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Human immune responses to lymphatic filariasis in Papua New Guinea

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SUMMARY

The following review highlights our current knowledge about the host immune response to lymphatic filariasis. Our understanding of how the host immune response influences the risk of developing disease has changed dramatically over the past decade. Previously the spectrum of disease associated with lymphatic filariasis was largely attributed to the nature of the host immune response. Now we appreciate that the duration and intensity of infection and possibly the direct influence of parasite-derived molecules also determine the risk of disease. The review highlights recent studies examining the influence of transmission intensity on the host immune response, and how this might be mediated by defects in antigen-presenting cell function, and also the role of basophils and mast cells.

Introduction

The human immune response to lymphatic filariasis is complex and combines elements of immunopathology, tolerance and the consequences of extended infection. The broad diversity of clinical responses to filarial infection has been generally considered to reflect the intensity and type of immune response to parasite or parasite products (1). Factors other than the host immune response are likely to contribute to whether an individual develops clinically apparent disease or not. Since the parasite does not replicate within the human host, the cumulative and/or temporal pattern of exposure to infective larvae determines the infection load and may therefore increase the likelihood of developing disease. This implies that parasites themselves, their soluble products and/or their anatomical location may directly affect lymphatic function apart from the host immune response, and increase the risk of developing disease. Genetic polymorphisms in the parasite and/or host may also influence the susceptibility to infection and/or disease. It has also been hypothesized that secondary infection by bacteria of lymphatics already damaged by preexisting *Wuchereria bancrofti* infection may accelerate or exacerbate development of chronic lymphatic disease (2). These hypotheses are probably not mutually exclusive and each, to varying degrees, may contribute to the heterogeneity in infection and disease with lymphatic filariasis infection.

This review examines the interrelationship between the first two hypotheses: the impact of transmission intensity on the host immune response. Because of the limited studies on filarial immunology in Papua New Guinea, most of the background studies presented review literature from other filarial endemic regions of world. The study of immune responses to lymphatic filariasis in Papua New Guinea, however, provides a unique opportunity to explore the relationship of transmission and host immune responses for several reasons. Transmission levels are highly heterogeneous in nearby geographic areas and because of the remoteness of many filarial endemic areas few if any individuals have ever received any treatment for this disease that could potentially alter the host immune response to *W. bancrofti*.

This link between transmission and host immunity derives from the complex life cycle of lymphatic-dwelling filariae and the

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likelihood that shared antigens exist between the different stages of the parasite (3,4). Like other nematodes, *W. bancrofti* has five distinct developmental stages and four of these stages occur within the human host beginning with inoculation of the infective third-stage larvae, or L3. L3 are deposited on the skin during blood feeding by the mosquito vector. The larvae enter the host at the puncture site and penetrate the dermis to establish residence in the local lymphatics, a tissue niche that is absolutely essential for their survival and subsequent development (5-7). 9 to 14 days later and after L3 have shed their cuticle (ie, moulted) fourth-stage larvae appear and differentiate over 6 to 9 months into sexually mature adult worms. Once an adequate number of female and male worms accumulate in the lumen of afferent lymphatic vessels, microfilariae (mf) are produced and released by fecund female worms. Infective-stage larvae release proteolytic enzymes that facilitate the penetration process (8). These molecules can also be highly antigenic and have the potential to influence the type of immune response (9).

Immediate hypersensitive responses and the Th1/Th2 paradigm

Immediate hypersensitivity reactions, characterized by the presence of IgE antibody, eosinophils, mast cells and basophils, are the hallmark of helminth infections, including lymphatic filariasis. Each of these immediate hypersensitivity responses has been implicated in the resistance to infection and in the various clinical manifestations of disease (10). Patients with lymphatic filariasis in spite of frequently having elevated IgE and eosinophils, rarely have clinically apparent allergic reactions to these parasites, although their basophils and mast cells can be highly sensitized with specific anti-parasitic IgE (11). This effector arm of the immediate hypersensitivity response is thought, in part, to be modulated by the production of blocking antibodies that are abundant in the serum of filarial infected patients (11,12). These blocking antibodies, by definition, can inhibit IgE-mediated activation of basophils. Blocking antibodies are primarily of the IgG4 isotype (11) and show a pattern of antigen recognition that parallels IgE (13), which suggests that blocking antibodies bind to parasite allergens in the fluid phase, thereby preventing access of the allergens to IgE-coated mast cells. Filarial infected individuals also produce large amounts of polyclonal IgE that does not recognize filarial antigens (12,14). This nonspecific IgE can also bind to the high affinity FcεRI on mast cells and basophils and reduce the amount of filaria-specific IgE bound to the cell surface. Therefore, only high amounts of filaria antigens can cross-link the widely spaced filaria-specific IgE on the cell surface which is necessary for cell activation.

