

The Innate Allergenicity of Helminth Parasites

**Franco H. Falcone,^{*1} Alex Loukas,² Rupert J. Quinnell,³
and David I. Pritchard⁴**

¹Immune Modulation Research Group, Boots Science Building, School of Pharmacy, University of Nottingham, Nottingham, UK; ²Department of Microbiology and Tropical Medicine, George Washington University Medical Center, Washington, DC; and ³School of Biology, University of Leeds, Leeds LS2 9JT, UK

Abstract

Helminth parasites are well known to induce an immune response in their hosts characterised by elevated IgE, peripheral blood or local tissue eosinophilia, and in some cases, intestinal mastocytosis. This immunological response has a strong T-helper 2 (Th2) cytokine bias and is reminiscent of the immunological constellation found in allergic diseases. However, the molecular forces driving the Th2 response to helminth parasites are still not understood. By using the human hookworm parasite *Necator americanus* as an example, the authors of the current article propose that in the course of its life cycle, this parasite becomes innately allergenic through the secretion of a molecular array designed to promote tissue migration and homing, feeding and survival against immunological attack. This complex array comprises proteases, lectins and other classes of molecules. Subsequent immunological and physiological events seemingly protect the host from both the allergic sequelae of exposure to environmental allergens and, moreover, from the parasite itself.

Index Entries:

Helminth; Th2; *Necator americanus*; superallergen; proteases; lectins.

*Author to whom all correspondence and reprint requests should be addressed. E-mail: Franco.Falcone@nottingham.ac.uk

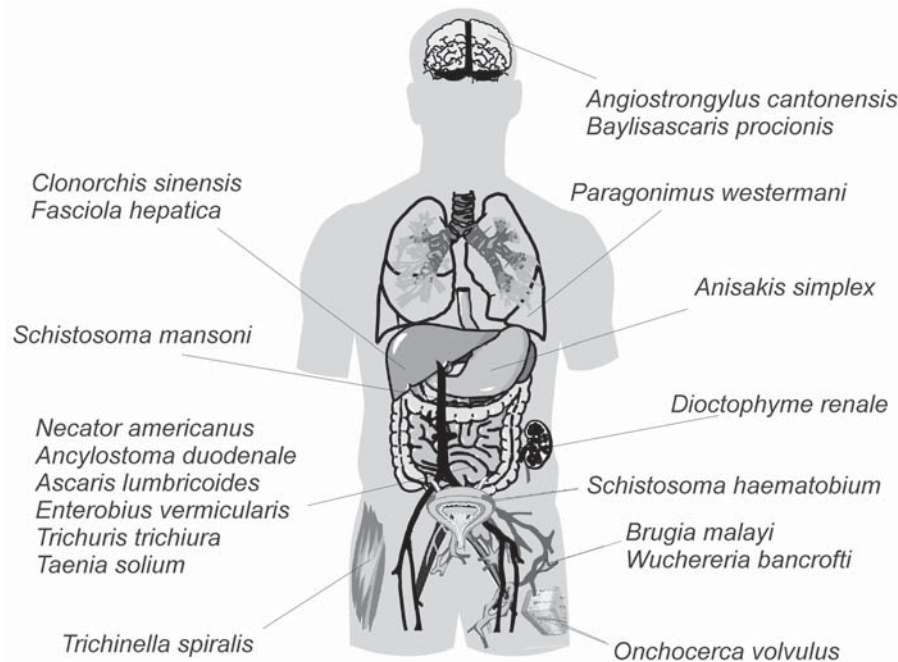


Fig. 1. In humans, helminths can parasitize the lymphatic system (*Brugia malayi*, *Wuchereria bancrofti*), blood vessels in the intestine and bladder (*Schistosoma mansoni*, *Schistosoma haematobium*), lungs (*Paragonimus westermani*), liver (*Clonorchis sinensis*, *Fasciola hepatica*), kidney (*Dioctophyme renale*), central nervous system (*Angiostrongylus cantonensis*, *Baylisascaris procionis*), skin (*Onchocerca volvulus*), stomach (*Anisakis simplex*), or muscle tissue (*Trichinella spiralis*). A common anatomical location for parasitism, perhaps not surprisingly, given the ease of oral-fecal transmission, is the host's gastrointestinal tract (*Ascaris lumbricoides*, *Taenia solium*, *Taenia saginata*, *Necator americanus*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Trichuris trichiura*, and *Strongyloides stercoralis*).

Parasite Habitats in Humans

Helminth parasites have evolved necessarily complex life cycles, which frequently involve temporary habitation in different tissues within the hosts, to transmit infection. Therefore, the different stages of many parasites display a high level of specialization and adaptation to their hosts, and, within these hosts, to specific tissues or locations. The interim and final habitats in mammalian hosts, where the parasites reach maturity and reproduce (see Fig. 1), can be very diverse.

The homing of the parasite to a specific tissue or final location implies that the larval stage of the parasite has to actively reach its target, while at the same time negotiating attack by the host's immune response to avoid recognition and destruction. The migratory route of larvae in many cases involves the skin

and includes an obligatory lung stage (*Schistosoma*, *Necator*, *Strongyloides*, and *Ascaris*). In order to migrate through the tissues, parasites almost inevitably produce and release a set of proteases and other molecules finely tuned to the specific host and capable of digesting host tissue (1,2), facilitating homing and immune evasion. Adult stages, having reached their final location, are faced with a completely different microenvironment from that initially encountered during invasion (in the physical, biochemical, and immunological sense), but also have very different tasks to fulfill, notably feeding (some feeding also occurs in the larval stage [3]), reproduction, and immune evasion. Overall, one would expect a complementary (or completely different) set of genes to be expressed and molecules to be secreted by the different life stages. This principle is adequately demonstrated by the comparative analysis of

parasitic secretions of larval and adult stages of *N. americanus* hookworms (4,5).

