Toward an understanding of the interaction between filarial parasites and host antigen-presenting cells

Summary: Lymphatic filarial infection, from an immunologic point of view, is one of the most complex parasite infections. Not only are there different clinical manifestations that reflect differing immune responses, but the parasite’s multiple stages, each with distinct anatomic tropism, add a compartmental layer of complexity to an already complicated process. Moreover, these parasites have finely tuned immune evasion strategies that enable escape from the innate immune system. As different stages of the parasite interact with different types of antigen-presenting cells that, in turn, may play a significant role in shaping the subsequent adaptive immune response, the focus of this review is to provide insight into the interaction between filarial parasites and antigen-presenting cells with an eye toward understanding how they influence parasite antigen-driven T-cell responses.

Introduction

Downregulation of an antigen-specific proliferative response is a hallmark of several different parasitic infections (1–3) and is suggested to be a mechanism by which parasite survival is promoted. The mechanisms underlying T-cell hyporesponsiveness vary from organism to organism, but in filarial and other helminth infections, factors such as regulatory cytokines (4), altered function of antigen-presenting cells (APCs) (5–8), T-cell apoptosis (9), inducible nitric oxide (NO) synthase (iNOS) (3, 10), and pro- and anti-inflammatory cytokines have been implicated (11, 12). This T-cell proliferative hyporesponsiveness extends to the production of interferon (IFN)-γ, as well. Of interest, despite the lack of IFN-γ (and some other cytokines), there is no lack of antibody reactivity (13), although those human isotypes dependent on type 2 cytokines [e.g. immunoglobulin (Ig) E, IgG4] predominate (14, 15).

Among the many hypotheses used to explain the mechanism of hyporesponsiveness seen in filarial infections (14), one that has been given little credence is the alteration of APC function. This concept, however, has gained some experimental footing...
lately, based on work showing that individuals with patent filarial infection have monocytes capable of producing large amounts of interleukin (IL)-10 spontaneously ex vivo (16) and more recent data that live worm-exposed human dendritic cells (DCs) function less well in presenting antigens to CD4$^+$ T cells (7, 8). Of interest, there are flow cytometric data from freshly isolated unperturbed peripheral blood mononuclear cells from microfilaria-positive (mf$^+$) South Indians demonstrating diminished expression of CD80 on CD14$^+$ cells (17). In addition, peripheral blood mononuclear cells from mf$^+$ patients were found to express low levels of CD80 mRNA compared with those of exposed but uninfected individuals (18).

The immunology of lymphatic filariasis is complex and is complicated by the anatomical compartmentalization of the immune response and the differing responses induced by the multiple stages of the parasite (Fig. 1). The infection is initiated by mosquito-derived third-stage larvae (L3) deposited in the skin, itself an immunologic organ, containing Langerhans’ cells (LC) and keratinocytes among other cells. The larvae enter through the skin into the body where, if successful, the parasite evades the primary line of defense. Between 9 and 14 days after infection, the parasites undergo a molt to the fourth larval stage (L4) and then mature into lymphatic tissue-dwelling adult male and female worms during the subsequent 3–12 months. The adult female worm, once fertilized, develops into a fully formed, sheathed, first-stage larvae (L1 or mf), a stage believed to mediate many of the systemic immunologic defects associated with chronic lymphatic filarial infection. Through this circuitous route from the skin to the lymphatics and lymph nodes (LNs) and ultimately to the systemic circulation, the various parasite stages interact with very different types of APCs that may induce quite distinct immune responses.

As the initiation of infection occurs through the skin and likely conditions the subsequent immune response, understanding the interaction between the filarial L3 and the relevant APC in the skin is imperative. In addition, it is important to understand how these parasites evade the first line of defense without being fully recognized by the host cells at the entry level. Once the infection is fully established and microfilariae are produced, the nature of the APCs that come into contact with the parasites and the responses these APCs mediate are likely very different from what occurs initially. The focus of this review is how DCs, macrophages (Mo$^+$), and monocytes may interact with the filarial parasites and what role these APCs play in shaping the immune response that follows.