Filarial antigen-specific T cells are thought to regulate the induction and down modulation of the immediate hypersensitivity response and blocking antibodies (1,15,16). The Th1/Th2 paradigm has been an attractive model to understand this T-cell response in human lymphatic filariasis. Typically filarial infected individuals stimulate an expanded population of antigen-specific T cells that produce varying amounts of IL-4, IL-5, IL-10 and IL-13 (17-21). Most of these cytokines are associated with a Th2 type immune response and participate in the generation of the immediate hypersensitivity responses described above. IL-4 and IL-13 produced by these T cells stimulate polyclonal and antigen-specific IgE and IgG4 (22,23). The cytokine regulation of IgG4, although it can parallel that of IgE, is also distinct (24). IL-12, for example, can inhibit IgE expression through enhanced IFN-γ release by NK cells, but has been shown to augment IgG4 release (25). This is consistent with a model that Th1 type immunity, as defined by increased IL-2 and IFN-γ production by T cells and IL-12 by NK cells and monocytes, can cross-regulate the Th2 type immunity at the T-cell level and by enhanced blocking antibody production. Although the Th1/Th2 paradigm has been widely criticized as being too simplistic for understanding the immune response for many diseases (26), the striking Th2 orientation of the immune response to filarial infection continues to make this model attractive. The Th1/Th2 model, however, fails to adequately explain the mechanisms of immune hyporesponsiveness observed in many filarial infection patients (see below) and attention should be paid to specific cytokines, other molecules and a wider range of cell types.

Relationship between filarial infection and immune hyporesponsiveness

Immunological studies of human lymphatic filariasis throughout endemic areas of the world consistently show that filarial antigen-specific immunity differs according to the microfilarial status of the individual. Adults who are microfilaraemic have significantly lower adult-worm antigen-driven lymphocyte proliferation (27,28) and diminished IFN-γ production (17,29). These studies also established that humoral immune responses, including filaria-specific IgG and IgE, are relatively depressed among microfilaraemic individuals (30,31). In contrast, mf-negative persons generally have strong parasite-specific proliferation responses and type 1 immunity (17). More recent observations indicate that impaired lymphocyte proliferation and IFN-γ responses correlate more closely with infection status defined by the presence of circulating antigen (CAg) as detected by *W. bancrofti*-specific monoclonal antibody Og4C3 than by microfilaraemia per se (29,32). Studies of *W. bancrofti*-infected subjects in Papua New Guinea confirm the association between CAg status and filaria-specific proliferation and IFN-γ responses (Figures 1 and 2). The immune hyporesponsiveness in filarial infected subjects from India corresponded to an absolute reduction in the frequency of filaria-specific T and B lymphocyte precursors (33). These studies showed that the frequency of proliferating CD3+ T cells responding to extracts of adult *Brugia malayi* was significantly lower among microfilaraemic (1/3757) than amicrofilaraemic subjects (1/1513). Similarly, the proportion of B cells

