The relationship between immunological responsiveness controlled by T-helper 2 lymphocytes and infections with parasitic helminths

D. I. PRITCHARD[1]* C. HEWITT[1] *and* R. MOQBEL[2]

[1] *Centre for the Mechanisms of Human Toxicity, The Hodgkin Building, Lancaster Road, Leicester LE1 9HN, UK*
[2] *Pulmonary Research Group, University of Alberta, Edmonton, T6G 2S2, Canada.*

SUMMARY

It should have been difficult until relatively recently for immunologists to ascribe a sound biological reason for the continued possession of the allergic phenotype in human populations. Nevertheless, for the past 20 years or so textbooks of immunology have routinely exhibited fanciful and perhaps exaggerated diagrams as to how IgE and eosinophils killed all helminth parasites. These diagrams were largely based on perhaps selective *in vitro* observations, and it is only now that immunoparasitologists, working on human populations under arduous conditions in the field, are able to provide data to corroborate these findings, and perhaps ascribe a useful purpose for a generally pathological immune response termed Type I *hypersensitivity*. The present paper reviews much of this recent literature, and asks a number of pertinent questions relating to the relationship between what we now know to be T-helper 2 lymphocyte-driven immunological responsiveness and infections with parasitic helminths.

Key words: T-helper 2 lymphocyte, helminth infection, IgE, eosinophil, immunity, allergy, allergen, cytokine.

INTRODUCTION

For clinical reasons, and for the sake of simplifying a complex picture, the mammalian immune response was categorized decades ago into 4 types of 'hypersensitivity' (Coombs & Gell, 1963). The types of hypersensitivity (there are now 5) described the molecular and cellular components of immune responses which led in many cases to the clinical manifestation of disease, in which the immune response was over-reactive or hyper-sensitive, leading to tissue damage. The immune response which was categorized as a type 1 immediate hypersensitivity is characterized by the production of immunoglobulin ϵ or IgE following exposure to allergens, and the sensitization of mast cells or basophils for the release of vasoactive mediators on subsequent exposure to the allergenic insult. Later, T cells were shown to be pivotal in the control of IgE synthesis, and sub-sets called Th1 and Th2 counter regulate each other through the secretion of cytokines which either switch on (Th-2, IL4)† or switch off (Th1, IFN-γ) B cell IgE synthesis. B cells also require a second signal to differentiate into IgE-secreting plasma cells, and this signal is classically provided by the T cell, but can also be provided by cells of the mast cell/basophil lineage, which also produce IL4 and IL13 (Ochensberger *et al*. 1996) (other sources of 'early' IL4 apparently include $CD4^+$ NK $1{\cdot}1^+$ cells, utilizing CD1 in the antigen recognition process), although there are also data to suggest that $NK1{\cdot}1^+$T cells are *not* involved in initiating Th2 responses (Brown *et al*. 1996).

This second signal is triggered by an interaction between the CD40 ligand, previously called gp (glycoprotein) 39, on the cooperative cell, and CD40 on the B cell. Once secreted, IgE attaches to the mast cell and basophil surface by interacting with its high-affinity receptor, FcϵRI. Cross-linkage of adjacent IgE molecules by allergen triggers mediator release. More recently a low-affinity receptor for IgE, called FcϵRII or CD23, was described and localized on a range of leucocyte types, including lymphocytes and eosinophils. It is through high and low affinity receptors that IgE mediates its biological and immunological activity, leading to the manifestation of allergic disease or, as is now believed, interference with the physiological activity of parasitic helminths. This latter point will be developed in the present article.

The allergic phenotype is considered to be of little benefit to humans in 'developed' countries; indeed, anti-allergic therapies targeted at the pivotal Th2 lymphocyte are justified on this basis. However, some believe that this type of immune response remains beneficial to a large proportion of the global population, people living in areas of high parasite endemicity. This statement is based on several reports in the literature which indicate that individuals best equipped to mount Th2-biased responses

* Corresponding author, on leave from Dept. of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD.

† There is recent evidence, in mice, that class switching to IgE can be initiated, by retroviral infection, using IL-4 independent pathways (Morawetz *et al*. 1996). Helminths apparently do *not* use this pathway.

https://doi.org/10.1017/S0031182097001996 Published online by Cambridge University Press

to infection are offered a degree of protection against helminths. In this context, allergy could be regarded as an evolutionary 'hangover from parasitism', and that those demonstrating a predisposition toward Type 1 hypersensitivity in relatively parasite-free societies (notwithstanding *Enterobius vermicularis*) would fare better than their non-hypersensitive neighbours if returned permanently to areas of high parasite endemicity. This would indeed be an interesting experiment to perform! However, in the absence of data from experiments of this sort, one is left to assess the current literature relating to atopy and parasitism, to decide whether allergic responses can be regarded as beneficial under certain circumstances. Indeed, the data supporting this concept are far stronger than those in favour of a parasite-protective role for Th2-driven immune responses. Thus a review based on the latter subject remains untenable in the continued presence of such sparse literature on the subject. However, in the context of host-protective Th2 responses, a number of questions can be asked, given the relative richness of the literature. (1) Do Th2 driven immune responses protect the host against parasitic helminths? (Alternatively do they protect the parasite?) (2) If Th2 responses protect the host, how are parasites damaged by these immune responses? (3) Can Th2 responses protect against tissue damage? (4) Why are parasitic helminths so allergenic? (5) How can study design be advanced/optimized to provide definitive answers?

IgE AND HUMAN HELMINTHIASES: EVIDENCE THAT TH2-DRIVEN RESPONSES ARE PROTECTIVE

The strongest evidence that allergic responses may be beneficial in areas of parasite endemicity comes from field studies of human schistosomiasis and necatoriasis (Hagan *et al*. 1991; Rihet *et al*. 1991; Dunne *et al*. 1992; Hagan, 1993; Pritchard, Quinnell & Walsh, 1995). These studies compellingly demonstrated a link between the ability to exhibit a Th2-biased response and resistance to infection or reduced parasite fitness and fecundity. For example, Hagan's studies of *S. haematobium* infection in The Gambia indicated that individuals with high levels of specific IgE against the parasite were less likely to become reinfected after successful chemotherapeutic intervention. This was an age-related phenomenon, with IgE levels and increased resistance to reinfection both associated with increasing age under conditions of equal exposure. Rihet's study of *S. mansoni* infections in Brazil, and Dunne's later study, support the link between high IgE responses and resistance to reinfection with schistosomes. Furthermore, undifferentiated Th0 cells derived from individuals with a resistant phenotype exhibited a high ratio of IL4 to IFN-γ production. This bias may be maintained by IL10, a Th1-blocking cytokine which has been implicated in the modulation of T cell responsiveness in patients infected with *S. mansoni* (Araujo *et al*. 1996).

Additional supportive data come from a study of necatoriasis in Papua New Guinea, where significant correlations were seen between the Th2-mediated phenomena of high IgE levels and eosinophilia and reduced parasite weight and fecundity (Pritchard *et al*. 1995). Although other isotypes were shown to have anti-parasitic effects in this study (Quinnell *et al*. 1995), evidence is mounting to support the hypothesis that type-1 hypersensitivity is useful when it manifests itself in the context of parasitism. Intriguingly, there are also data which indicate that schistosomes may interfere with the activity of antigen-reactive IL4-producing T cells (Grogan *et al*. 1996), reinforcing the belief that this response is host-protective, and demonstrating that the co-evolution of host–parasite relationships continues. However, one should not ignore the fact that Th1 responses seem to be successful in controlling the invasive larval stages of some human parasites (Pritchard & Wilson, 1997). It is therefore likely that Th1 and Th2 responses act together in possibly a stage-specific manner and perhaps at different anatomical locations to control human parasitic infection.

IgE responses also seem to be more reliable and specific indicators of current infection status with hookworms (Ganguly *et al*. 1988; Pritchard & Walsh, 1995) and filarial parasites (Weiss, Hussain & Ottesen, 1982; Cabrera, Cooper & Parkhouse, 1986), indicating the additional value of this type of immune response to human medicine.

What is the biological significance of the polyclonal IgE response so often seen following helminth infection?