Macrophages and DCs are specialized phagocytic cells that are widely dispersed throughout the body and play an important role in defense against infection. These APCs are involved in initial capture and processing of antigens (innate immunity) and then activation of specific T- and B-lymphocyte effector mechanisms (adaptive immunity). What makes DCs unique and distinguishes them from Mo$^+$s is their capacity to capture antigens from the periphery and deliver them to the secondary lymphoid organs, bridging the innate and adaptive immune systems. They are involved in actively responding to microbial antigens, as they can produce cytokines such as IL-12 and both type I and type II interferons. Precursor DCs that encounter pathogenic organisms induce the production of chemokines and cytokines that can attract other cell types such as eosinophils, Mo$^+$s, and natural killer cells (19). Immature DCs, which reside in the peripheral tissue, can capture antigen and, in response to microbial stimuli, migrate to lymphoid organs, the spleen, and the LNs, and undergo maturation. Mature DCs express high levels of surface major histocompatibility complex (MHC) class I and II, CD40, CD80, CD86, and early activation markers such as CD83 (20). Moreover, these cells do not proliferate and ultimately undergo apoptosis. In the lymphoid organs, DCs can present the nonself peptide–MHC complexes to naïve T cells, and they are able to deliver specific costimulatory signals essential for T-cell activation, to prime and activate T cells into distinct classes of effector cells (20, 21).

Dendritic cells are a very heterogeneous group of APCs (22). CD34$^+$ hematopoietic stem cells differentiate into common lymphocyte or common myeloid progenitors (23) that can be distinguished on the basis of surface markers [CD11c$^+$CD1a$^+$ or CD11c$^+$CD1a$^-$ immature DCs (imDCs) respectively] (24). CD11c$^+$CD1a$^+$ imDCs migrate into the skin dermis and other tissues and become interstitial imDCs, while the
CD11c⁺CD1a⁻ cells migrate into the skin epidermis and become LCs (25). In addition to the two subsets of imDCs, stem cells can also give rise to two types of DC precursors (preDCs)–monocytes (preDC1) and plasmacytoid cells (preDC2) (26). PreDC1s differentiate into immature myeloid DCs in culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 or after exposure to bacterial stimuli. PreDC2s differentiate into imDC2s in culture with IL-3 or following a response to viral infection. While DC1s produce a large amount of IL-12 and induce strong T helper (Th1) 1 and cytotoxic T-lymphocyte responses, DC2s induce Th2 responses and result in the generation of IL-10-producing CD4⁺ suppressor cells. Furthermore, DC2s are not capable of producing IL-12 in large amounts (27, 28).

Macrophages play a key role in immunity by helping to initiate the immediate innate response to infection. Macrophages have various functions, some of which are phagocytosis, bactericidal activity, antigen presentation, tumor cytotoxicity, and removal of damaged and dead cells. Macrophages originate from hematopoietic stem cells in bone marrow and predominantly derive from circulating blood monocytes. They then enter various tissues and differentiate into tissue-specific Mφ populations (29, 30).

Macrophages display a wide variety of phenotypes depending on the localized cytokine environment and inflammatory process. Although they have the ability to produce IL-12, thereby driving Th1 responses, their unique ability is to produce proinflammatory cytokines, such as IL-8, tumor necrosis factor (TNF)-α, and IL-1β. Because of their intrinsic microbicidal function, Mφs can be distinguished from DCs. There are at least two different activation pathways of Mφs, classical and alternatively activated, the former involving Toll-like receptor (TLR) recognition and signaling (31). Such Mφ activation depends particularly on the IFN-γ (32) produced by Th1 and natural killer cells and on cytokines, such as IL-12 and IL-18, produced by other APCs. These classically activated Mφs also secrete proinflammatory chemokines that recruit other inflammatory effector cells to the site of infection. These inflammatory Mφs produce NO as a result of upregulating iNOS (33).

The alternatively activated Mφs – apparently activated by IL-4 and IL-13 that are generally produced in Th2-type responses (34) – selectively utilize arginase 1, an enzyme that mediates the metabolism of L-arginine to NO (35) and, in so doing, shunts the metabolism of L-arginine to L-ornithine and urea (36).

In this study, we review the role played by various APC populations in their response to filarial parasites in both animals and humans to gain insight into how these APCs promote both parasite survival and the downregulated T-cell responses that are the hallmarks of patent lymphatic filariasis.

