligands 114, 115
classification 114
function 114, 115
pathology 115, 116
prospects for study 124
Th2 inflammatory response in asthma
mechanisms 118, 122–124
overview 117, 118
Nematodes, *see also* Helminth Th2-polarizing immune response; *Strongyloides venezuelensis*
epidemiology 14, 15
mast cell response, *see* Mast cell

Protease allergens
allergenicity criteria 45, 46
helminth proteases 54, 58–61
mechanisms
basophil degranulation 57
dendritic cell function modulation 50
epithelial barrier disruption 49
lymphocyte function modulation 50, 58
mast cell activation 50, 51, 57
protease/antiprotease balance disruption 51, 52
prospects for study 61, 62
protease activity importance in allergy induction 52, 57
sensitization via cross-reactive immunoglobulin E 54–56
structural classes 46, 48
types 46–49

Regulatory T cells, *see* CD4+CD25+ regulatory T cells

Schistosoma mansoni
atopy and asthma protection
infection treatment and reversal of protection 186
mechanisms
immunoglobulin G4 antibodies 169
interleukin-13 decoy receptors 169, 171
overview 38–42
regulatory T cells 168, 169
skin test reactivity studies 36–38, 167, 168, 185, 186
autoimmune disease damage protection 34
double-stranded RNA response from eggs 160
glycan antigens, *see* Glycan antigens, schistosome eggs
immune response in schistosomiasis
adaptive immune response 160–162
downregulation in chronic infection
adaptive immunity 165
innate immunity 162–164
regulatory T cells 165–167
window of immunosuppression 171, 172
innate immune response 158–160
overview 30, 31, 66, 67, 158
interleukin-10-inducing antigens 42
life cycle 30, 93, 94, 157, 158
natural killer cell response 119–121
pathology 31, 157, 158
Th1 response downregulation 31–35, 160, 161
Th2 polarization pathways
dendritic cell role
downregulation in negative pathway 71–73
evidence against default pathway 69–71
prospects for study 77
selective activation in positive pathway 73, 74

soluble egg antigen interactions 70, 71, 74–77, 85–87
Toll-like receptors 72–74, 87–89
interleukin-4 role 67–69, 74–77, 165
viral susceptibility studies 32–34
Soluble egg antigen, *see* Glycan antigens, schistosome eggs; *Schistosoma mansoni*
STAT6, polymorphism in asthma and *Ascaris* resistance 192
Stem cell factor (SCF), mast cell regulation 129, 130
Strongyloides stercoralis, mouse model of infection 186, 187
Strongyloides venezuelensis
allergy protection
airway hyperreactivity cessation 20
overview 15–17
parasite burden reduction
overview 20–22
pulmonary function response 22
Th2 response role 22–25
eosinophilic airway inflammation induction 18, 19
rodent infection model 17, 18
transient airway hyperreactivity induction 19, 20
Surfactant proteins, schistosome egg glycan antigen and allergen binding 105

T helper balance
helminth response and allergy protection, *see* Helminth Th2-polarizing immune response
hygiene hypothesis 2
natural killer cell Th2 inflammatory response in asthma 117, 118
subsets of cells 65, 66, 84
Th3 regulatory T cells 180
Toll-like receptor (TLR)
adapter molecules 161
classification 159
double-stranded RNA response from schistosome eggs 160
glycan antigen interactions 101, 102
mast cell expression 135, 136
Th2 polarization role in schistosomiasis 72–74, 87–89, 164, 165
Transforming growth factor-β (TGF-β)
allergy protection in helminth infection 190
immune response downregulation in schistosomiasis 165, 167
mast cell regulation 131, 132