Some consider polyclonal IgE responses, induced by helminth parasites, to be parasite-protective (reviewed by Pritchard, 1993*a*). The concept that polyclonal IgE protects the parasite from potentially damaging specific IgE by saturating Fcϵ receptors with 'irrelevant' antibody is difficult to support experimentally (Moqbel & Pritchard, 1990), and explains the apparent bias of the present article, which is largely supportive of a host-protective role for Th2, and IgE responses. In fact, the existence of an excess of blocking polyclonal IgE could explain why adverse allergic reactions are rare following the chemotherapeutic treatment of systemic helminths such as schistosomes. In this instance, a polyclonal response would have to be seen as host-protective, preventing possible lethal episodes of systemic anaphylaxis following drug/immunologically-induced parasite destruction and concomitant allergen release.

Therefore, the true biological significance of polyclonal IgE is difficult to evaluate given the evidence available, and it can be seen as parasite- or host-protective, or both, depending on your viewpoint.

IF TH2-DRIVEN RESPONSES ARE HOST-PROTECTIVE, HOW IS THE PARASITE DAMAGED?

Mechanisms of damage in vitro

Studies in this area have largely concentrated on the role of eosinophils and antibodies (particularly IgE) in cytotoxicity reactions to larval stages. Initial *in vitro* experiments using the antibody-dependent cellular cytotoxicity (ADCC) reaction demonstrated that human peripheral blood granulocytes (particularly eosinophils) from patients infected with *Schistosoma mansoni* were able to adhere to and kill larvae of this trematode (Butterworth, 1984; Butterworth & Richardson, 1985; Butterworth & Thorne, 1993). These observations were confirmed using rat and human eosinophils against schistosome larvae (McLaren *et al*. 1977, 1978, 1981, 1984; Ramalho-Pinto, McLaren & Smithers, 1978). Similar antibody-dependent, eosinophil-mediated *in vitro* cytotoxicity was reported for a number of other helminth parasites. The targets employed in these systems included the eggs of *S. mansoni* using murine eosinophils (James & Colley 1976; Hsu *et al*. 1980; Feldman, Dannenberg & Seed, 1990), invading larvae of *Fasciola hepatica* (Duffus & Franks, 1980) and *M. corti* (Cook, Ashworth & Chernin, 1988). Newborn larvae of *T. spiralis*, nematode larvae that, although less susceptible because of their thick collagen-rich cuticle, were also killed following adherence to eosinophils (Kazura & Aikawa, 1980). These studies were extended by others to demonstrate eosinophil cytotoxicity *in vitro* against microfilariae of *Onchocerca volvulus* (Greene, Taylor & Aikawa, 1981; Mackenzie *et al*. 1981; Williams *et al*. 1987), larval stages of *Dictyocaulus viviparus* (Butterworth & Thorne, 1993), *Toxocara canis* (Fattah *et al*. 1986; Badley *et al*. 1987; Lombardi *et al*. 1990), *Brugia malayi* (Sim, Kwa & Mak, 1982; Chandrashekar *et al*. 1986) and *N. americanus* (Desakorn *et al*. 1987).

Eosinophils adhere readily and firmly to opsonized worms or larvae and release their granule contents which can be detected by electron microscopy as thick layers of electron-dense deposits on the surface of the organism (Mclaren *et al*. 1977, 1978, 1981; Glauert *et al*. 1978; Caulfield, 1980; Caulfield *et al*. 1980). Such deposition results in damage to the parasite in the vicinity of contact with the attached eosinophil. Damage was associated with the appearance of vacuoles in the syncytial tegument of the larvae and was followed by detachment of the tegumental membrane leading to the exposure of the underlying muscle layers. It has been suggested that tegumental membrane detachment may be mediated in part by worm-derived lysophospholipids (Golan *et al*. 1986; Furlong & Caulfield, 1989; Caulfield & Chiang, 1990) and by released eosinophil granule proteins including EPO, MBP and ECP. These products have the capacity to damage schistosomula directly when incubated as isolated proteins with parasitic larvae at very low molar concentrations. MBP and ECP both produced ballooning in the tegument in a similar pattern to that observed with whole eosinophils (Butterworth *et al*. 1979*a*, *b*; McLaren *et al*. 1984). ECP was 10 times more active on a molar basis than MBP. However, because MBP is present in the granule in larger amounts, it may account for a higher proportion of the toxicity observed (Ackerman *et al*. 1985). That eosinophil cationic proteins induce damage to schistosomula of *S. mansoni* through their basic charge was confirmed by use of a variety of synthetic polycations (Butterworth *et al*. 1979*b*; Jones, Helms & Kusel, 1988) and its inhibition by polyanions such as heparin (Young *et al*. 1986). Purified eosinophil cationic proteins were also toxic *in vitro* for the newborn larvae of *T. spiralis* (Wassom & Gleich, 1979; Hamann *et al*. 1987), the eggs of *S. mansoni* (Sher *et al*. 1980; Kephart, Andrade & Gleich, 1988) and larvae of both *B. malayi* and *B. Pahangi* (Hamann *et al*. 1990). These cytotoxic effects may, therefore, be due primarily to the intensely basic nature of these proteins rather than to other properties such as the ribonuclease activity of ECP (Barker *et al*. 1989). This is particularly relevant, since worm tegument and cuticle were shown to be strongly polyanionic (Pritchard *et al*. 1985). Recent evidence suggests that only 1 isoform of ECP, out of a total of 7, possesses any cytotoxic activity (P. Venge, personal communication). In contrast, EPO and EDN are relatively inactive on their own in causing direct damage to the parasite (Pincus *et al*. 1981; Ackerman *et al*. 1985). EPO action was enhanced in the presence of hydrogen peroxide and a halide. The effects of these polycations were inhibited by polyanions such as heparin (Gleich *et al*. 1980; Venge *et al*. 1983).

Although eosinophils can release higher concentrations of superoxide radicals than neutrophils in response to stimulation, Pincus *et al*. (1981) demonstrated that damage to schistosomula by normal eosinophils can occur under strictly nonaerobic conditions, suggesting that such oxidative metabolism is not necessary for *in vitro* killing. However, oxygen may be required for degranulation (Baskar & Pincus, 1988). Eosinoplasts (experimentally) generated granule-containing eosinophils devoid of a nucleus) elaborated oxygen metabolites and synergized with ECP in helminth toxicity (Yazdanbakhsh *et al*. 1987). Oxidative mechanisms also appear to be essential in killing of newborn larvae of *T. spiralis* by

eosinophils (Bass & Szejada, 1979; Buys *et al*. 1981, 1984) while a reduction in oxygen tension limited the capacity of intact eosinophilic granulomas or isolated granuloma cells to kill eggs of *S. mansoni in vitro* (Feldman *et al*. 1990). Adherence of eosinophils to both live and fixed *S. mansoni* larvae induced *de novo* synthesis and release of lipid mediators including LTC_4 (Moqbel *et al*. 1990). The precise role of this mediator in parasite damage is not yet known. The ligands that mediate killing by both normal and activated eosinophils include immunoglobulins, particularly IgG, IgA and IgE. In addition, complement components C3b and C3bi were shown to facilitate adherence and killing in the absence of any immunoglobulin and this may be achieved through eosinophil adherence by CR1 and CR3 respectively (Anwar, Smithers & Kay, 1979; Moqbel *et al*. 1983; Fischer *et al*. 1986). Antibody-dependent killing may also be enhanced by LFA-1 associated mechanisms. Monoclonal antibodies against the α-chain of this β-1 integrin partly blocked the killing of *S. mansoni* larvae (Capron *et al*. 1987). That eosinophils utilize the receptor for IgA (Abu-Ghazaleh *et al*. 1989) to induce eosinophil degranulation (following incubation with either anti-IgA-coated sepharose beads, particularly sIgA (Fujisawa *et al*. 1990)) suggests that this ligand is an important receptor for mediator release. However, the involvement of sIgA in eosinophil-mediated cytotoxic response against parasitic helminthic targets is not yet established.