**Dendritic cell/filarial infection**

How extracellular parasites interact with APCs is not well understood nor can it be extrapolated from the information gleaned from the study of intracellular pathogen/APC interaction. As these extracellular parasites cannot enter the APC directly, they must interact with the APCs through receptors expressed on the cell surface or through other mechanisms that allow for the soluble factors that are excreted/secreted by these worms to be internalized. Although there are limited studies exploring the interaction between filarial parasites and murine DCs, studies using ES-62, a secreted phosphorylcholine (PC)-containing glycoprotein from the rodent filarial nematode *Acanthochelonus vitae*, have provided some very interesting insights.

In filarial nematodes, PC has been found attached to both glycolipids (37) and glycoproteins (38). Some of the PCs attached to glycoproteins can be secreted by the worms and indeed can be detected in the bloodstream of infected individuals (39). Phosphorylcholine is a potent immunogen that can act as a dominant hapten during nematode infections. It has been shown that some patients with lymphatic filariasis have significant anti-PC responses (40–42). It was found that ES-62 was able to inhibit polyclonal (anti-CD3) activation of the Jurkat T-cell line (43), an effect mainly due to the PC (44). Furthermore, it was shown that PC-containing molecules isolated from whole worm extracts of the human filarial nematode *Brugia malayi* could also inhibit activation of human T lymphocytes induced by the mitogen phytohemagglutinin (45).

To gain insight into the role played by DCs exposed to ES-62 in influencing Th-cell subset differentiation, naïve DO.11.10 transgenic CD4⁺ T cells were cultured with bone marrow-derived DCs exposed to ES-62. The ES-62-exposed DCs matured into DC2s with the ability to induce an IL-4-dominated Th2 response (6) without altering the DC production of IL-12p70. Furthermore, it was shown that while DCs exposed to lipopolysaccharide (LPS) increased the expression of CD40, CD80, CD86, and CD54; DCs exposed to ES-62 did not show an increased expression of any of these markers. In vivo data corroborated the in vitro data that subcutaneous inoculation of ES-62 resulted in a dramatic increase in serum levels of ES-62-specific IgG1 but not IgG2a (38).
Previous reports indicated that PC-containing molecules appear to downregulate Th1 responses, at least in part, by inducing IL-10 by B1 cells (46). However, this finding does not seem to be the general rule, as DCs matured with ES-62 resulted in a small but significant production of IL-12 but not IL-10.

Human dendritic cells and filarial infection

The mechanisms underlying the antigen uptake, processing, and presentation of filarial parasites by DCs or Mφs (or less conventional APCs) have not been well studied. While it has been shown that monocyte-derived DCs are capable of taking up antigens from live mf or live L3 as well as from the crude antigen products, how these soluble products are recognized by DCs and how they are presented to T cells have not been investigated fully. Confocal microscopy and immunofluorescent staining have, in preliminary data, demonstrated that parasite antigen is seen both in the DC cytoplasm and on the plasma membrane (Fig. 2).

Monocyte-derived dendritic cells in response to L3

All the work in human systems studying the role of DCs in filarial infections has been performed using in vitro-generated, monocyte-derived DCs or epidermal LCs. We and others have suggested that different stages of the filarial parasite are likely to interact with different APCs that, in turn, may result in very distinct intracellular signals. To explore this possibility directly, we initiated studies using monocyte-derived DCs exposed to either L3 or mf at physiologically relevant numbers. As the L3 actually enter the human through the skin, we also developed a system to examine the interaction between L3 and epidermal LCs, the first APCs likely to encounter filarial parasites.

Using microarray analysis, there was shown to be minimal alteration in gene expression by the infective stage of B. malayi in monocyte-derived DCs. In general, L3s, at either high (n = 50) or low (n = 5) numbers, altered fewer genes and with lower fold changes than any of the intracellular pathogens to which this gene expression was compared (47). Indeed, only a small number of genes (23 genes) were altered following 16-h exposure of DCs to either of the different doses of L3. Because the mf stage (blood borne) must encounter DCs at different stages of differentiation as they travel from the afferent lymphatic vessels to the peripheral circulation, we contrasted the effect of live mf on the gene expression on monocyte-derived DCs to that induced by L3 (Fig. 3). Performing kinetic studies (6–48 h of exposure to parasites), we observed a maximum alteration in gene expression after 48 h of DC exposure to either stage of the parasites. In fact, clusters that were similarly regulated in DCs by both live mf and live L3 after 24 h of exposure were identified. At 48 h of exposure, however, there were clear clusters of repressed genes that were regulated very differently in response to various stages of parasites (Fig. 3). For example, genes involved in signaling (MAP3K11, MAPKAPK3, IKBKB, and STAT1) or genes involved in apoptosis (BCL2L1, BCL2, RELA, and BAX) were repressed by 48-h exposure of DCs to L3 but not to mf. Conversely, IL-8Rb, MAX, RAB11B, and MAFK were downregulated after exposure of DCs to mf but not to L3.