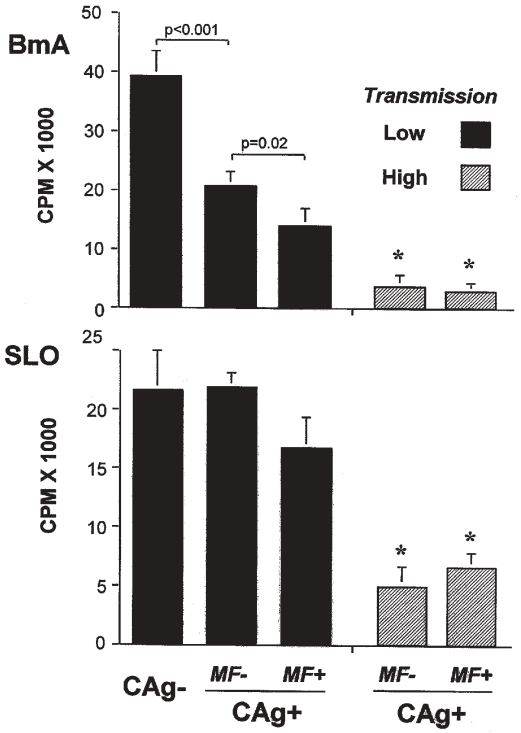

Figure 1. The presence of *W. bancrofti* infection and exposure correlates with impaired lymphocyte proliferation responses by Papua New Guinean study subjects. Solid bars represent individuals from the low transmission village and hatched bars those from the high transmission village. Infection status was determined by measurement of circulating antigen (CAg) by Og4C3 sandwich ELISA and peripheral blood microfilaraemia (mf) by Nuclepore filtration. Lymphocyte proliferation (measured in counts per minute) represents the net proliferative response (antigen-induced – spontaneous) of peripheral blood mononuclear cells (PBMC) stimulated with BmA (soluble extract of *Brugia malayi* adult worms) or SLO (streptolysin-O). Bars represent the mean ±SEM of each group. The mean cpm for PBMC from residents of the high transmission village was significantly less (denoted by an asterisk) than that of every group from the low transmission village ($p<0.001$–$p<0.01$).

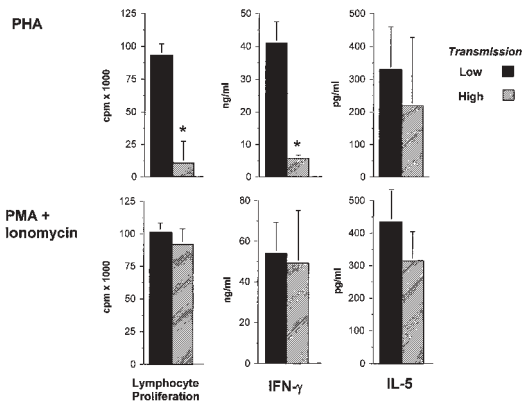

Figure 2. Heavy exposure to infective larvae results in impaired PHA- but not PMA+ionomycin-driven lymphocyte proliferation responses and IFN-γ production suggesting that frequent exposure to developing larvae induces a defect in antigen-presenting cell (APC) function. Solid bars indicate circulating antigen-positive (CAg+) residents of the low transmission village and hatched bars residents of the high transmission village. Bars represent mean ± SEM for lymphocyte proliferation and geometric mean with confidence limits for cytokine production. Asterisks denote a significant difference between two groups, $p<0.001$ for both comparisons. PHA and PMA+ionomycin are mitogens which are respectively APC-dependent and APC-independent.

producing parasite-specific IgG and IgE was lower in the microfilaraemic group.

The mechanism of hyporesponsiveness or partial anergy among infected individuals is in part related to the dominance of the Th2 subset of CD4+ T cells. If IL-4 is used as a marker of a Th2 type response (and IFN-γ as an indicator of a Th1 type response), microfilaraemic subjects had significantly fewer IFN-γ secreting cells than amicrofilaraemic subjects (17). The absolute number of IL-4 secreting cells, however, was equivalent between the two groups. Overall microfilaraemic individuals had 8-fold more filaria-specific IL-4 relative to IFN-γ secreting cells. By contrast, amicrofilaraemic individuals had a predominance of IFN-γ relative to IL-4 secreting cells. It should be pointed out that the absolute number of filaria-specific lymphocytes, both Th1 and Th2 type cells, were diminished in the microfilaraemic compared to amicrofilaraemic individuals. The relative reduction in Th1 type cells is in part due to IL-10 and/or TGF-β since in vitro neutralization of these cytokines partially reversed lymphocyte hyporesponsiveness among microfilaraemic subjects as measured by proliferation and cytokine production (15,17,34). To attribute this antigen-specific hyporesponsiveness among infected patients to immune deviation towards Th2 is probably an oversimplification for a number of reasons. First, impaired lymphocyte proliferation should affect both Th1 and Th2 type cells, although it appears that Th1 type lymphocytes may be more affected. Second, Th1 and Th2 type cells as well as monocytes produce IL-10 and its suppressive effect on lymphocyte proliferation cannot be explained by a simple Th2/Th1 dichotomy. Third, the depressed production of parasite-specific IgE is discordant with increased IL-4 and IgG4 production characteristic of infected individuals.