In vivo *correlates of* in vitro *damage*

The precise regulatory and functional roles of IgE and eosinophils in the progression of human helminthiases is unknown. Information in man is largely limited to measurements of blood and tissue eosinophilia and IgE during the migration of helminth(s) in various tissue sites (Wardlaw & Moqbel, 1992). There is some evidence of direct contact between eosinophils and adult worms during infections. Eosinophil-rich granulomas surrounding dead fragments of skin invading larvae of *Strongyloides ratti* were found in hyper-immune rats after challenge with infective larvae (Moqbel, 1980). Eosinophils were also found in close contact with the surface tegument of schistosomula of *S. haematobium* in the cutaneous tissue of immune monkeys. This was associated with the presence of large numbers of dead larvae in eosinophil-rich sites (Hsu *et al*. 1980). Similar observations were made in other host/parasite systems (Gleich & Adolphson, 1986; Butterworth & Thorne, 1993). Using appropriate antibodies, eosinophil-derived toxic proteins were identified on worm targets, *in vivo*. Immunofluorescent staining for MBP revealed the deposition of this eosinophil-derived product onto the surface of microfilariae of *Onchocerca volvulus* in skin biopsies of patients with onchocerciasis following treatment with diethylcarbamazine (Kephart *et al*. 1988). The presence of an eosinophilic infiltrate in association with human onchocerciasis was shown to be correlated with microfilarial production from pregnant female adult worms but not with the host's immune status (Wildenburg *et al*. 1995). Adult *O. volvulus* elicited tissue eosinophilia only if microfilariae appear in the surrounding tissue. The levels of blood ECP were elevated in patients with filariasis suggesting the activation and degranulation of eosinophils (Spry, 1981). The rate of reinfection in African children with *S. haematobium* indicated that both IgE and eosinophils appear to influence resistance in that age group (Hagan *et al*. 1991; Woolhouse *et al*. 1991). Thus, much of our existing knowledge about the possible *in vivo* role of eosinophils in helminth-induced inflammation arises from studies in laboratory animals, although data are being gradually obtained on the sequence of events that may regulate human eosinophil-mediated responses to helminths (Mahanty *et al*. 1992).

While rat and human eosinophils have been shown to possess IgE-dependent anti-parasitic effector functions both *in vivo* and *in vitro*, BAL eosinophils from lungs of mice infected with *T. canis* were devoid of receptors for sIgM, sIgA, sIgE, but were positive for receptor for sIgG1, although FcεRII and FcγRII were absent in mouse eosinophils (Jones *et al*. 1994). This suggests that there is a heterogeneity in the profile of eosinophils in different host species thus raising doubts concerning the role of eosinophils in helminth disease. It has been suggested that the presence of Th2-type responses (i.e. IgE-dependent mechanisms with its associated eosinophilia) to helminth infection may either contribute to host protection or lead to prolonged parasite survival. This appears particularly true in mouse models of schistosomiasis, in which the presence of IFN-γ and IgG have a more prominent protective role than IL-4 and IL-5, at least against larval stages of infection.

Reassessment of the leak–lesion hypothesis

Given that components of a Th2 driven response can be shown to damage parasite larvae *in vitro*, and that some *in vivo* correlates can be demonstrated, it is appropriate to reassess briefly the merits of the 'leak–lesion' hypothesis. This hypothesis was first mooted in the late 1960s, in an attempt to explain how type I hypersensitivity responses could effect parasite expulsion. Central to the hypothesis is the ability of IgE to sensitize mast cells and basophils specifically for the release of vasoactive amines, increasing vascular permeability and consequently flooding any site of parasitic challenge with blood rich in potentially host-protective cells and molecules. This phenomenon is most dramatically demonstrated following *Nippostrongylus brasiliensis* infection in rats, where the challenge of a sensitized

small intestine results in 'gut shock', which is manifested by the engorgement of the tissues with vascular components. This type of reaction would certainly make the gut environment inhospitable to parasite colonization, and inflammatory responses are certainly implicated in worm expulsion in other systems, e.g. *Trichinella spiralis* (Wakelin, 1993). Also mast cell proteinases are known to digest nematode cuticle collagens (McKean & Pritchard, 1989) suggesting that mast cell degranulation and associated proteinase release could have a direct and physically damaging effect on the helminth. Such reactions would also be beneficial in anatomical locations such as the skin following challenge with hookworm larvae. Anti-larval IgG responses appear to be beneficial in necatoriasis, and occur in tandem with IgE responses (Quinnell *et al*. 1995). The leakage of plasma and inflammatory cells onto the site of parasite invasion, mediated by a type 1 response (the cause of 'ground itch'?) should be beneficial to the host; but, as we know, the parasite seems to have evolved evasion strategies (Hotez & Pritchard, 1995). The 'leak–lesion' hypothesis does not command overwhelming support (Ahmad, Wang & Bell, 1991). Nevertheless, the immunological components for leakage are certainly induced by infection and would be activated upon exposure to parasite allergens. It is certainly a hypothesis that warrants resurrection. Readers are directed to a recent review by Bell (1996) who expresses some interesting views on the role of IgE in helminth infection.

CAN TH2 RESPONSES PROTECT THE HOST FROM PATHOLOGY BY SUPPRESSING POTENTIALLY TISSUE-DAMAGING HOST IMMUNE RESPONSES?

As T cell responses can counter-regulate each other, depending on the balance of cytokines in the milieu surrounding the interactive components (Abbas, Murphy & Sher, 1996; Anderson & Coyle, 1994), it is conceivable that Th2-driven responses may suppress the potentially tissue-damaging Th1-driven responses, known to occur in some parasitic infections. The prime manifestation of this type of interaction would seem to occur in lymphatic filariasis, where microfilariaemic yet asymptomatic and hyporesponsive individuals demonstrate Th2 responses to crude antigen preparations, although the literature on this subject matter is highly confusing and apparently contradictory in places. Nevertheless, because these individuals are considered to be tolerating the infection, they are described as being in an antigen-specific anergic state. When anergy is overcome, and Th1 responses come to the fore, the state of hypersensitivity of the patient would appear to swing from the immediate (Type 1) to the delayed (Type IV) phenotype, leading to the clearance of microfilariae, but the development of clinical disease (chronic lymphatic obstruction).

There is also evidence that IgG4, an isotype also under the control of Th2 cytokines and able to compete with IgE for both allergen epitopes and Fcϵ receptors, may play an important role in modulating IgE-mediated allergic responses *in vivo*. IgG4 is, thus, often described as a blocking antibody, and it is becoming apparent that it may act, in microfilariaemic patients, to suppress the manifestation of TPE or tropical pulmonary eosinophilia (reviewed by King & Nutman, 1993). Consequently, individuals predisposed to mount Th2-biased responses in areas of high endemicity for lymphatic filariasis, particularly those with a high IgG4 to IgE ratio, could be considered to be less prone to the pathological sequelae of infection. In this sense, the Th2 response would be seen as beneficial to the individual.

WHY ARE HELMINTH INFECTIONS SO ALLERGENIC?

Helminth parasites are particularly adept at stimulating IgE synthesis, and a number of parasite allergens have recently been cloned and characterized (McReynolds, Kennedy & Selkirk, 1993). Do certain parasite antigens have molecular properties which support their allergenicity? This is a question which has puzzled immunologists for decades and a number of theories have been put forward. One popular theory at present is based on the fact that many allergens have enzymic activity (e.g. *Der p* I from *Dermatophagoides pteronyssinus*), and that enzymic activity somehow deviates antigen processing in a way that supports the generation of Th2 lymphocytes. It is, therefore, of interest that highly allergenic parasites such as schistosomes and hookworms are rich sources of secreted proteinases (Smith *et al*. 1994; Brown *et al*. 1995). How do these enzymes promote IgE synthesis? One clue to a possible mechanism comes from an observation made in a population in Papua New Guinea infected with the hookworm *N. americanus*. This population was noted to have exceedingly high levels (on average, 40 times that of normal) of soluble CD23 (the low-affinity receptor for IgE) and IgE in its plasma (up to 17000 IU/ml) (Pritchard, Kumar & Edmonds, 1993). This observation led to the hypothesis that parasite proteases might accelerate the natural proteolytic cleavage of CD23 from the leucocyte surface, leading to an upregulation of immediate-type hypersensitivity through the generation of soluble CD23 (a pro-allergic cytokine) and the removal of a negative feedback signal for IgE synthesis (Pritchard, 1993*a*). IgE-containing immune complexes bind to CD23, to send a feedback inhibition signal to the B cell, which reduces IgE synthesis (reviewed by Delespesse *et al*.

https://doi.org/10.1017/S0031182097001996 Published online by Cambridge University Press