Fig. 2. Parasite antigen internalization. Confocal (top) and light (bottom) microscopy images of dendritic cells (DCs) after culture with live microfilariae (mf) of Brugia malayi. The confocal image was obtained following the use of using rabbit polyclonal antimf antisera.
Monocyte-derived dendritic cells in response to microfilaria

Exposure of monocyte-derived DCs to live mf for 48 h resulted in a number of changes in DCs. First, live mf-exposed DCs induced a contact-dependent cell-parasite aggregate formation. More profound was the finding that live mf induced cell death in DCs (8) but not in the precursor monocytes or M/MCs that were generated using M-CSF from the same donor (unpublished data). This cell death was in large part caused by parasite-induced apoptosis, the exact mechanism of which is still under study. What is clear, however, is that neither Fas/FasL expression nor NO activation of caspases (48) underlies the apoptosis seen. Given that IL-1β, TNF-related apoptosis-inducing ligand, CXCR4, and TNF ligand 14 mRNA are induced by mf, a TNF-related (and perhaps caspase-independent) mechanism is currently being explored.

Apoptosis induced by mf is not a new concept, although few studies have focused on APCs. For example, microfilarial sheath proteins have been shown to induce apoptosis in the human epithelial cell line Hep2 through an apoptotic pathway that involves IL-6 and IL-8 (49). In a murine model of Brugia infection, in response to in vitro restimulation with filarial antigen, CD4+ T cells from mf-infected mice undergo apoptosis (9). What may tie these findings together are the data from filarial infection of immunodeficient mice, which results in the development of lymphedema in the absence of T cells (50) and appears to be related to the production of proinflammatory cytokines, such as IL-1α, IL-6, TNF-α, and GM-CSF, within the lymphatic vessels (51). That B. malayi contains a homolog of human Mφ inhibitory factor (12) with a known ability to activate monocytes/Mφs to produce IL-8 and TNF-α (52) suggests that parasite-derived products may also serve to induce apoptosis indirectly through induction of cytokines and chemokines.

Not only do mf induce apoptosis in DCs, but also those DCs that remain alive are functionally impaired. Pre-exposure of DCs to mf suppresses the production of IL-12 and IL-10 in response to either SAC/IFN-γ or CD40/IFN-γ (8). This impairment of DC function prevents them from inducing autologous T cells to produce IFN-γ in response to either staphylococcal enterotoxin B or anti-CD3 and to mf themselves. This observation closely follows what has been found ex vivo in mf+ patients (53) and from more recent observations demonstrating that monocytes from mf+ individuals have an inability to respond to LPS compared with endemic normal individuals (54).

Epidermal Langerhans’ cells in response to L3

As the initiation of infection occurs through the skin and likely conditions the subsequent immune response, understanding the interaction between the filarial L3 and the APCs in the skin (particularly epidermal LCs) is crucial. Langerhans’ cells are bone marrow-derived cells that are present in all epithelial tissues (55) and are essential for the initiation and propagation of immune responses against foreign antigen in the skin. Prior to contact with antigen, LCs express low levels of MHC class I
and II and costimulatory molecules, and are poor stimulators of unprimed T cells. Upon contact with antigen, these cells become activated and migrate to the regional LN, where they act as mature APCs (20, 56). It has been suggested that TNF-α and IL-1β are the two independent cytokine signals required for migration of LCs. Both are upregulated following various forms of skin trauma and result in necessary physiologic changes to allow for migration from the skin to the draining LNs (57). Langerhans’ cells produce a variety of mediators, including cytokines such as IL-1β, IL-6, IL-12, and IL-18 that are capable of playing a role in the initiation and modulation of immune responses in the skin (58).