The mechanisms associated with partial anergy observed in filarial infected patients may be considered in the context of 'central tolerance' versus 'peripheral tolerance'. Central tolerance relates to the selective depletion or anergy that may develop as a consequence of in utero exposure to filarial antigens or anti-idiotypic antibodies. This idea has been well addressed by several reviews and papers and will not be considered further here (33,35,36). Peripheral tolerance may be considered as the effect of persistent or intermittent release of high amounts of antigen by the parasite on the host immune response, through the direct effect of the parasite itself or by parasite-derived molecules that may mimic or block host cytokines or receptors (37,38). Peripheral tolerance is likely to be only partial and can be reversed with elimination of the parasite infection after chemotherapy (39,40). Increasing evidence, both from older studies and more recent experiments, indicates that a defect in antigen presentation is critical for the development of peripheral tolerance (41,42).

Effect of exposure on immune responses to lymphatic filariasis

Development of a Th2 bias and suppression of the host proliferative response begins at the earliest stages of infection. Subcutaneous infection of mice with infective-stage larvae (L3) of *Brugia pahangi* suppresses lymphocyte proliferation and IFN-γ production by splenocytes in response to mitogens and parasite antigens within 12 days (43). This corresponds to a time when infective larvae have migrated to the lymphatics and begin to undergo their moult to L4 larvae. This active suppression has been shown to involve IL-10 and resident antigen-presenting cells (APCs) (44). The animals were not unresponsive, however, and they mounted a concomitant Th2 response to the parasite, characterized by elevated levels of IL-4, IL-5, IL-10 and parasite-specific serum immunoglobulin G (IgG), in particular IgG1, and IgE (43). The elevation of IL-4 appears within 24 hours after L3 injection and is produced by a population of CD4– CD8– T cells in draining lymph nodes at the infection site (45).

To better understand the mechanisms by which L3 larvae produce this immuno-suppression, L3 have been implanted in the murine peritoneum. Within 7 days after implantation, peritoneal exudate cells developed a marked capacity to suppress various T-cell lines in vitro (46). The precise mechanisms by which these cells mediate this immune suppression remains unclear, but it requires IL-4, although IL-4 itself is not directly suppressive, and is not dependent on

IL-10 nor on the presence of macrophages (46). In this model, excretory-secretory products from the adult *B. malayi* worms, as well as from other common nematode parasites, also induce this suppression (47). These observations differ from the role of IL-10 and APC in modulating this immunosuppression observed after subcutaneous infection with larvae. These models of infection are clearly different, but they may not be mutually exclusive. Overall these observations indicate that frequent exposure of mammalian hosts to developing larvae plays a critical role in Th2 bias and the development of immune hyporesponsiveness. This is not surprising since L3 parasites must also evade immune defenses on penetration of the host.

These murine studies appear to mirror a similar induction of immune hyporesponsiveness by infective larvae and their contribution to the initiation and maintenance of Th2 bias in filarial infected subjects. The immune responses of individuals residing in remote areas of Papua New Guinea highly endemic for *W. bancrofti* differ greatly depending on the levels of their exposure. Here transmission intensity – quantified as the annual transmission potential (the number of L3 to which an individual is theoretically exposed per year) – of the local mosquito vector *Anopheles punctulatus* varies tremendously among nearby villages within same linguistic groups (48). Transmission intensity can vary between 50- and 100-fold between hamlets only several kilometres apart in this rugged and geographically diverse country. Such differences were exploited to compare T-cell and cytokine responses among children and adults of two villages within 20 km of each other where transmission intensity of *Wuchereria bancrofti* differed by 63-fold (37 vs 2355 L3 per person per year). Residents of the high transmission village had 4- to 11-fold lower proliferation and IFN-γ responses to filarial antigens, even when subjects were matched for the intensity of infection (all study subjects in the high transmission village were infected with *W. bancrofti*) (Figure 1). Residents of the high transmission village also had a markedly impaired lymphocyte proliferation and IFN-γ production to the nonparasite antigen streptolysin-O (SLO) (Figure 1) and to the antigen-presenting-cell-dependent mitogen, phytohaemagglutinin (PHA) (Figure 2). By contrast, lymphocyte proliferation and IFN-γ production in response to the mitogen combination PMA+ionomycin (which directly activates protein kinase C and facilitates influx of Ca++ into the cell and does not require APC help) were similar between residents of the high and low transmission villages (Figure 2). Moreover, enriched CD4+ cells could also be activated with immobilized anti-CD3 that cross-links the T-cell receptor and anti-CD28, which provides an important costimulatory requirement. These results suggest that the incoming and developing larvae nonspecifically depress lymphocyte proliferation and IFN-γ production, analogous to that observed in murine models. Because the T cells are capable of full activation if the requirement for APC costimulation is bypassed, this suggests that the larvae or excretory-secretory production impair APC function. Further investigation of this issue will require isolation of APC from the skin of infected individuals and assessment of their level of activation and expression of costimulatory molecules such as CD40, CD80 and CD86. It may also be informative to determine whether filarial larvae themselves or molecules released during the moulting process modify the function of APC isolated from the dermis of uninfected individuals.