In the following section, we will attempt to illustrate how some of these secretions are thought to assist parasites in fulfilling their varying tasks "at hand" in their hosts, i.e., migration to the final location, feeding, growth and development, reproduction, and immune evasion. In subsequent sections, we shall outline how, in our view, these molecular arrays cannot help but innately elicit a pro-allergic immune response, while at the same time, perhaps paradoxically, exerting antiinflammatory, protective effects.

The Molecular Array of Hookworms

The molecular array of parasites is necessarily diverse, given their complex life cycles, and now includes a number of reasonably well-defined entities for which examples can be given. For an insight into the known molecular array of *Necator*, see NemBase= (<http://nema.cap.ed.ac.uk/nematodeESTs/nembase.html>).

Tissue Migration and Feeding

N. americanus infective larvae have to migrate through the skin, where key developmental cues appear to be received (3,6). Skin penetration is assisted by the secretion of proteinases with elastin-cleaving properties, and other (aspartyl) proteinases, which digest collagens (types I, II, IV, and V), fibronectin, elastin, and laminin (7,8). The importance of parasitic proteinases to infection is convincingly demonstrated by experiments showing that penetration of hookworm larvae is significantly inhibited by treatment with pepstatin A (selectively inhibiting the aspartyl proteinase activity) (7), and by antisera raised to hookworm aspartyl proteinases (2). Another member of the parasite's aspartyl proteinase repertoire is involved in the later digestion of hemoglobin by adult stages, and has also been shown to digest fibrinogen, imparting an antihemostatic property to what could be an important feeding enzyme, given the hemato-

phagous life style of this parasite (1). The antihemostatic strategy is supported by the secretion of molecules that inhibit coagulation factors and platelet activity (9).

It may also be proposed that nematodes secrete and express lectins and glycoproteins designed to assist parasite homing in the manner described for leucocyte adhesion to, and margination from, selected tissues (10,11). Lectins, in particular C-type lectins (C-TLs)¹ were recently shown to be the major family of proteins secreted by tissue-stage larvae of the parasitic nematode of dogs *Toxocara canis* (14). *T. canis* larvae remain developmentally (but not metabolically) arrested in tissues of vertebrate hosts for many years without succumbing to immune responses, and it has been suggested that the major secreted antigens of this stage of the parasite, C-TLs and mucins (15,16), are aimed at immune evasion. In published (17,18) and unpublished work (A. Loukas), we have found that C-TLs are an abundant family of secreted molecules in hookworms, other strongylid, and ascarid nematodes. Some of these nematode lectins such as TES-32 and TES-70 from *T. canis* are more similar in sequence and predicted tertiary structure to host lectins involved in immune responses than they are to *Caenorhabditis elegans* lectins, implying roles in a parasitic existence. Indeed, a recent analysis of pseudogenes in *C. elegans* revealed that the most common pseudofold (determined by the presence of disablements such as insertions/deletions, frame shifts, and truncations) was that of C-TLs (19). Therefore, given their apparent demise in free-living nematodes and their relative abundance in some parasitic species (as defined by EST frequencies [17,18,20]), it appears that C-TLs might be a requisite for a parasitic existence in tissue- and gut-dwelling nematodes at least.

¹C-type lectins (C-TLs) are proteins with a carbohydrate recognition domain (CRD) that bind to glycoprotein ligands in a calcium-dependent manner. They are pivotal in a multitude of physiological processes in animals, including innate and acquired immunity (12) and venom-induced hemostasis (13).

Immune Defense

The secretion of anti-oxidants (glutathione-S-transferase [GST] [21,22] and superoxide dismutase [SOD] [23,24]) to potentially inhibit lipid peroxidation of parasite membranes, combined with an ability of parasite secretions to selectively induce apoptosis in activated T cells, and the secretion of proteinases, which digest the eosinophilic chemokine eotaxin (25) and IgA (26), have been proposed to establish a site of immune privilege around hookworms, which may explain the longevity of infection in humans in the presence of a highly activated immune system. Furthermore, a secreted calreticulin has been shown to interfere with complement activation and, potentially, integrin-mediated cell activation (27). Calreticulin also possesses a biologically active lectin domain (see previous section), and has been implicated as an antihemostatic molecule (28), suggesting the presence of multifunctional molecules in nematode parasites.

Therefore, we can conclude at this stage that *N. americanus* is adequately adapted to life in humans, in terms of the production of a molecular array designed to promote the passage of worms through the tissues to sites of ultimate feeding and reproduction, and to establish sites of immune privilege. Another result of the presentation of this molecular array to the host immune system is the generation of the allergic or T helper 2 (Th2) phenotype. This is reflected by the appearance of IgE (29), eosinophilia (30), high levels of soluble CD23 (31), interleukin-4 (IL-4) secretion by activated T cells (32), and mast-cell sensitization (author's unpublished observations). The aims of the next sections of this article will be to link the parasite molecular array described earlier with the establishment of the allergic phenotype, suggesting a combined role for lectins, proteinases, and pro-apoptotic factors in the generation of this phenotype. First, evidence will be presented to support the appearance of this phenotype in necatoriasis.

Immune Responses to Helminth Parasites

Most helminth parasites induce a Th2 host immune response at some stages of their life cycles, and are seemingly potent adjuvants for the development of polyclonal IgE responses to bystander antigens, which are associated with local and systemic eosinophilia and basophilia. There are obvious immunological parallels to atopy and asthma, suggesting an etiological link between the molecular stimulation and control of allergy and helminth infections, but the precise nature of this link still remains elusive. There is, however, an important difference in terms of sensitization and control: helminth parasites appear to be "obligate" inducers of the allergic phenotype, because they will effectively raise a Th2-biased immune response in most infected hosts (although the intensity may vary [30,33]), whereas only a subpopulation of people exposed to the same common allergens will become sensitized and display an allergic phenotype.