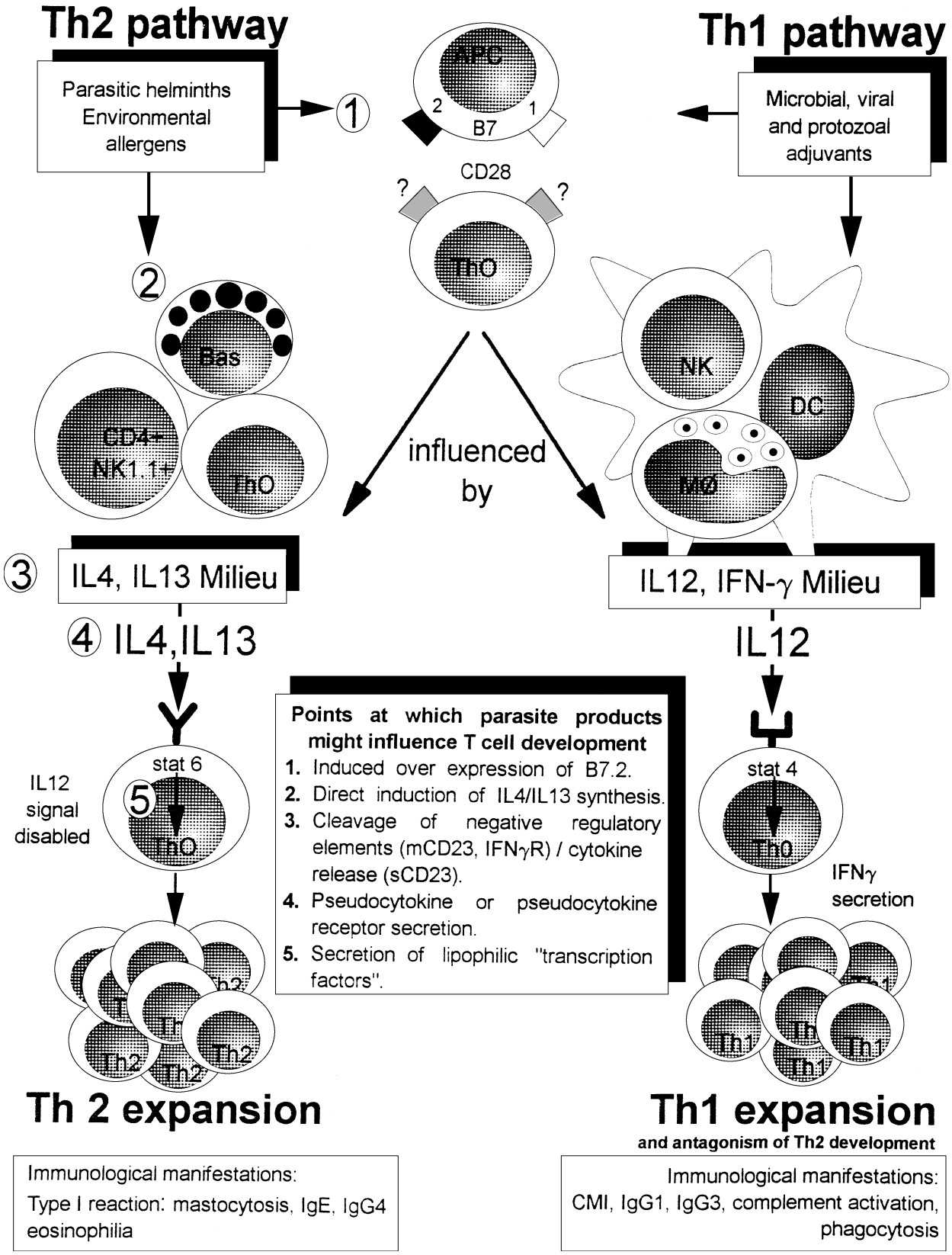

Fig. 1. T cell development can be influenced by a number of factors in the immunological environment. The points in T cell development where parasitic helminths and their ES products could conceivably exert an influence are shown here. For example, it is possible that helminth ES, particularly secreted proteinases and proteinase inhibitors (doubling as anticoagulants?), could influence the proteinase-rich antigen processing milieu to an extent that a pathway favouring Th2 development is selected. The mechanism by which CD28 controls IgE synthesis is sketchy. Some data support a positive role for CD28 (King *et al.* 1996) through an interaction with the B7 marker (Corry

https://doi.org/10.1017/S0031182097001996 Published online by Cambridge University Press

1992). Consequently, allergens which selectively cleave CD23 would theoretically be pro-allergic. It is therefore of interest that *Der p* I, a proteolytically active allergen of the house mite *D. pteronyssinus*, selectivity cleaves CD23 from the human B cell surface (Hewitt *et al*. 1995). This is the first and most convincing experimental evidence that dust mites dysregulate the IgE network by directly interfering with the control of IgE synthesis; these observations have since been confirmed by other workers.

This explanation may not hold for all allergens. For example, many fatty acid binding proteins (FABP) are now proven allergens (McReynolds *et al*. 1993). However, it provides an experimental basis for testing the pro-allergic activity of at least a major group of common environmental toxins. Given the ability of FABPs to sequester vitamins, and the fact that some vitamins (e.g. D3) promote Th-2 development (Rook, Hernandez-Pando & Lightman 1994), it is possible that some allergens promote the allergic response through selective vitamin uptake. A résumé of recent developments in our understanding of the development of helper T cells at this point wold help to indicate other points in the development processes where parasites and their products could exert influence on Th-2 bias.

Recent advances in our understanding of the development of helper T cell subsets

The early cytokine milieu seems to be of critical importance to the differential development of T cells. Both Th1 and Th2 cells can develop from the same T cell precursor, with IL-12 from macrophages and activated dendritic cells the predominant Th1 promoting cytokine, and Il-4, possibly from basophils, T cells themselves or CD4$^+$ NK1·1$^+$, equivalents in man, promoting Th2 development. However, a number of caveats exist. Although IL-4-independent pathways of class-switching for IgE synthesis have been demonstrated, initiated by retroviral infection (Morawetz *et al*. 1996), nematode infection does not seem to influence this pathway. Also there are data to suggest that β_2 microglobulin-independent NK1·1$^+$ T cells are not involved in Th2 responses (Brown *et al*. 1996).

The downstream effects of these cytokines on further T cell development would appear to be mediated through a number of transcription factors belonging to the *stat* family. Of these, *stat* 4 would appear to be activated by IL-12 (with IFNγ playing a supportive role), and *stat* 6 would appear to be activated by IL-4 (Kaplan *et al*. 1996*a*, *b*). The other points in T cell development in which parasite products would be active are illustrated (in Fig. 1) and described below. Table 1 lists known and putative helminth secretions which could polarize the development of T cells.

Are there other possible explanations for the allergenicity of helminth parasites?

The CD23 effect described would be largely mediated through B cells committed to IgE synthesis and it has already been suggested that helminth parasites may promote their own survival by stimulating polyclonal IgE synthesis, although clear evidence for this hypothesis is still being found. As well as cleaving CD23 to promote IgE synthesis, it is also possible that parasitic helminths secrete molecules which act as pseudocytokines, to maintain the Th2 bias. Molecules with the activities of IL-4, Il-10 and sCD23 would fulfil these criteria.

Alternatively, if Th2 responses, in a specific sense, begin to damage the parasite, as has been inferred from a number of field studies described above, then it would be in the interest of the parasite to induce a Th1 bias. This could be done through the secretion of Th1-promoting pseudocytokines such as IFNγ and IL-12. This may indeed be the case in *Trichuris muris* infection, (see Grencis and Entwistle, this volume). Work by Birgit Helm and her colleagues (Dudler *et al*. 1995; Machado *et al*. 1996) would also suggest that enzymically active allergens induce IL-4 synthesis by cells of the basophil lineage in the absence of sensitization with IgE. This kind of

---

et al. 1994), but it may not always stimulate through B7 (Life *et al*. 1995). Other data suggest that co-stimulation of T cells via CD28 inhibits IgE production (Van der Pouwkraan *et al*. 1996). Interestingly, the latter effect was reversed by pertussis toxin. Proteinases could also effect IL-4 secretion directly, in a non-antigenic fashion, in a manner similar to that already described for other enzymically active allergens. It is also already known that another proteolytically active environmental allergen (*Der p* I) has the ability to dysregulate the IgE network; formal proof demonstrating a similar effect by helminth proteases is lacking, although the suggestion that they might potentiate IgE synthesis by cleaving molecules such as CD23 was first muted in the early 90s (Pritchard, 1993*a*). The pro Th2 developmental pathway would then be propagated by the possible release of cytokines (IL-4, sCD23) or secretion of pseudocytokines or pseudocytokine receptors (e.g. IFN-γR) and even lipophilic homoserine lactones similar to those already shown to be active in chemical communication between bacteria (Williams, 1994). Another parasite secretion with the potential to modulate T cell development is acetylcholinesterase. Cholinergic influences on T cell development/activation have been recorded, yet it remains to be established whether extraneous sources of AChE, which would compete with T cell membrane AChE, exert any polarizing effect on T cell development (Pritchard, 1993*b*). Abbreviations: APC, antigen presenting cell; BAS, basophil; DC, dendritic cell; ES, excretory/secretory product; MO, macrophage; NK, natural killer; ThO, undifferentiated T cell; STAT, signal transducer and activator of transcription.