Several studies have addressed the interaction of LCs with parasites and the consequences of these interactions. It has been shown that when LCs are loaded with Leishmania major antigen, they become highly efficient in inducing protective immunity against a cutaneous form of leishmaniasis (59). In addition, uptake of L. major amastigotes by mouse skin-derived DCs results in both activation and IL-12 release from these cells (60). In murine infection with schistosomula, LCs were shown to become activated, although their migration to the LNs was impaired, an effect that was mediated by excreted/secreted lipophilic factors produced by parasite larvae such as prostaglandin D2 (61). Other investigators have shown that percutaneous exposure of guinea-pigs to attenuated or normal larvae of Schistosoma mansoni results in marked changes in distribution and morphology of epidermal LCs (62).

Considering the role played by LCs both in initiation of the immune response and in presentation of parasite antigen in the regional LN, we have examined the consequences of interaction between the L3 stages of filarial parasites and LCs through a system that relies on human epithelium-derived LCs obtained in a physiologically native state (63). Using human epithelial explants exposed to live parasite L3s, we have demonstrated that epidermal LCs are capable of internalizing L3 antigens (64). Furthermore, this interaction resulted in an increased migration of viable LCs from the explants into the culture. The LCs exposed to L3s, however, had markedly diminished expression of MHC class I and II. By microarray and realtime reverse-transcriptase polymerase chain reaction, we were also able to demonstrate that L3s induced diminished expression of IL-8 and a multitude of genes involved in antigen presentation. This downregulation translated into a reduced capacity to stimulate a mixed lymphocyte reaction or to activate autologous CD4+ T-cell activation and to produce cytokines/chemokines (e.g. TNF-α, IL-1β, IL-16, transforming growth factor-β, or IL-10). In contrast, IL-18, a cytokine structurally similar to IL-1β (65, 66), can mediate LC migration through mechanisms dependent on TNF-α and IL-1β (67). Whether this cytokine plays a role in migration of LCs from the explants by live L3s remains to be determined.

On balance, the filarial L3 clearly has a suppressive or non-activating effect on epidermal LCs. Using microarray analysis, we compared the response of monocyte-derived DCs to those from epidermal LCs using skin explants to the L3 stage of Brugia (Fig. 4). Notably, DCs and LCs showed very different responses to L3. After 16 h of exposure to L3, monocyte-derived DCs appeared to be less responsive than the skin explants. We identified clusters of both repressed and involved genes, but mainly in the explants. For example, 65 genes were repressed in the explants while only two were repressed in DCs by L3.

**Macrophage/filarial infection**

Macrophages are the key effector cells in many bacterial, protozoan, and viral infections. They have long been recognized as important cells associated with filarial infection. Our understanding of the role of these APCs in lymphatic filariasis comes primarily from in vitro studies and mouse models of filarial infection. The fact that there are large numbers of MoCs at the site of infection in experimental models of lymphatic filariasis...
has long implicated Mφs as being important cells in the killing of parasites and perhaps as one of the candidate APCs associated with the hyporesponsiveness seen in this infection.

Hyporesponsiveness and macrophages
As mentioned previously, one of the most consistent findings in both human (68, 69) and animal (70–74) studies is that lymphocytes from filaria-infected individuals exhibit a profound defect in antigen-driven proliferation. Macrophages have been shown to have a downregulatory role in this regard. In the experimental jird model (71, 75), removal of a plastic-adherent cell population could reverse the proliferative defect. Furthermore, intraperitoneal infection of B. pahangi in jirds led to deactivation of Mφs, as measured by their inability to kill the intracellular pathogen Toxoplasma and produce TNF (76). This deactivation correlated with a reduction in the systemic granulomatous inflammatory response that was observed in these animals. Osborne and Devaney (77) demonstrated that IL-10 produced by splenic adherent cells suppress T-cell proliferative responses in B. pahangi-infected mice, suggesting that IL-10 may be a mediator through which Mφs exert their suppressive effect. In another in vivo study, a population of adherent cells was recruited to the site of the filarial parasite that, when used as APCs in vitro, prevented the proliferation of a T-cell clone (78).

Macrophages and killing
Two separate groups have shown that Mφs activated in vitro by IFN-γ can kill B. malayi mf through a mechanism that may be mediated by NO (79, 80); however, filarial nematodes are remarkably resistant to a lethal hit and require sustained exposure to NO for killing (80). Of interest, in vivo, B. pahangi L3s induced activation of Mφs with increased phagocytic and microbicidal activity in the peritoneal cavity of jirds (81). In addition, in both the lymphatics and the peritoneal cavity in jirds, granulomatous lesions that form around the adult parasites and mf are associated with Mφs that are adherent to the parasite surface (82). Although there is cumulative evidence that Mφs are important in parasite killing, there is not much known about in vivo factors that activate these cells.