This may suggest that there are subtle differences in the pathways of induction of an allergic response or, alternatively, indicate that helminths are particularly powerful inducers of allergy, over-riding intrinsic control mechanisms. This makes them an interesting study object not only for the immunoparasitologist, but also for the allergist. Paradoxically, studies are appearing where allergenic parasites downregulate atopic and allergic responses to environmental aerosol allergens (34–38).

Why and How Are Helminth Parasites So Efficient at Inducing an Allergic Phenotype in Their Host?

We are still far from understanding the intricate network of interactions from the moment of infection to the establishment of the full-fledged allergic phenotype. Any approach that is likely to explain allergic sensitization will also have to take into consideration not only the products released by the

parasites (as well as other physical properties of the parasite, such as cuticular components), but also the specific context of the immunological environment encountered during the parasite's migration. In the following section, continuing to use the example of hookworm infection, we project a scenario of how the milieu generated by this parasite may interact with the local microenvironment, resulting in the induction of an allergic phenotype, which in turn may protect against subsequent development of allergy to environmental aerosol allergens.

The Allergenic Nature of Hookworm Infection

Human hookworms are at all stages of their life cycles in intimate contact with components of the immune system, in the skin, lungs, and gut, prime sites for allergic sensitization (39,40). The first contact with the host's immune system in the skin is likely to involve the activation of Langerhans cells, as shown for the infective skin stage of *S. mansoni* (41). This will induce priming against the parasite, unless, as in the case of *S. mansoni*, the parasite has evolved specific strategies interfering with leukocyte trafficking (41,42).

Successive reinfection such as trickle infection will boost the initial immune response. In a sensitized host, the invading larva will also encounter and activate effector cells such as eosinophils and mast cells sensitized with IgE. In *N. americanus* infection, invasion of the skin is accompanied by an urticarial reaction called "ground itch." This condition tends to be much more severe when hookworms infect a nonpermissive host (e.g., *Ancylostoma braziliense* in humans) and are not able to penetrate the basal layer ("creeping eruption"). This suggests that *N. americanus* in its natural host, despite being powerfully immunogenic (43–45), somehow manages to downregulate or elude the effector part of the immune response. Partial protection may be conferred to the skin stage by the antigenic sheath (46), or the deposition of collagen-binding molecules that may

leave a false antigenic trail behind the migrating worm (47,48). It is currently unknown whether skin-stage hookworms, by releasing proteases or other substances with pro-allergic properties, and interacting with, for example, skin mast cells or basophils recruited from peripheral blood, can skew the immune response to the Th2 phenotype already at this early stage. Other parasites such as *S. mansoni* and *T. spiralis* are known to induce a Th1-like response at this stage (49,50).

Despite a varying degree of attrition that is difficult to quantify, some hookworm larvae will gain entry to lymphatic or blood vessels and through the heart, finally reaching the lungs. In experimental infections, entry of *Necator* larvae into the lung triggers a rapid influx of eosinophils (51). This situation is akin to eosinophilic pulmonary syndromes associated with the lung stage of other helminth parasites, such as infection with *Ascaris lumbricoides* (Loeffler's syndrome) and *Brugia malayi* (tropical pulmonary eosinophilia [TPE] [52]) and resembles the pathophysiological features of chronic asthma (53). Previous immunization through exposure to irradiated larvae or multiple infections resulted in a more pronounced, faster eosinophil recruitment, higher IL-5 and IgE levels in the serum, and marked lung pathology. In this experimental setting, the allergic phenotype established during repeated infection is clearly host-protective, because none of the immunized animals progressed to patency (51). These results also point out the lung as the main site of parasite attrition, as described in lung stages of other helminth parasites such as *S. mansoni* (54). In the natural human host, however, evidence suggests that parasites have evolved strategies targeted at downregulating and modulating potentially lethal arms of the immune response, thus displaying anti-allergic, anti-inflammatory properties. An example of such properties is the selective cleavage and inactivation of the chemokine eotaxin by adult *N. americanus* metalloproteinases (25).

Indeed, this type of molecular protection may account for the apparent absence of distinct pathology seen in the gut of people infected with *N. americanus* (40), compared with zoonotic infections with canine hookworms, where the type 2 phenotype is seemingly uncontrolled, manifesting itself as eosinophilic gastroenteritis in a number of patients (55). In contrast, the classical symptoms of eosinophilic gastroenteritis are clearly not seen in communities with significant levels of human hookworm infection.

Therefore it is apparent that necatoriasis does result in the expression of the Th2 phenotype and indeed there is evidence that Th2 responses to hookworm infection are host-protective, because anti-hookworm IL-5 responses are negatively correlated with worm burdens after reinfection (32), and total and specific IgE are negatively correlated with hookworm weight and fecundity (30). The "allergenic" nature of hookworm infection is also demonstrated by cytokine responses to hookworm antigens in infected individuals. The majority of infected individuals produce IL-4 and IL-5 in response to stimulation of peripheral blood mononuclear cells (PBMC) with adult *N. americanus* ES products (32), and both IL-5 and IL-10 are produced in response to adult homogenate (56). However, in this in vitro analysis, cytokine responses are better described as mixed Th1/Th2 than Th2, because significant amounts of interferon- γ (IFN- γ) and IL-12 are also produced. This mixed response is typical of cytokine responses to helminth infections, including *Trichuris trichiura* (57), *Schistosoma*, and filarial infections (58), though responses to *Ascaris lumbricoides* have been reported to be more Th2-biased (59). Mixed Th1/Th2 responses to aeroallergens are also typically seen in asthmatics (60).