https://doi.org/10.1017/S0031182097001996 Published online by Cambridge University Press

Table 1. Examples of known and putative parasite secretions with the potential to support the development and growth of Th2 lymphocytes

ES product	Putative pro-Th2 effect
Proteinases e.g. cysteinyl proteinases, glycosidases, lipases (cf. phospholipase A2)	(i) cleavage of negative regulatory elements (CD23, IFN-γR/IL-12R); (ii) release of cytokines (sCD23) and 'anti-cytokines' (sIFN-γR/IL-12R) (iii) direct induction of IL-4 and IL-13 synthesis.
Proteinases/inhibitors e.g. serpins	Interference with cytosolic pathways of antigen processing/presentation.
Acetylcholinesterase	Interference with muscarinic (+ve) and nicotinic (−ve) influences on T cell function, and T-cell mAChE.
Pseudocytokines/receptors/ligands	Supportive of IL-4 milieu, sequestration of negative regulatory cytokines. Presentation of CD40 ligand-like molecules.
Superantigens	Induction of CD40 ligand expression and class switching to IgE.
Mitogens	Expansion of IL-4-receptive B (as found in *Ascaris* body fluid) and T cell sub-populations.
Protein kinases	Deviation of developmental pathways to Th-2 via intracellular signalling processes.
Lipophilic 'transcription factors'	Conversion of cytosolic regulating proteins into transcription factors.

direct activity would generate the early IL-4 milieu necessary for Th2 development. It is also intriguing that a number of pathogenic and non-pathogenic bacteria signal to each other through the secretion of homoserine lactones, which are lipophilic and enter cells to interact with proteins; the complex in turn becomes a transcription factor (Williams, 1994). Could parasitic helminths do the same? B cell mitogens which support the growth and division of ϵ B cells would also be important (Lee, 1995). Finally, superantigens can induce CD40 ligand expression, to modulate class-switching to IgE (Jabara & Geha, 1996). The possibility that helminths secrete superantigens should be fully explored. A full list of parasite molecules with the putative ability to control T cell development is shown in Table 1.

A PROSPECTIVE VIEW

When you ask the question, 'does a Th2 response protect against helminth infection?', you are almost asking whether atopics are protected against nematodes. These are thorny questions, which are difficult to answer in a definitive fashion (Moqbel & Pritchard, 1990) because many of the studies designed to answer these questions have been incomplete. How can study design be improved? The simplest experiments would involve the deliberate trickle infection of large numbers of human volunteers exhibiting a range of abilities to mount type 1 responses, or the transfer of such individuals from areas of low to areas of high parasite endemicity. Failing this, high-quality longitudinal studies should be conducted in which children of known atopic predisposition (judged by a combination of skin tests, IgE levels and symptomology) living in areas of high parasite endemicity and exhibiting similar behavioural profiles are followed over a 15-year period. For example, hookworm infection is acquired in a cumulative form from 3 years to adolescence, where infection intensity levels off (Pritchard *et al*. 1990). The assessment of atopic status would be supported by genetic analysis. However it is likely that atopy/asthma has a polygenic basis, involving genes in the 5q31–q33 region, the 11q13 region and other genetic loci (Marsh *et al*. 1994; Herwerden *et al*. 1995; Levitt & Holroyd *et al*. 1995; Daniels *et al*. 1996). We currently have a collection of DNA samples from hookworm infected individuals under analysis in the context of total IgE levels induced by infection, but larger-scale individual studies will be required to produce definite answers.

ACKNOWLEDGEMENTS

The authors are indebted to Barbara Williams for her excellent secretarial support over the years, to the Wellcome Trust for their generous and consistent financial support, to an anonymous referee for a very useful critique, and to Ms Mari Nowell for the artwork. Redwan Moqbel is an Alberta Heritage Medical Senior Scholar.

REFERENCES

ABBAS, A. K., MURPHY, K. M. & SHER, A. (1996). Functional diversity of helper T lymphocytes. *Nature* **383**, 787–793.

ABU-GHAZALEH, R. I., FUJISAWA, T., MESTECKY, J., KYLE, R. A. & GLEICH, G. J. (1989). IgA-induced eosinophil degranulation. *Journal of Immunology* **142**, 2393–2400.

ACKERMAN, S. J., KEPHART, G. M., HABERMAN, T. M., GRIEPP, P. R. & GLEICH, G. J. (1985). Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **34**, 735–745.

AHMAD, A., WANG, C. H. & BELL, R. G. (1991). A role for IgE in intestinal immunity. Expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. *Journal of Immunology* **146**, 3563–3570.

ANDERSON, G. P. & COYLE, A. J. (1994). T_H2 and 'T_H2-like' cells in allergy and asthma: pharmacological

perspectives. *Trends in Pharmacological Sciences* **15**, 324–332.

ANWAR, A. R. E., SMITHERS, S. R. & KAY, A. B. (1979). Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leucocytes *in vitro*: requirement for complement in preferential killing by eosinophils. *Journal of Immunology* **122**, 628–637.

ARAUJO, M. I., RIBEIRO DE JESUS, A., BACELLAR, O., SABIN, E., PEARCE, E. & CARVALHO, E. M. (1996). Evidence of a T helper type 2 activation in human schistosomiasis. *European Journal of Immunology* **26**, 1399–1403.

BADLEY, J. E., GRIEVE, R. B., ROCKY, J. G. & GLICKMAN, L. T. (1987). Immune mediated adherence of eosinophils to *Toxocara canis* infective larvae: the role of excretory–secretory antigens. *Parasite Immunology* **9**, 133–143.

BARKER, R. L., LOEGERING, D. A., TEN, R. M., AMANN, K. J., PEASE, L. R. & GLEICH, G. J. (1989). Eosinophil cationic protein cDNA. Comparison with other toxic cationic proteins and ribonucleases. *Journal of Immunology* **143**, 952–955.

BASKAR, P. & PINCUS, S. H. (1988). Oxidative requirements for degranulation of human peripheral blood eosinophils. *Infection and Immunity* **56**, 1907–1911.

BASS, D. A. & SZEJADA, P. (1979). Mechanisms of killing of newborn larvae of *Trichinella spiralis* by neutrophils and eosinophils. Killing by generators of hydrogen peroxide *in vitro*. *Journal of Clinical Investigation* **64**, 1558–1564.

BELL, R. G. (1996). IgE, allergies and helminth parasites: A new perspective on an old conundrum. *Immunology and Cell Biology* **74**, 337–345.

BROWN, A., BURLEIGH, J. M., BILLETT, E. E. & PRITCHARD, D. I. (1995). An initial characterization of the proteolytic enzymes secreted by the adult stage of the human hookworm *Necator americanus*. *Parasitology* **110**, 555–563.

BROWN, D. R., FOWELL, D. J., CORRY, D. B., DYNN, T. A., MOSKOQUITZ, N. H., CHEEVER, A. W., LOCKSLEY, R. M. & REINER, S. L. (1996). β_2 microglobulin independent NK1·1$^+$ T cells are not essential for T helper cell and immune responses. *Journal of Experimental Medicine* **184**, 1295–1304.

BUTTERWORTH, A. E. (1984). Cell-mediated damage to helminths. *Advances in Parasitology* **23**, 143–235.

BUTTERWORTH, A. E. & RICHARDSON, B. A. (1985). Factors affecting the levels of antibody- and complement-dependent eosinophil-mediated damage to schistosomula of *Schistosoma mansoni in vitro*. *Parasite Immunology* **7**, 119–131.

BUTTERWORTH, A. E. & THORNE, K. J. I. (1993). Eosinophils and parasitic diseases. In *Immunopharmacology of Eosinophils* (ed. Smith, H. & Cook, R. M.), pp. 119–150. London: Academic Press.