This ability of the parasite to modify the host immune response has obvious advantages to the parasite to enhance its survival. However, the impact that impaired APC function might have on the host's response to other infections has not been well investigated. Other filarial infections, particularly human onchocerciasis, have also been shown to develop mitogen and nonparasite antigen-specific suppression of lymphocyte proliferation and IFN-γ production (49-52). Although these studies did not correlate transmission intensity to levels of immune hyporesponsiveness, larvae infective to the insect vector persist in the skin where their excretory-secretory production may also modify the host immune response, analogous to that observed by infective larvae of lymphatic filariasis.

Frequent exposure to infective-stage larvae, however, enhanced the Th2 type immune

response. Filarial antigen-driven IL-5 production was 5.5-fold greater ($p<0.001$) and plasma IL-4 levels were elevated in residents of the high transmission village (Figure 3). IL-4 and IL-10 responses by peripheral blood mononuclear cells (PBMC) differed little according to village, however. Furthermore the increased production of the counter-regulatory cytokines IL-10 or TGF-β did not correlate with weak proliferation and IFN-γ responses. Plasma IL-5, IFN-γ, IL-10 and TGF-β levels were similar in the two villages. This observation contrasts with a previous report which showed that IL-10 and TGF-β contribute to lymphocyte hyporesponsiveness in microfilaria-positive subjects (17), although other studies have also failed to demonstrate that IL-10 modulates T-cell responses to filariae (53). The molecular basis of the propensity for filariae and other helminths to induce bias toward type 2 immunity is poorly understood. Helminths have abundant 'ladder' proteins with amino acid repeat sequences similar to those of environmental and venom allergens (54). They also contain carbohydrates that preferentially induce IL-10 production by innate immune cells and upregulate CD28-CTLA4 or other costimulatory pathways that favour type 2 T-cell differentiation (55,56). In this context, a secreted product of the animal filarial parasite *Acanthocheilonema viteae* denoted ES-62 has been reported to signal murine dendritic cells to drive differentiation of ovalbumin-specific transgenic T cells to the type 2 cytokine phenotype (57).

An additional biological feature of human filariasis that may predispose to the establishment of type 2 bias relates to the temporal profile of exposure to parasite antigens in the skin. Prolonged and continuous administration of soluble antigens into the subcutaneous tissue of genetically predisposed mice resulted in preferential induction of CD4+ Th2 cells (58,59). If the intensity or