It may be of immunological significance that heavy parasite burdens are associated with downregulated cellular responses, which increase again after successful chemotherapy. High *N. americanus* burden was associated with lowered IFN- γ responses (32), whereas both

Th1 and Th2 responses are downregulated in chronic schistosomal and filarial infection (58). The mechanisms underlying downregulation have not been investigated in *N. americanus* infection, but evidence from schistosomal and filarial infections suggests that IL-10 and transforming growth factor- β (TGF- β) are important mediators of hyporesponsiveness (58,61). Therefore, it can be concluded in this section that, although a Th2 phenotype is highly evident in necatoriasis, in vitro experiments would indicate that the driving force is not over-riding an ability to recruit the Th1 phenotype, which is of considerable importance to immunity to other pathogenic organisms. Nevertheless, the induction of the Th2 phenotype by nematode infections is unequivocal, and in the following section we will discuss the molecular forces we believe to be driving the event.

The Molecular Basis for the Generation of the Superallergenic Milieu in Necatoriasis

Given our knowledge of the parasite molecular array presented to the host in necatoriasis, and the response elicited, the potential contribution of life-style molecules to the generation of the immunological phenotype is discussed here. In this section, we use the term "superallergen"² to highlight the innate nature of the pro-allergic parasitic molecular array. The pathways described in this section are summarized in Fig. 2.

The Contribution of Proteases

As described in the initial section of this review, *N. americanus* secretes numerous pro-

²The term "superallergen" was used by Patella et al. (62) in describing molecules that, like allergens or anti-IgE antibodies, can cross-link Fc ϵ R1-bound IgE and trigger degranulation of mast cells and basophils. Unlike allergens, however, superallergens mediate this effect in a nonantigen-specific manner, e.g., by binding to the VH3 regions of receptor-bound IgE. Examples of superallergens are the endogenous protein Fv (62), the HIV glycoprotein gp120 (63), and *Peptostreptococcus magnus* protein L (64).

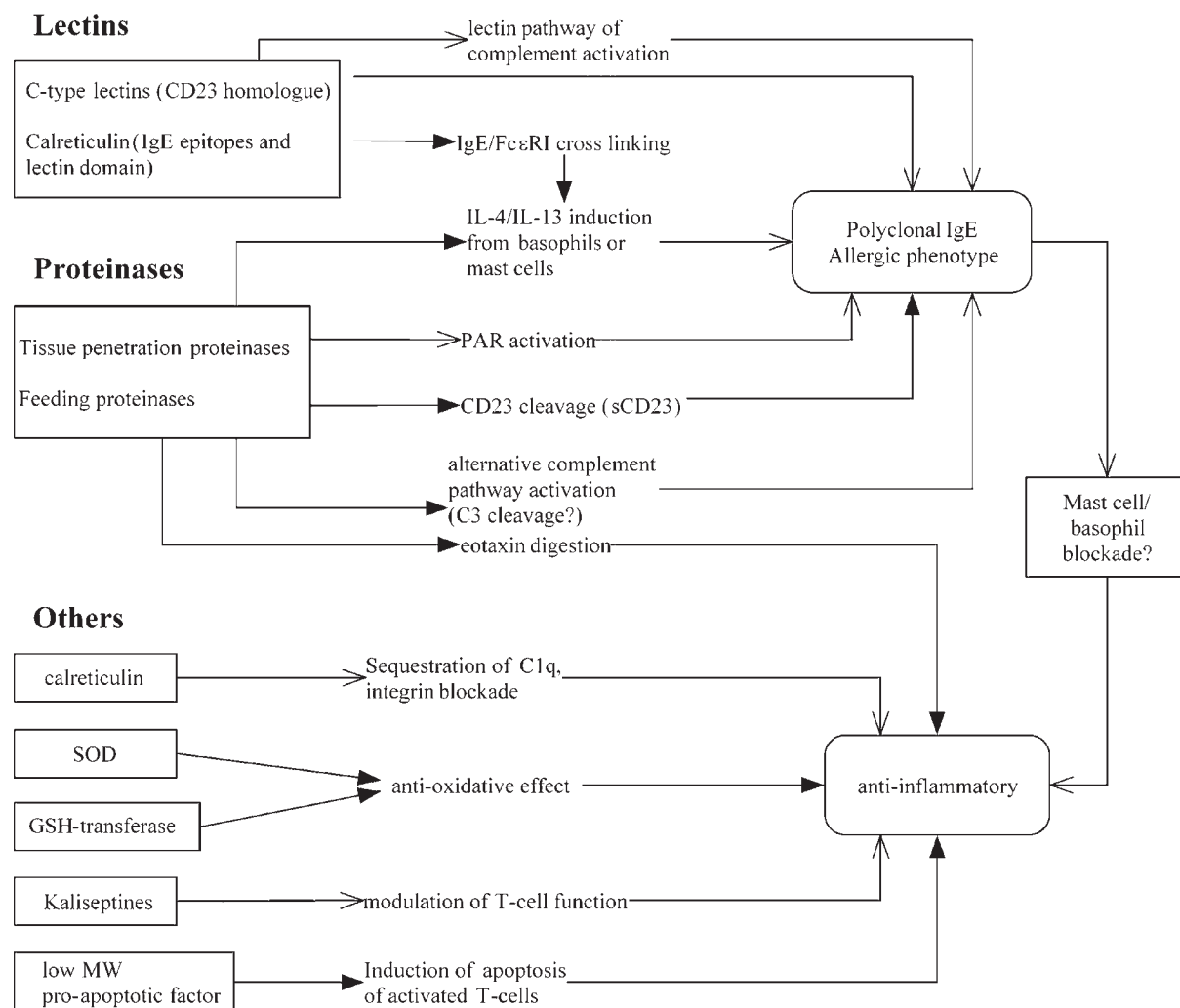


Fig. 2. The "superallergenic" (see Footnote 2) milieu generated by hookworms. The biology of hookworm infection (tissue migration, skin and lung stages, hematophagous gut life stage) entails the generation of a superallergenic environment, rich in proteinases and lectins, which arguably results in the induction of a protective allergic phenotype. However, the parasite has also evolved defense mechanisms that control potentially lethal effector pathways, such as the induction of apoptosis of activated T cells by parasitic kaliseptines, the inactivation of host eotaxin by a metalloproteinase, or the sequestration of C1q by hookworm calreticulin. Hypothetical pathways still in need of experimental backing are indicated by open arrows.