BUTTERWORTH, A. E., WASSOM, D. L., GLEICH, G. J., LOEGERING, D. A. & DAVID, J. R. (1979*a*). Interactions between human eosinophils and schistosomules of *Schistosoma mansoni*. II. The mechanisms of irreversible eosinophil adherence. *Journal of Experimental Medicine* **150**, 1456–1471.

BUTTERWORTH, A. E., VADAS, M. A., WASSOM, D. L., DESSEIN, A., HOGAN, M., SHER, A. & GLEICH, J. (1979*b*). Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *Journal of Immunology* **122**, 221–229.

BUYS, J. WEVER, R. & RUITENBERG, E. J. (1984). Myeloperoxidase is more efficient than eosinophil peroxidase in the *in vitro* killing of newborn larvae of *Trichinella spiralis*. *Immunology* **51**, 601–607.

BUYS, J., WEVER, R., VAN STIGT, R. & RUITENBERG, E. J. (1981). The killing of newborn larvae of *Trichinella spiralis* by eosinophil peroxidase, *in vitro*. *European Journal of Immunology* **11**, 843–845.

CABRERA, Z., COOPER, M. D. & PARKHOUSE, R. M. E. (1986). Differential recognition patterns of human immunoglobulin classes to antigens of *Onchocerca gibsoni*. *Tropical Medicine and Parasitology* **37**, 113–116.

CAPRON, M., KAZATHCHKINE, M. D., FISCHER, E., JOSEPH, M., BUTTERWORTH, A. E., KUSNIERZ, J.-P., PRIN, L. & PAPIN, J. (1987). Functional role of the alpha-chain of complement receptor type 3 in human eosinophil-dependent antibody-mediated cytotoxicity against schistosomes. *Journal of Immunology* **139**, 2059–2065.

CAULFIELD, J. P. (1980). The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. *Journal of Cell Biology* **86**, 46–63.

CAULFIELD, J. P. & CHIANG, C. P. (1990). How does the schistosome evade host defenses? *Gastroenterology* **98**, 1712–1713.

CAULFIELD, J. P., KORMAN, G., BUTTERWORTH, A. E., HOGAN, M. & DAVID, J. R. (1980*a*). Partial and complete detachment of neutrophils and eosinophils from schistosomula: evidence for the establishment of continuity between a fused and normal parasite membrane. *Journal of Cell Biology* **86**, 64–76.

CHANDRASHEKAR, R., RAO, U. R., PARAB, P. B. & SUBRAHMANYAN, D. (1986). *Brugia malayi*: rat cell interactions with infective larvae mediated by complement. *Experimental Parasitology* **62**, 362–369.

COOK, R. M., ASHWORTH, R. F. & CHERNIN, J. (1988). Cytotoxic activity of rat granulocytes against *Mesocestoides corti*. *Parasite Immunology* **10**, 97–109.

COOMBS, R. R. A. & GELL, P. G. H. (1963). The classification of allergic reaction underlying disease. In *Clinical Aspects of Immunology* (ed. Gell, P. G. H. & Coombs, R. R. A.), pp. 317–337. Oxford: Blackwell Scientific Publications.

CORRY, D. B., REINER, S. L., LINSLEY, P. S. & LOCKSLEY, R. M. (1994). Differential effects of blockage of CD28–B7 on the development of Th1 and Th2 effector cells in experimental leishmaniasis. *Journal of Immunology* **153**, 4142–4148.

DANIELS, S. E., BHATTACHARRYA, S., JAMES, A., LEAVES, N. I., YOUNG, A., HILL, M. R., FAUX, J. A., RYAN, G. F., LE SÖUEF, P. N., LATHROP, G. M., MUSK, A. W. & COOKSON, W. O. C. M. (1996). A genome-wide search for quantitative trait loci underlying asthma. *Nature* **383**, 247–250.

DELESPESSE, G., SARFATI, M., WU, C. Y., FOURNIER, S. & LETELLIER, M. (1992). The low affinity receptor for IgE. *Immunology Review* **125**, 77–97.

DESAKORN, V., SUNTHARASAMAI, P., PUKPITTAYAKAMEE, S., MIGASENA, S. & BUNNAG, D. (1987). Adherence of

human eosinophils to infective filariform larvae of *Necator americanus in vitro*. *Southeast Asian Journal of Tropical Medicine and Public Health* **18**, 66–72.

DUDLER, T., CANTARELLI-MACHADO, D., KOLBE, L., ANNAND, R. R., RHODES, N., GELB, M. H., KOELSCH, E., SUTER, M. & HELM, B. A. (1995). A link between catalytic activity, IgE-independent mast cell activation, and allergenicity of bee venom phospholipase A_2. *Journal of Immunology* **155**, 2605–2613.

DUFFUS, W. P. & FRANKS, D. (1980). *In vitro* effect of immune serum and bovine granulocytes on juvenile *Fasciola hepatica*. *Clinical and Experimental Immunology* **41**, 430–440.

DUNNE, D. W., BUTTERWORTH, A. E., FULFORD, A. J. C., KARIUKI, H. C., LANGLEY, J. G., OUMA, J. H., CAPRON, A., PIERCE, R. J. & STURROCK, R. F. (1992). Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *European Journal of Immunology* **22**, 1483–1494.

FATTAH, D. I., MAIZELS, R. M., McLAREN, D. J. & SPRY, C. J. F. (1986). *Toxocara canis*: interaction of human blood eosinophils with the infective larvae. *Experimental Parasitology* **61**, 421–431.

FELDMAN, G. M., DANNENBERG, A. M. & SEED, J. L. (1990). Physiology oxygen tensions limit oxidant-mediated killing of schistosome eggs by inflammatory cells and isolated granulomas. *Journal of Leukocyte Biology* **47**, 344–354.

FISCHER, E., CAPRON, M., PRIN, L., KUSNIERZ, J.-P. & KAZATCHKINE, M. D. (1986). Human eosinophils express CR1 and CR3 complement receptors for cleavage fragments of C3. *Cellular Immunology* **97**, 297–306.

FUJISWA, T., ABU-GHAZALEH, R., KITA, H., SANDERSON, C. J. & GLEICH, G. J. (1990). Regulatory effect of cytokines on eosinophil degranulation. *Journal of Immunology* **144**, 642–646.

FURLONG, S. T. & CAULFIELD, J. P. (1989). *Schistosoma mansoni*: synthesis and release of phospholipids, lysophospholipids, and neutral lipids by schistosomula. *Experimental Parasitology* **69**, 65–77.

GANGULY, N. M., MAHAJAN, R. C., SEHGAL, R., SHETTY, P. & DILAWARI, J. B. (1988). Role of specific immunoglobulin E to excretory–secretory antigen in diagnosis and prognosis of hookworm infection. *Journal of Clinical Microbiology* **26**, 737–742.

GLAUERT, A. M., BUTTERWORTH, A. E., STURROCK, R. G. & HOUBA, V. (1978). The mechanism of antibody-dependent, eosinophil-mediated damage to schistosomula of *Schistosoma mansoni in vitro*: a study by phase-contrast and electron microscopy. *Journal of Cell Science* **34**, 173–192.

GLEICH, G. J. & ADOLPHSON, C. R. (1986). The eosinophil leukocyte: structure and function. *Advances in Immunology* **39**, 177–253.

GLEICH, G. J., LOEGERING, D. A., FRIGAS, F., WASSOM, D. L., SOLLEY, G. O. & MANN, K. (1980). The major basic protein of the eosinophil granule: physicochemical properties, localization and function. In *The Eosinophil in Health and Disease* (ed. Mahmoud, A. A. F. & Austen, K. F.); pp. 79–94. New York: Grune Stratton.

GOLAN, D. E., BROWN, C. S., GIANCI, C. M., FURLONG, S. T. & CAULFIELD, J. F. (1986). Schistosomula of *Schistosoma mansoni* use lysophosphatidylcholine to lyse adherent human red blood cells and immobilize red cell membrane components. *Journal of Cell Biology* **103**, 819–828.

GREENE, B. M., TAYLOR, H. R. & AIKAWA, M. (1981). Cellular killing of microfilariae of *Onchocerca volvulus*: eosinophil and neutrophil-mediated immune serum-dependent destruction. *Journal of Immunology* **127**, 1611–1618.

GROGAN, J. L., KREMSNER, P. G., DEELDER, A. M. & YAZDANBAKHSH, M. (1996). Elevated proliferation and interleukin-4 release from $CD4^+$ cells after chemotherapy in human *Schistosoma haematobium* infection. *European Journal of Immunology* **26**, 1365–1370.