Macrophages and filarial pathology
It has been thought that Mφs may be responsible for some of the pathology associated with lymphatic filariasis. For example, in both human and animal studies of filarial infection, inflammatory lesions in lymphatic vessels of infected individuals can be observed (73). In fact, the granulomatous lesions that develop around dying worms are composed predominantly of eosinophils and Mφs, suggesting that activated Mφs may damage the tissue (83, 84). Taylor et al. (85) have also suggested that the inflammatory pathology seen in filarial patients could be due in part to the intracellular bacteria (Wolbachia) present in B. malayi. They showed that soluble worm debris from B. malayi could induce in vitro production of proinflammatory cytokines such as TNF, IL-1, and NO in Mφs. Using a CD14-deficient Mφ cell line and a TLR4-deficient mouse strain, they have demonstrated that production of some of the parasite-induced proinflammatory cytokines was related to LPS from the intracellular Wolbachia, a finding leading to the hypothesis that dying (but not live) worms release LPS and have a role in the pathology.

Natural resistance and macrophages
It has also long been thought that the B cell is the cell type responsible for clearance of mf. This idea stems from studies with CBA/N mice that have a defect in the Bruton’s tyrosine kinase (Btk) gene. Using this model, studies have shown that these mice have a reduced ability to clear mf (46, 86); however, Btk can also be expressed in other APCs that originate from myeloid precursors. Furthermore, Mφs from CBA/N mice have been shown to have a reduced capacity to produce NO but an enhanced ability to produce IL-12 (87). This finding could be due to the fact that NO has downregulatory effects on IL-12 induction. In CBA/N mice, T-cell responses to mf antigens were shown to be skewed toward a Th1 response, an effect that was mainly due to the Mφs (88).

Nematode-elicited macrophages
It has been previously shown that when B. malayi parasites are implanted into the peritoneal cavity of mice, alternatively activated Mφs are induced and recruited to the site of infection (78). Of interest, the generation or recruitment of these cells is dependent on host production of IL-4 (89). Recently, Nair et al. (90), using a murine model of nematode infection injected with adult female B. malayi, discovered Mφs with novel properties. These so-called nematode-elicited Mφs (NeMφs) are able to suppress the proliferation of T cells and certain tumor cells, an effect that was shown to be reversible (91). In addition, analysis of expressed sequence tags from a cDNA library of NeMφs showed that greater than 10% of the transcriptional activity was for two genes, Ym1 and Fizz1 (92); Fizz1, in addition, was shown to require IL-4 for its induction (93).

These NeMφs share some features of Mφs activated by Th2 cytokines in vitro. When NeMφs were compared with in vitro IL-4-activated thioglycolate-elicited or J774 Mφs, arginase 1, a Mφ
alternative activation marker, was shown to be induced in NeMΦs, as were Fizz1 and Ym1 (90). Nematode-elicited MΦs appear to have a higher suppressive effect on T-cell or thymoma cell proliferation than do IL-4-treated thioglycolate-elicited MΦs, an effect not mediated by IL-10, prostaglandins, NO, or changes in surface expression of costimulatory molecules (89, 94, 95). These findings are corroborated by the finding that suppression by NeMΦs is contact dependent (91).

Exposure of MΦs to soluble nematode products has also provided insights into parasite/APC interaction. For example, ES-62 suppresses the production of IL-6 and TNF-α in IFN-α+, LPS-activated, thioglycolate-elicited, peritoneal MΦs (96). ES-62 also suppressed LPS-induced synovial TNF-α and IL-6 production in a model of rheumatoid arthritis (97). In addition, ES-62 inhibited IL-12p40 and p35 transcripts in these cells without altering NO production or cell viability (96). Combining these findings with the data from ES-62 and DCs (see above), the authors concluded that ES-62 helps polarize the immune response toward a Th2 phenotype by limiting the ability of APCs to produce IL-12. Whether ES-62 alone can induce alternatively activated MΦs in vitro or can elicit NeMΦs in vivo has not yet been addressed.