teases with nutritional or other functions. Many allergens, including *Der p1* from house dust mites (65) and *Fel d1* from domestic cats (66), are also proteinases, and although proteolytic activity is not a *conditio sine qua non* for allergenicity, deletion of proteolytic activity abolishes allergenicity, as shown with papain as a proof of principle (67). Also, a recent paper by Kheradmand and colleagues highlights the potency of exogenous proteinases as allergens

even in the absence of adjuvants by bypassing the normal tolerogenic mechanisms of the lung (68). This work also implies that proteinases act as powerful allergenic adjuvants. An important aspect in this scenario may be the disruption of epithelial tight junctions by proteinases, resulting in better access of allergens to antigen-presenting and other immunologically relevant cells (69,70). Evidence also exists for the cleavage of the allergy regulatory low-affinity

IgE receptor by *N. americanus* secretions (unpublished data), supporting our early work on CD23 cleavage by *Der p1* (65), a process that perhaps contributes to the high levels of sCD23 observed in the plasma of hookworm patients (31). Furthermore, polyclonal antisera to human CD23 cross-react with products in *N. americanus* secretions (unpublished data), raising the possibility that pro-allergic sCD23 homologs exist in hookworm secretions. Indeed, some homology exists between CD23 and lectins identified in or on *N. americanus*.

It is therefore reasonable to assume that the proteolytic secretions of parasites also may have similar pro-allergic effects, and by compromising the integrity of the respiratory epithelium, gain access to other immunologically important compartments in the lung. This may in turn result in the activation of specific, pro-allergic pathways of innate immunity. This is supported by our recent data showing that *Der p1* and the proteinases in *N. americanus* secretions induce a Th2 cytokine profile (IL-4, IL-5, and IL-13) in the KU812 human basophilic cell line and the production of IL-4 from purified human peripheral-blood basophils (71), thus generating an environment that is conducive to an allergic phenotype. A similar response may be triggered in resident mast cells, resulting not only in the release of IL-4 and IL-13, but also mast cell-derived proteinases such as tryptase and chymase, TNF- α , histamine, and the *de novo* synthesis of leukotrienes C4 and B4 as well as prostaglandin D2 (72), although this effect of hookworm proteases remains hypothetical. Recent data by Asokanathan et al. (73) show an involvement of protease-activated receptor (PAR)-2 in *Der p1*-induced inflammatory cytokine release from respiratory epithelial cells. Because mast cells derived from human lung and other tissues are known to express PAR-1 and PAR-2 on their surface (74), it may well be that PAR activation of mast cells by parasitic proteinases, with the resulting release of endogenous pro-inflammatory and pro-allergic mediators and cytokines, ini-

tiates a cascade of events contributing to the establishment of an allergic phenotype.

Drouin et al. suggest an important role for a C3a/C3aR-mediated pathway for the development of an allergic phenotype in a murine model of pulmonary allergy (75). In murine schistosomiasis, C3 deficiency is associated with an impaired ability to mount a Th2-like response to the parasite (76). Thus, generation of C3a by proteolytic cleavage of the precursor C3 by parasitic proteinases may be an alternative route of Th2 activation in helminth infection certainly worth studying. There is evidence coming from our lab (77) and other labs (78) showing that nematode cuticles convert (or at least attract) C3b, lending further support to older literature showing that nematode cuticles can activate the alternative pathway (79–82). We are investigating the role of the C1q binding and inhibitory secretion of calreticulin in modifying this process.

The Contribution of Lectins

Because IgE is a heavily glycosylated immunoglobulin with a carbohydrate content of 11.7% (83), lectins specific for the carbohydrate side-chains could also cross-link membrane-bound IgE on basophils and mast cells, thus inducing IL-4 and IL-13 release in an antigen-independent manner. In fact this has been shown for plant lectins such as concanavalin A (Con A), lentil lectin, phytohemagglutinin, *Pisum sativum*, and *Sambucus nigra* agglutinin (84). IgE binding has been demonstrated for animal lectins, such as Galectin-3, which had originally been described as IgE-binding protein ϵ BP (85). In particular, tandem-type galectins with two separate carbohydrate recognition domains and a specific affinity for β -galactoside sugar structures are interesting candidates as IgE cross-linkers and Th2-cytokine inducers. It is therefore interesting to note that such tandem-repeat type galectins have been cloned from intestinal parasites such as *Teladorsagia circumcincta* (Tci-gal-1 and Tci-gal-2) (86), *Haemonchus contortus* (Hco-gal-1),

and *Trichostrongylus colubriformis* (Tco-gal-2) (87,88), as well as filarial worms (OvGalBP) (89). Indeed OvGalBP, a galectin from *Onchocerca volvulus*, was found to bind to IgE but not other immunoglobulins, in a lactose-inhibitable manner, and thus might potentially cross-link cell surface-bound IgE; this filarial lectin, however, did not bind to sera obtained from noninfected individuals (89). Nematode lectins that bind IgE might directly induce basophil or mast cell-derived Th2 cytokines (as well as histamine and other active mediators) in non-immune hosts, thus skewing the early immune response to the allergic phenotype. It is interesting to note in this context that histamine is known to stimulate feeding in helminths (90).