HAGAN, P. (1993). IgE and protective immunity to helminth infections. *Parasite Immunology* **15**, 1–4.

HAGAN, P., BLUMENTHAL, U. J., DUNN, D., SIMPSON, A. J. G. & WILKINS, H. A. (1991). Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* **349**. 243–245.

HAMANN, K. J., BARKER, R. L., LOEGERING, D. A. & GLEICH, G. J. (1987). Comparative toxicity of purified human eosinophil granule proteins for newborn larvae of *Trichinella spiralis*. *Journal of Parasitology* **73**, 523–529.

HAMANN, K. J., BARKER, R. L., LOEGERING, D. A. & GLEICH, G. J. (1987). Comparative toxicity of purified human eosinophil granule proteins for newborn larvae of *Trichinella spiralis*. *Journal of Parasitology* **73**, 523–529.

HAMANN, K. J., TEN, R. M., LOEGERING, D. A., JENKINS, R. B., HEISE, M. T., SCHAD, C. R., PEASE, L. R. & GLEICH, G. J. (1990). Structure and chromosomal localization of the human eosinophil derived neurotoxin and eosinophil cationic protein genes: evidence for intron-less coding sequences in the ribonuclease gene superfamily. *Genomics* **7**, 535–546.

HERWERDEN, L., HARRAP, S. B., WONG, Z. Y. H., ABRAMSON, M. J., KUTIN, J. J., FORBES, A. B., RAVEN, J., LANIGAN, A. & WALTERS, E. H. (1995). Linkage of high-affinity IgE receptor gene with bronchial hyperactivity, even in absence of atopy. *Lancet* 346, 1262–1265.

HEWITT, C. R. A., BROWN, A. P., HART, B. J. & PRITCHARD, D. I. (1995). A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *Journal of Experimental Medicine* **182**, 1537–1544.

HOTEZ, P. & PRITCHARD, D. I. (1995). Hookworm infection. *Scientific American* **272**, 42–48.

HSU, S. Y., MITROS, F. A., HELMS, C. M. & SOLOMON, R. I. (1980). Eosinophils as effector cells in the destruction of *Schistosoma mansoni* eggs in granulomas. *Annals of Tropical Medicine and Parasitology* **74**, 179–183.

JABARA, H. H. & GEHA, R. S. (1996). The superantigen toxic shock syndrome toxin 1 induces CD40 ligand expression and modulates IgE isotype switching. *International Immunology* **10**, 1503–1510.

JAMES, S. L. & COLLEY, D. G. (1976). Eosinophil-mediated destruction of *Schistosoma mansoni* eggs. *Journal of the Reticuloendothelial Society* **20**, 359–374.

JONES, J. T., HELMS, C. N. & KUSEL, J. R. (1988). Variation in susceptibility of *Schistosoma mansoni* to damage by polycations. *Molecular and Biochemical Parasitology* **30**, 35–44.

JONES, R. E., FINKLEMAN, F. D., HESTER, R. B. & KAYES, S. G. (1994). *Toxocara canis*: failure to find IgE receptors (Fc epsilon R) on eosinophils from infected mice suggests that murine eosinophils do not kill helminth larvae by an IgE-dependent mechanism. *Experimental Parasitology* **78**, 64–75.

KAPLAN, M. H., SCHINDLER, U., SMILEY, S. T. & GRUSBY, M. J. (1996*a*). *Stat* 6 is required for mediating responses to IL-4 and for the development of Th-2 cells. *Immunity* **4**, 313–319.

KAPLAN, M. H., SUN YA-LIN, HOEY, T. & GRUSBY, M. J. (1996*b*). Impaired IL-12 responses and enhanced development of Th2 cells in *Stat* 4-deficient mice. *Nature* **382**, 174–177.

KAZURA, J. W. & AIKAWA, M. (1980). Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil-mediated destruction of newborn larvae *in vitro*. *Journal of Immunology* **124**, 355–361.

KEPHART, G. M., ANDRADE, Z. A. & GLEICH, G. J. (1988). Localization of eosinophil major basic protein onto eggs of *Schistosoma mansoni* in human pathologic tissue. *American Journal of Pathology* **133**, 389–396.

KING, C. L. & NUTMAN, T. B. (1993). Cytokines and immediate hypersensitivity in protective immunity to helminth infections. *Infectious Agents and Disease* **2**, 103–108.

KING, C. L., XIANLI, J., JUNE, C. H., ABE, R. & LEE, K. P. (1996). CD28 deficient mice generate an impaired Th2 response to *Schistosoma mansoni* infection. *European Journal of Immunology* **26**, 2448–2455.

LEE, T. D. G. (1995). IgE regulation by nematodes: the body fluid of *Ascaris* contains a B cell mitogen. *Journal of Allergy and Clinical Immunology* **95**, 1246–1254.

LEVITT, R. C. & HOLROYD, K. J. (1995). Fine-structure mapping of genes providing susceptibility to asthma on chromosome 5q31–q33. *Clinical and Experimental Allergy* **25**, supplement 2, 119–123.

LIFE, P., AUBRY, J. P., ESTOPPEY, S., SCHNURIGER, V. & BONNEFOY, J. Y. (1995). CD28 functions as an adhesion molecule and is involved in the regulation of human IgE synthesis. *European Journal of Immunology* **25**, 333–339.

LOMBARDI, S., VEGNI TALLURI, M., BANCHIERI, L. & ESPOSITO, F. (1990). The *in vitro* adherence of murine eosinophils, neutrophils and non-induced and induced macrophages to infective larvae of *Toxocara canis* (Nematoda, Ascarididae). *International Journal for Parasitology* **20**, 603–613.

MACHADO, D. C., HORTON, D., HARROP, R., PEACHELL, P. T. & HELM, B. A. (1996). Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitisation with antigen-specific IgE. *European Journal of Immunology* **26**, 2972–2980.

MACKENZIE, C. D., JUNGERY, M., TAYLOR, P. M. & OGILVIE, B. M. (1981). The *in vivo* interaction of eosinophils, neutrophils, macrophages and mast cells with nematode surfaces in the presence of complement or antibodies. *Journal of Pathology* **133**, 161–175.

MAHANTY, S., ABRAMS, J. S., KING, C. L., LIMAYE, A. P. & NUTMAN, T. B. (1992). Parallel regulation of IL-4 and IL-5 in human helminth infections. *Journal of Immunology* **148**, 3567–3571.

MARSH, D. G., NEELY, J. D., BREAZEALE, D. R., GHOSH, B., FRIEDHOFF, L. R., EHRLICH-KAUTZKY, E., SCHOU, C., KRISHZAWAMY, G. & BEATY, T. H. (1994). Linkage analysis of IL-4 and other chromosome 5q31·1 markers and total serum immunoglobulin E concentrations. *Science* **264**, 1152–1155.

MCKEAN, P. G. & PRITCHARD, D. I. (1989). The action of a mast cell protease on the cuticular collagens of *Necator americanus*. *Parasite Immunology* **11**, 293–297.

MCLAREN, D. J., MACKENZIE, C. D. & RAMALHO-PINTO, F. J. (1977). Ultrastructural observations on the *in vitro* interaction between rat eosinophils and some parasitic helminths (*Schistosoma mansoni*, *Trichinella spiralis* and *Nippostronglyus brasiliensis*). *Clinical and Experimental Immunology* **30**, 105–118.

MCLAREN, D. J., MCKEAN, J. R., OLSEN, I., VENGE, P. & KAY, A. B. (1981). Morphological studies on the killing of schistosomula of *Schistosoma mansoni* by human eosinophil and neutrophil cationic proteins *in vitro*.; *Parasite Immunology* **3**, 359–373.

MCLAREN, D. J., PETERSON, C. G. & VENGE, P. (1984). *Schistosoma mansoni*: further studies of the interaction between schistosomula and granulocyte-derived cationic proteins, *in vitro*. *Parasitology* **88**, 491–503.

MCLAREN, D. J., RAMALHO-PINTO, F. J. & SMITHERS, S. R. (1978). Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of *Schistosoma mansoni* by rat eosinophils *in vitro*. *Parasitology* **77**, 313–324.

MCREYNOLDS, L. A., KENNEDY, M. W. & SELKIRK, M. E. (1993). The polyprotein allergens of nematodes. *Parasitology Today* **9**, 403–406.