Nematode-elicited MΦs have only been generated by surgically implanting with live adults intraperitoneally, because exposure to mf did not generate antiproliferative cells. One explanation for this finding is that mf initially because exposure to mf did not generate antiproliferative

 TNF-α, IL-1β, IL-6, macrophage inflammatory protein (MIP)-1b, MIP-3b, and IL-8 as well as CD44 and intercellular adhesion molecule 1, genes involved in inflammation and adhesion. Unlike in other pathogen studies, L3s did not alter the expression of IFN-related genes involved in signaling, antiviral activities, or proliferation; however, some of the genes related to the nuclear factor-κB family were slightly upregulated by this L3 stage of Brugia parasite.

We are currently investigating the effect of the mf stage of B. malayi on monocyte-derived MΦs in their gene expression, cytokine profile, and ability to act as APCs. Using an in vitro system, we have generated human MΦs by culturing elutriated monocytes in M-CSF for 7 days, at which time point they are exposed to live mf. Using microarray analysis, and similar to what has been found following exposure to L3s, mf can also induce expression of inflammatory genes such as IL-8, RANTES (Regulated upon activation, Normal T cell Expressed, and presumably secreted), and IL-1 (unpublished data). Although this finding was similar to the effect of live mf on human monocyte-derived DCs (8), regulation of the genes in DCs compared with MΦs had quite different kinetics, with expression/repression of mRNA in MΦs by mf lagging behind that seen in DCs by as much as 48 h. Another difference between these two cell types in response to live mf is in their production of IL-12 and IL-10 in response to stimuli. While mf-exposed DCs failed to respond to stimuli such as SAC/IFN-α or CD40L/IFN-α to produce IL-12 or IL-10, preliminary data suggest that mf-exposed MΦs are capable of producing both IL-12 and IL-10.

Human macrophages and filarial infection

Utilizing microarray analysis, we compared the response of MΦs to L3 to the infective stages of intracellular parasites Toxoplasma gondii, L. major, L. donovani, and Mycobacterium tuberculosis, all involved in chronic infection (47). We found that MΦs and DCs have 96% homology in expressed genes at the basal level (Fig. S); however, each cell type was found to respond very distinctly to pathogen exposure. In general, changes in gene expression were more profound and diversified in DCs, although MΦs were found to respond to a wider range of pathogens and responded more to the filarial parasites than did the corresponding DCs. L3s used either at high or low numbers, altered the expression of 72 genes in MΦs compared with only 23 genes in DCs. Moreover, proinflammatory genes in MΦs predominated in the response to L3. For example, exposure of MΦs to L3s for 16 h resulted in the induction of

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**Fig. 5.** Venn diagrams illustrating the transcriptional differences between resting dendritic cells (DCs) and macrophages (MΦ). The number of genes commonly expressed by both cell types or unique to either cell type is shown.
Concluding remarks

From the collective data from in vivo and in vitro studies, we have examined the complex interactions that occur between host APCs and filarial parasites with an emphasis on physiologically relevant numbers of parasites and types of APCs that are likely to encounter them. We are still left with the following questions: (i) do filarial parasites evade the immune system or do they confront it? (ii) how does the interaction of different filarial stages with APCs change the outcome of immune response? (iii) do these parasites need to evade the immune system at an early time point in their life cycle for survival? and (iv) how do the cells of the immune system see these parasites?

From the human data, we propose a model of evasion and confrontation. (Fig. 6) L3s deposited on the skin of an individual after a mosquito bite enters the body and interacts with epidermal LCs; however, L3s fail to activate these LCs and, if anything, shut off gene transcription. These L3 filarial parasites cause the downregulation of genes involved in antigen processing and presentation and fail to induce many of the inflammatory cytokines/chemokines apart from IL-18, a cytokine that may play a role in increased migration of these cell types from the epidermis. The LCs that have migrated from the epidermis, however, have a diminished ability to activate CD4⁺ T cells.

Once the L3s have successfully entered the body, they make their way to the lymphatics, where development to L4 and adulthood occurs. From these adults, the mf are produced in large numbers, armed to confront the immune system. They clearly interact with DCs and MΦs, but they target DCs. They can impair DCs either by inducing apoptosis or by affecting their ability to produce cytokines essential for Th1 development. While by no means only the mechanisms implicated in downregulated parasite-specific T-cell responses seen in filarial infection, parasite interference with APC function must be considered as central to this process.

Fig. 6. Model for filarial parasite/antigen-presenting cell (APC) interaction. DC, dendritic cells; KC, keratinocytes; LC, Langerhans’ cells; MΦ, macrophages.

References


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