Helminth Superallergens?

A parasite molecule that could fit the description of a superallergen (see Footnote 2) is an extracellular calreticulin. This was discovered in *N. americanus* following the screening of cDNA libraries with IgE from infected humans, and has subsequently been shown in the secretions/saliva of *N. americanus*, *Meloidogyne incognita* (nematodes), fleas, and ticks (27,91–93). Calreticulin is a multifunctional molecule, with anticomplementary and integrin-binding activity. It is also implicated in the clearance of apoptotic cells via CD91 (94), which may be relevant to the antiinflammatory strategy of *N. americanus*, the secretions of which induce apoptosis in activated human T cells (95). However, for the purpose of this review, it is the lectin domains of hookworm calreticulin that attract interest, given their specificity for sugars expressed by the human IgE molecule. This potentially confers on calreticulin the ability to cross-link IgE allergenically, and via the lectin domains. Given that calreticulin is monovalent in its native form, the ability of calreticulin to readily complex with human proteins such as C1q and MBL would confer the multivalency required for mast-cell and basophil activation via engagement of IgE Fab and Fc regions. It is of rel-

evance that the C1q binding domains of calreticulin do not fall into the highly antigenic domains of the molecular sequence, albeit following PEPSCAN on linear sequences (96); this may allow calreticulin to bind C1q despite being targeted by the immune system.

Additional Potential Molecular Contributors

Very recently, another parasitic factor inducing production and release of Th2-promoting cytokines (IL-4 and IL-13) from purified human basophils in vitro has been cloned from an *S. mansoni* egg-stage cDNA library (97) and expressed as a fusion protein. IPSE is an IgE-binding factor that strongly activates basophils of nonimmune donors, probably by cross-linking membrane-bound IgE in an antigen-independent manner. As such, IPSE may be the first parasite-derived representative of a new category of molecules, which, in analogy to superantigens and their effects on T-cell activation, have been termed “superallergens” (see Footnote 2) (98). As such, IPSE may be a further candidate for a parasite-derived representative of the suggested category of superallergens (see Footnote 2) (98).

Summary: The Parasite Paradox

Parasites are potent inducers of a pro-allergic immune response, and parasite products such as proteinases and lectins, which fulfill nutritional or other tasks of central importance, could mediate this effect. Our general view is that the helminth parasite, by generating a (superallergenic) milieu that is rich in proteases, lectins, and other biologically active molecules, is bound to interact with cells of the innate immune system such as mast cells and basophils as described, resulting in a Th2 promoting cytokine milieu and the establishment of an allergic phenotype. Specific mechanisms of immune evasion displayed by the different life stages may guarantee a survival of the parasite, but also reduced pathology in the

host. In the case of human necatoriasis—and this may be applicable to other parasitic infections—this overall phenotype will generate an environment that will protect the host, but also preserve the integrity of the parasite (30).

The downstream effect of this molecular balance between parasite and host is down-regulation of allergic manifestations to aerosol allergens, such as those generated by dust mites (35,36). This then calls into question the true nature of parasitism with hookworms, if low-intensity infections can be tolerated and provide some medical benefit to the host (40). It also illuminates the paradoxical nature of this relationship, where an allergenic parasite protects the host against the manifestations of respiratory wheeze to dust mites in the absence of an effect on sensitization to environmental allergen (indicated by the retention of skin-test positivity to dust mites [35]). This leads to a number of interpretations, the best-favored at present being an organ-specific suppression of the allergic phenotype in the lung. The molecular basis for this immune modulation is under active investigation.

Acknowledgments

The authors sincerely thank Helmut Haas for revision and helpful discussion of the manuscript.