MOQBEL, R. (1980). Histopathological changes in rats following primary, secondary and repeated infections with *Strongyloides ratti*, with special reference to tissue eosinophils. *Parasite Immunology* **2**, 11–27.

MOQBEL, R., MACDONALD, A. J., CROMWELL, O. & KAY, A. B. (1990). Release of leukotriene C_4 (LTC_4) from human eosinophils following adherence to IgE- and IgG-coated schistosolula of *Schistosoma mansoni*. *Immunology* **69**, 435–442.

MOQBEL, R. & PRITCHARD, D. I. (1990). Parasites and allergy: evidence for a 'cause and effect' relationship. *Clinical and Experimental Allergy* **20**, 611–618.

MOQBEL, R., SASS-KUHN, S. P., GOETZL, E. J. & KAY, A. B. (1983). Enhancement of neutrophil- and eosinophil-mediated complement-dependent killing of schistosomula of *Schistosoma mansoni in vitro* by leukotriene B_4. *Clinical and Experimental Immunology* **52**, 519–527.

MORAWETZ, R. A., GABRIELE, L., RIZZO, I. V., NOBENTRAUTH, N., KUHN, R., RAJEWSKY, K., MULLER, W., DOHERTY, T. M., FINKELMAN, F., COFFMANN, R. L. & MORSE, H. C. (1996). Interleukin (IL 4-independent immunoglobulin class switch to immunoglobulin (Ig) E in the mouse. *Journal of Experimental Medicine* **184**, 1651–1661.

OCHENSBERGER, B., DAEPP, G. C., RIGS, S. & DAHUNDEN, C. A. (1996). Human blood basophils produce

interleukin 13 in response to IgE receptor dependent and independent activation. *Blood* **88**, 3028–3037.

PINCUS, S. H., BUTTERWORTH, A. E., DAVID, J. R., ROBBINS, M. & VADAS, M. A. (1981). Antibody-dependent eosinophil-mediated damage to schistosomula of *Schistosoma mansoni*: lack of requirement for oxidative metabolism. *Journal of Immunology* **126**, 1794–1799.

PRITCHARD, D. I. (1993*a*). Immunity to helminths: is too much IgE parasite – rather than host-protective? *Parasite Immunology* **15**, 5–9.

PRITCHARD, D. I. (1993*b*). Why do some parasites need to secrete acetylcholinesterase (AChE)? *International Journal for Parasitology* **3**, 549.

PRITCHARD, D. I., CRAWFORD, C. R., DUCE, I. R. & BEHNKE, J. M. (1985). Antigen stripping from the nematode epicuticle using the cationic detergent cetyltrimethylammonium bromide (CTAB). *Parasite Immunology* **7**, 575–585.

PRITCHARD, D. I., KUMAR, S. & EDMONDS, P. (1993). Solubles(s) CD23 levels in the plasma of a parasitized population from Papua New Guinea. *Parasite Immunology* **15**, 205–208.

PRITCHARD, D. I., QUINNELL, R. J., SLATER, A. F. G., MCKEAN, P. G., DALE, D. D. S., RAIKO, A. & KEYMER, A. (1990). Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to excretory–secretory and cuticular collagen antigens. *Parasitology* **100**, 317–326.

PRITCHARD, D. I., QUINNELL, R. J. & WALSH, E. A. (1995). Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunology* **17**, 71–75.

PRITCHARD, D. I. & WALSH, E. A. (1995). The specificity of the human IgE response to *Necator americanus*. *Parasite Immunology* **17**, 605–607.

PRITCHARD, D. I. & WILSON, R. A. (1997). Helminth infections. In *Pulmonary Defences* (ed. Stockley, R. A.), pp. 305–319. Chichester: John Wiley and Sons Ltd.

QUINNELL, R. J., WOOLHOUSE, M. E. J., WALSH, E. A. & PRITCHARD, D. I. (1995). Immunoepidemiology of human necatoriasis: correlations between antibody responses and parasite burdens. *Parasite Immunology* **17**, 313–318.

RAMALHO-PINTO, F. J., MCLAREN, D. J. & SMITHERS, S. R. (1978). Complement mediated killing of schistosomula of *Schistosoma mansoni* by rat eosinophils *in vitro*. *Journal of Experimental Medicine* **147**, 147–156.

RIHET, P., DEMEURE, C. E., BOURGOIS, A., PRATA, A. & DESSEIN, A. J. (1991). Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *European Journal of Immunology* **21**, 2679–2686.

ROOK, G. A. W., HERNANDEZ-PANDO, R. & LIGHTMAN, S. L. (1994). Hormones, peripherally activated prohormones and regulation of the Th1/Th2 balance. *Immunology Today* **15**, 301–303.

SHER, A., WADEE, A. A., MASON, P. R. & FRIPP, P. J. (1980). Cytotoxic effect of human eosinophils and eosinophil lysate on *Schistosoma haematobium* ova. *South African Journal of Science* **76**, 266–268.

SIM, B. K., KWA, B. H. & MAK, J. W. (1982). Immune response in human *Brugia malayi* infections: serum-dependent cell-mediated destruction of infective larvae *in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 362–370.

SMITH, A. M., DALTON, J. P., CLOUGH, K. A., KILBANE, C. L., HARROP, S. A., HOLE, N. & BRINDLEY, P. J. (1994). Adult *Schistosoma mansoni* express cathepsin L proteinase activity. *Molecular and Biochemical Parasitology* **67**, 11–19.

SPRY, C. J. F. (1981). Alterations in blood eosinophil morphology, binding capacity for complexed IgG and kinetics in patients with tropical (filarial) eosinophilia. Parasite Immunology **3**, 1–11.

VAN DER POUWKRAAN, C. T. M., RENSINK, H. J. A. M., RAPPUOLI, R. & AARDEN, L. A. (1996). Co-stimulation of T cells via CD28 inhibits human IgE production, reversal by pertussis toxin. *Clinical and Experimental Immunology* **99**, 473–478.

VENGE, P., DAHL, R., FREDENSK, K., HAUGREN, R. & PETERSON, C. (1983). Eosinophil cationic proteins (ECP and EPX) in health and disease. In *Immunobiology of the Eosinophil* (ed. Yoshida, T., Torisu, M.) pp. 163–179. New York: Elsevier.

WAKELIN, D. (1993). Allergic inflammation as a hypothesis for the expulsion of worms from tissues. *Parasitology Today* **9**, 115–116.

WARDLAW, A. J. & MOQBEL, R. (1992). The eosinophil in allergic and helminth-related inflammatory responses. In *Allergy and Immunity to Helminths. Common Mechanisms or Divergent Pathways*? pp. 154–186. (London: Taylor & Francis

WASSOM, D. L. & GLEICH, G. J. (1979). Damage to *Trichinella spiralis* newborn larvae by eosinophil major basic protein. *American Journal of Tropical Medicine and Hygiene* **28**, 860–863.

WEISS, N., HUSSAIN, R. & OTTESEN, E. A. (1982). IgE antibodies are more species-specific than IgG antibodies in human onchocerciasis and lymphatic filariasis. *Immunology* **45**, 129–137.

WILDENBURG, G., KRÖMER, M., BONOW, I. & BÜTTNER, D. W. (1995). Distribution of effector cells in nodules of *Onchocerca volvulus*. *Zentralblatt für Bakteriologie* (Gustav Fischer, Verlag) **282**, 221 (abstr.).

WILLIAMS, J. F., CHALIB, H. W., MACKENZIE, C. D., ELDHALIFA, M. Y., AYUYA, J. M. & KRON, M. A. (1987). Cell adherence to microfilariae of *Onchocerca volvulus*: a comparative study. *Ciba Foundation Symposium* **127**, 146–163.

WILLIAMS, P. (1994). Compromising bacterial communication skills. *Journal of Pharmacology and Pharmacological Science* **46**, 252–260.

WOOLHOUSE, M. E. J., TALOR, P., MATANHIRE, D. & CHANDIWANA, S. K. (1991). Acquired immunity and epidemiology of *Schistosoma haematobium*. *Nature* **351**, 757–758.

YAZDANBAKHSH, M., TAI, P.-C., SPRY, C. J. F., GLEICH, G. J. & ROOS, D. (1987). Synergism between eosinophil cationic protein and oxygen metabolites in killing of schistosomula of *Schistosoma mansoni*. *Journal of Immunology* **138**, 3443–3447.

YOUNG, J. D. E., PETERSON, C. G. B., VENGE, P. & COHN, Z. A. (1986). Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* **321**, 613.