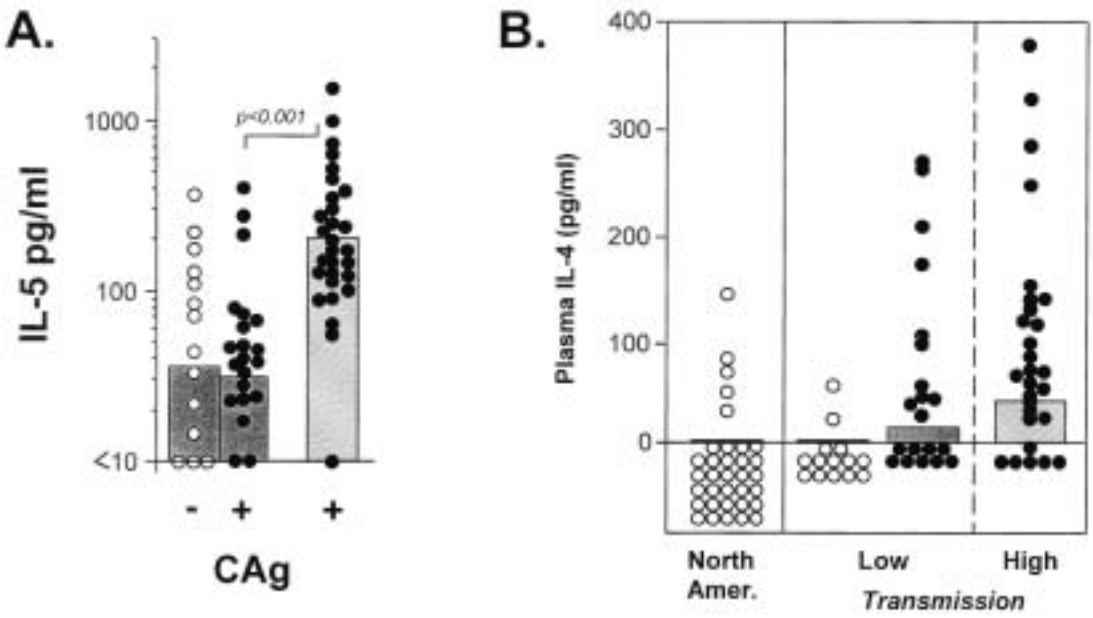

Figure 3. Heavy exposure promotes a Th2 bias indicated by increased filarial antigen-stimulated IL-5 production by peripheral blood mononuclear cells (PBMC) and elevated plasma IL-4 levels among Papua New Guinean residents of the high compared to low transmission villages. Plate A shows net BmA-driven IL-5 production with respect to the presence (CAg+) or absence (CAg-) of infection and either comparatively light exposure (shaded bars) or intense exposure (hatched bars). Bars indicate geometric means. The significance of the difference among infected subjects between the low and high transmission groups is shown in the figure. Plate B shows plasma IL-4 levels determined by immunoassay. Each circle represents the mean value for two or more determinations of serial two-fold dilutions of plasma from one individual. The significance of the differences in the mean level for CAg+ (closed circles) vs CAg– (open circles) subjects from the low transmission village and CAg+ persons from the low vs high transmission villages was $p<0.05$. BmA = soluble extract *of Brugia malayi* adult worms. CAg = circulating filarial antigen.

cumulative degree of exposure to L3 and developing larvae in the dermal lymphatics is an important determinant of the strength of type 2 immunity in human filariasis, such responses should wane following sustained reduction in transmission. Comparison of T-cell cytokine responses before and after reduction in transmission intensity should allow this hypothesis to be tested.

L3 activation of basophils in the development of Th2 immune bias and immune hyporesponsiveness

A striking finding was the elevated plasma levels of IL-4 in infected subjects compared to uninfected individuals from PNG. Overall plasma IL-4 levels were more than two-fold higher in the high transmission village than in the low transmission village. Particular care must be made in the interpretation of IL-4 in plasma, because it has a short in vivo half-life (IL-4 is a T-cell growth factor and may thus be rapidly consumed). In addition, much of the IL-4 may be bound to soluble IL-4 receptors and therefore interfere with its measurement by ELISA (enzyme-linked immunosorbent assay) or alter its biological activity. We therefore confirmed that plasma IL-4 detected by the two-site ELISA was able to drive the proliferation of an IL-4-dependent human T-cell clone.