References

- Williamson, A.L., Brindley, P.J., Abbenante, G., et al. (2002), *FASEB J.* **16**, 1458–1460.
- Williamson, A.L., Brindley, P.J., Abbenante, G., et al. (2003), *J. Infect. Dis.* **187**, 484–494.
- Hawdon, J.M., Volk, S.W., Rose, R., et al. (1993), *Parasitology* **106**(Pt 2), 163–169.
- Pritchard, D.I., Leggett, K.V., Rogan, M.T., et al. (1991), *Parasite Immunol.* **13**, 187–199.
- Girdwood, K. (1999), Aspartic proteinases and aspartic proteinase inhibitors from parasitic nematode, PhD thesis. University of Wales, Cardiff.
- Hawdon, J.M., Volk, S.W., Pritchard, D.I., and Schad, G.A. (1992), *J. Parasitol.* **78**, 1036–1040.
- Brown, A., Girod, N., Billett, E.E., and Pritchard, D.I. (1999), *Am. J. Trop. Med. Hyg.* **60**, 840–847.
- Williamson, A.L., Brindley, P.J., and Loukas, A. (2003), *Parasitology* **126**, 179–185.
- Furmidge, B.A., Horn, L.A., and Pritchard, D.I. (1996), *Parasitology* **112**(Pt 1), 81–87.
- Vestweber, D. and Blanks, J.E. (1999), *Physiol. Rev.* **79**, 181–213.
- McEver, R.P. (2002), *Curr. Opin. Cell Biol.* **14**, 581–586.
- Weis, W.I., Taylor, M.E., and Drickamer, K. (1998), *Immunol. Rev.* **163**, 19–34.
- Andrews, R.K. and Berndt, M.C. (2000), *Toxicon* **38**, 775–791.
- Loukas, A. and Maizels, R.M. (2000), *Parasitol. Today* **16**, 333–339.
- Loukas, A., Mullin, N.P., Tetteh, K.K., et al. (1999), *Curr. Biol.* **9**, 825–828.
- Loukas, A., Hintz, M., Linder, D., et al. (2000), *J. Biol. Chem.* **275**, 39,600–39,607.
- Loukas, A., Brown, A.P., and Pritchard, D.I. (2002), *DNA Seq.* **13**, 61–65.
- Jasmer, D.P., Roth, J., and Myler, P.J. (2001), *Mol. Biochem. Parasitol.* **116**, 159–169.
- Harrison, P.M., Echols, N., and Gerstein, M.B. (2001), *Nucleic Acids Res.* **29**, 818–830.
- Daub, J., Loukas, A., Pritchard, D.I., and Blaxter, M. (2000), *Parasitology* **120**(Pt 2), 171–184.
- Brophy, P.M., and Pritchard, D.I. (1994), *Exp. Parasitol.* **79**, 89–96.
- Brophy, P.M., Patterson, L.H., Brown, A., and Pritchard, D.I. (1995), *Acta Trop.* **59**, 259–263.
- Taiwo, F.A., Brophy, P.M., Pritchard, D.I., et al. (1999), *Eur. J. Biochem.* **264**, 434–438.
- Brophy, P.M., Patterson, L.H., and Pritchard, D.I. (1995), *Int. J. Parasitol.* **25**, 865–866.
- Culley, F.J., Brown, A., Conroy, D.M., et al. (2000), *J. Immunol.* **165**, 6447–6453.
- Kumar, S. and Pritchard, D.I. (1992), *Int. J. Parasitol.* **22**, 563–572.
- Kasper, G., Brown, A., Eberl, M., et al. (2001), *Parasite Immunol.* **23**, 141–152.
- Gray, A.J., Park, P.W., Broekelmann, T.J., et al. (1995), *J. Biol. Chem.* **270**, 26,602–26,606.
- Pritchard, D.I. and Walsh, E.A. (1995), *Parasite Immunol.* **17**, 605–607.
- Pritchard, D.I., Quinnell, R.J., and Walsh, E.A. (1995), *Parasite Immunol.* **17**, 71–75.
- Pritchard, D.I., Kumar, S., and Edmonds, P. (1993), *Parasite Immunol.* **15**, 205–208.
- Quinnell, R.J., Pritchard, D.I., Raiko, I., Brown, A.P., Shaw, M-A. (2003), *J. Infect. Dis.*, submitted.
- Pritchard, D.I., Shakib, F., Walsh, E.A., and Smith, S.J. (1994), *J. Invest. Allergol. Clin. Immunol.* **4**, 238–241.
- Araujo, M.I., Lopes, A.A., Medeiros, M., et al. (2000), *Int. Arch. Allergy Immunol.* **123**, 145–148.
- Scrivener, S., Yemaneberhan, H., Zevenigus, M., et al. (2001), *Lancet* **358**, 1493–1499.
- van den Biggelaar, A.H., van Ree, R., Rodrigues, L.C., et al. (2000), *Lancet* **356**, 1723–1727.
- Yazdanbakhsh, M., van den Biggelaar, A., and Maizels, R.M. (2001), *Trends Immunol.* **22**, 372–377.

38. Selassie, F.G., Stevens, R.H., Cullinan, P., et al. (2000), *Clin. Exp. Allergy* **30**, 356–358.
39. Hotez, P.J., Hawdon, J.M., Cappello, M., et al. (1995), *Infect. Agents Dis.* **4**, 71–75.
40. Pritchard, D. I. and Brown, A. (2001), *Trends Parasitol.* **17**, 169–172.
41. Angeli, V., Faveeuw, C., Roye, O., et al. (2001), *J. Exp. Med.* **193**, 1135–1147.
42. Trottein, F., Nutten, S., Angeli, V., et al. (1999), *Eur. J. Immunol.* **29**, 3691–3701.
43. Brown, A. and Pritchard, D.I. (1993), *Parasite Immunol.* **15**, 195–203.
44. Pritchard, D.I., Brown, A., Kasper, G., et al. (1999), *Parasite Immunol.* **21**, 439–450.
45. Pritchard, D.I., McKean, P.G., and Rogan, M.T. (1988), *Mol. Biochem. Parasitol.* **28**, 275–283.
46. Kumar, S. and Pritchard, D.I. (1992), *Int. J. Parasitol.* **22**, 573–579.
47. Viaene, A., Crab, A., Meiring, M., et al. (2001), *J. Parasitol.* **87**, 619–625.
48. Pritchard, D.I., Quinell, R.J., Hotez, P.J., et al. (2002), in *The Geohelminths: Ascaris, Trichuris and Hookworm*. (Holland, C.V. and Kennedy, M.W., eds.) Kluwer Academic Publishers, Amsterdam, pp. 143–165.
49. Pearce, E.J., Caspar, P., Grzych, J.M., et al. (1991), *J. Exp. Med.* **173**, 159–166.
50. Ishikawa, N., Goyal, P.K., Mahida, Y.R., et al. (1998), *Immunology* **93**, 257–263.
51. Culley, F.J., Brown, A., Girod, N., et al. (2002), *Eur. J. Immunol.* **32**, 1376–1385.
52. O'Bryan, L., Pinkston, P., Kumaraswami, V., et al. (2003), *Infect. Immun.* **71**, 1337–1342.
53. Maddox, L. and Schwartz, D.A. (2002), *Annu. Rev. Med.* **53**, 477–498.
54. Wilson, R.A., Coulson, P.S., and Mountford, A.P. (1999), *Immunol. Lett.* **65**, 117–123.
55. Walker, N.I., Croese, J., Clouston, A.D., et al. (1995), *Am. J. Surg. Pathol.* **19**, 328–337.
56. Pit, D.S., Polderman, A.M., Schulz-Key, H., and Soboslay, P.T. (2000), *Allergy* **55**, 732–739.
57. Faulkner, H., Turner, J., Kamgno, J., et al. (2002), *J. Infect. Dis.* **185**, 665–672.
58. Yazdanbakhsh, M. (1999), *Immunol. Lett.* **65**, 109–115.
59. Cooper, P.J., Chico, M.E., Sandoval, C., et al. (2000), *J. Infect. Dis.* **182**, 1207–1213.
60. Smart, J.M. and Kemp, A.S. (2002), *Clin. Exp. Allergy* **32**, 796–802.
61. Doetze, A., Satoguina, J., Burchard, G., et al. (2000), *Int. Immunol.* **12**, 623–630.
62. Patella, V., Giuliano, A., Bouvet, J.P., and Marone, G. (1998), *J. Immunol.* **161**, 5647–5655.
63. Patella, V., Florio, G., Petraroli, A., and Marone, G. (2000), *J. Immunol.* **164**, 589–595.
64. Genovese, A., Borgia, G., Bjorck, L., et al. (2003), *J. Immunol.* **170**, 1854–1861.
65. Hewitt, C.R., Brown, A.P., Hart, B.J., and Pritchard, D.I. (1995), *J. Exp. Med.* **182**, 1537–1544.
66. Ring, P.C., Wan, H., Schou, C., et al. (2000), *Clin. Exp. Allergy* **30**, 1085–1096.
67. Chambers, L., Brown, A., Pritchard, D.I., et al. (1998), *Biochem. Biophys. Res. Commun.* **253**, 837–840.
68. Kheradmand, F., Kiss, A., Xu, J., et al. (2002), *J. Immunol.* **169**, 5904–5911.
69. Wan, H., Winton, H.L., Soeller, C., et al. (1999), *J. Clin. Invest.* **104**, 123–133.
70. Wan, H., Winton, H.L., Soeller, C., et al. (2001), *Clin. Exp. Allergy* **31**, 279–294.
71. Phillips, C., Coward, W.R., Pritchard, D.I., and Hewitt, C.R. (2003), *J. Leukoc. Biol.* **73**, 165–171.
72. Hart, P.H. (2001), *Immunol. Cell Biol.* **79**, 149–153.
73. Asokanathan, N., Graham, P.T., Stewart, D.J., et al. (2002), *J. Immunol.* **169**, 4572–4578.
74. D'Andrea, M.R., Rogahn, C.J., and Andrade-Gordon, P. (2000), *Biotech. Histochem.* **75**, 85–90.
75. Drouin, S.M., Corry, D.B., Hollman, T.J., et al. (2002), *J. Immunol.* **169**, 5926–5933.
76. La Flamme, A.C., MacDonald, A.S., Huxtable, C.R., et al. (2003), *J. Immunol.* **170**, 470–476.
77. Nowell, M.A., De Pomerai, D.I., and Pritchard, D.I. (1999), *Parasite Immunol.* **21**, 495–505.
78. Meri, T., Jokiranta, T.S., Hellwage, J., et al. (2002), *J. Infect. Dis.* **185**, 1786–1793.
79. Prowse, S.J., Ey, P.L., and Jenkin, C.R. (1979), *Aust. J. Exp. Biol. Med. Sci.* **57**, 459–466.
80. Yates, J.A., Higashi, G.I., Lowichik, A., et al. (1985), *Acta Trop.* **42**, 157–163.
81. Preston, C.M., Jenkins, T., and McLaren, D.J. (1986), *Parasite Immunol.* **8**, 597–611.
82. Hong, Y., Kim, C.W., and Ghebrehiwet, B. (1992), *Exp. Parasitol.* **74**, 290–299.
83. Baenziger, J., Kornfeld, S., and Kochwa, S. (1974), *J. Biol. Chem.* **249**, 1889–1896.
84. Haas, H., Falcone, F.H., Schramm, G., et al. (1999), *Eur. J. Immunol.* **29**, 918–927.
85. Liu, F.T., Albrandt, K., Mendel, E., et al. (1985), *Proc. Natl. Acad. Sci. USA* **82**, 4100–4104.
86. Newton, S.E., Monti, J.R., Greenhalgh, C.J., et al. (1997), *Mol. Biochem. Parasitol.* **86**, 143–153.
87. Greenhalgh, C.J., Loukas, A., Donald, D., et al. (2000), *Mol. Biochem. Parasitol.* **107**, 117–121.
88. Greenhalgh, C.J., Beckham, S.A., and Newton, S.E. (1999), *Mol. Biochem. Parasitol.* **98**, 285–289.
89. Klion, A.D. and Donelson, J.E. (1994), *Mol. Biochem. Parasitol.* **65**, 305–315.
90. Bone, L.W. and Bottjer, K.P. (1985), *J. Parasitol.* **71**, 235–238.
91. Jaubert, S., Ledger, T.N., Laffaire, J.B., et al. (2002), *Mol. Biochem. Parasitol.* **121**, 205–211.
92. Jaworski, D.C., Higgins, J.A., Radulovic, S., et al. (1996), *J. Med. Entomol.* **33**, 482–489.
93. Ferreira, C.A., Da Silva Vaz, I., da Silva, S.S., et al. (2002), *Exp. Parasitol.* **101**, 25–34.
94. Ogden, C.A., deCathelineau, A., Hoffmann, P.R., et al. (2001), *J. Exp. Med.* **194**, 781–795.

95. Chow, S.C., Brown, A., and Pritchard, D. (2000), *Parasite Immunol.* **22**, 21–29.
96. Pritchard, D.I., Girod, N., Brown, A., et al. (2003), in *Calreticulin*, 2nd ed., Landes Bioscience, Georgetown, TX, p. 300.
97. Schramm, G., Falcone, F.H., Gronow, A., et al. (2003), *J. Biol. Chem.* **278**, 18,384–18,392.
98. Snow, R.E., Chapman, C.J., Holgate, S.T., and Stevenson, F.K. (1996), *Hum. Antibodies Hybridomas* **7**, 157–166.