An important source of this IL-4 is likely to derive from mast cells and basophils in the dermis. Both cell types are present in low numbers in peripheral blood but plentiful in dermal tissues where L3 are inoculated and larval development subsequently takes place. Persons exposed repeatedly to large numbers of L3 and pre-adult *W. bancrofti* may thus experience sustained increases in IL-4 production by activation and degranulation of cells located in the dermis, particularly mast cells bearing cytophilic filaria-specific IgE bound to FcεRI. This possibility is supported by the observation that soluble antigens from L3 larvae preferentially stimulate the mast cell's circulating counterparts, basophils, enriched from peripheral blood. More than 80% of individuals released IL-4 in response to L3 antigens compared to 51% and 36% of individuals that produced these same molecules in response to adult or microfilarial antigens respectively (Figure 4). Histamine was also preferentially produced by basophils in response to L3 antigens. This production of IL-4 by FcεR+ cells may contribute to the Th2 bias observed among heavily exposed individuals. Basophils and mast cells release other mediators such as prostaglandin D_2 that has been shown to participate in the development of a Th2 type inflammatory response (60). Most mediators released by basophils and mast cells promote a local inflammatory response, including histamine that causes a local increase in blood flow and vascular permeability. Histamine may also modulate this local inflammatory response. It can suppress pro-inflammatory mediators such as TNF-α (61) and has been shown to quell lymphocyte proliferation in human filariasis (62).

Mast cells and basophils play a critical role in host defense, particularly across epithelial barriers such as the skin. It has been increasingly recognized that these cells also regulate the local inflammatory responses, both by perpetuating the local allergic inflammatory

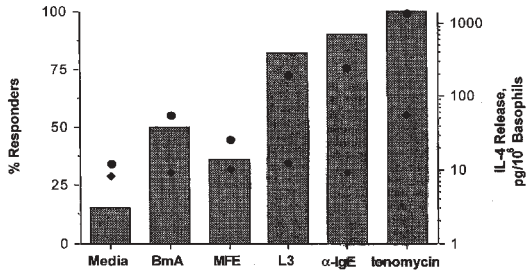

Figure 4. Infective stage larvae preferentially stimulate IL-4 release by basophils among filarial infected subjects in Papua New Guinea. Basophils were enriched from PBMC by Percoll-gradient centrifugation and adjusted to 10^6 basophils per millilitre. Cultures were stimulated with the amount of soluble stage-specific filarial antigen previously determined to stimulate the optimal amount of IL-4 release after 4 hours of incubation (ie, 30 µg/ml of BmA, 10 µg/ml of MFE and 1 µg/ml of L3). IL-4 levels were determined by immunoassay. These antigens stimulated no IL-4 release from enriched basophils from North Americans or Papua New Guineans who resided in an area not endemic for lymphatic filariasis (n=13). Bars show the percentage of filarial infected adults (all CAg+ males >20 years of age, n=20) that produced detectable IL-4 and circles (high) and diamonds (low) the range of IL-4 produced in the culture supernatants (see scale on the right side of the figure). Stimulation with anti-IgE and ionomycin in parallel cultures served as positive controls. PBMC = peripheral blood mononuclear cells. BmA = soluble extract of *Brugia malayi* adult worms; MFE = microfilarial extract; L3 = third-stage larval extract. CAg = circulating filarial antigen.

response and by modulating this inflammation (63). The role of these cells in regulating host inflammatory responses to filarial infections has been less well appreciated. Basophils and mast cells, for example, express CD40L and along with their release of IL-4 and IL-13 induce B cells to generate polyclonal IgE. This may be an important source of the extremely high levels of polyclonal IgE observed in filarial infected individuals. This polyclonal IgE, as indicated above, may act to downregulate further activation of these cell types by displacing parasite-specific IgE on the cell surface. Further, even if only a small percentage of basophils and mast cells had sufficient levels of antigen-specific IgE to trigger cell activation with antigen exposure, these cells are likely to exceed by far the number of antigen-specific T cells capable of making the same cytokine (64).

If the intensity or cumulative degree of exposure to L3 and developing larvae in the dermal lymphatics is an important determinant of the strength of type 2 immunity in human filariasis, such responses should wane following sustained reduction in transmission. Comparison of plasma IL-4 levels and T-cell cytokine responses before and after reduction in transmission intensity should allow this hypothesis to be tested. Given the existing global plan to control lymphatic filariasis through mass chemotherapy that reduces or even eliminates mosquito-borne transmission of *W. bancrofti* (65), such studies may be feasible in the near future. Although the interruption of transmission is the desired goal in the control of lymphatic filariasis, the absence of infection and lack of exposure would eliminate those factors that contribute to a suppressed host immune response. Subsequent reinfection may trigger a more vigorous host response and increase the risk for disease. Clearly a better understanding about the pathogenesis of lymphatic filariasis remains an important objective even as a worldwide program to eliminate this disease begins.

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