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Review

The third-stage larva (L3) of *Brugia*: its role in immune modulation and protective immunity

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ABSTRACT – In this review, we focus on the role of the L3 (third-stage larva) of lymphatic filarial nematodes in immunomodulation and in the development of protective immunity. Studies in the mouse models of *Brugia* have been fundamental to our understanding of the mechanisms by which infection with L3 results in Th2 responses and the active suppression of Th1 responses. The relevance of these phenomena to the human infection is discussed. © 2000 Éditions scientifiques et médicales Elsevier SAS

L3 / *Brugia* / T lymphocyte, double-negative / antigen-presenting cell / IL-4 / IL-10

1. Introduction

Lymphatic filariasis is a mosquito-borne nematode infection responsible for considerable pathology in the tropical regions of the world. The infection is caused by parasites of the genera *Brugia* and *Wuchereria*. A recent article estimated that on the order of 120 million individuals are infected with lymphatic filarial worms, with ~20% of the population of the world living in endemic areas [1], while The World Health Report in 1995 suggested that infection with these parasites was the second most important cause of permanent and long-term disability worldwide.

The adult parasites live in the afferent vessels of the lymphatic system and, following mating, the females release an abundance of first stage larvae (the microfilariae or mf) which make their way into the bloodstream. The life cycle proceeds when mf are ingested by a mosquito when taking a blood meal. Mf develop to infective third stage larvae (L3) in the vector, and can be transmitted into a new host when the infected mosquito next takes a blood meal. The L3 migrate to the lymphatics where they undergo two moults during their development to adults. Filarial nematodes have evolved a multiplicity of ways of evading/modulating the host immune response thus promoting their own survival. This review focuses on the role of the infective form, the L3, in the modulation of the immune response and in the development of protective immunity.

For an infectious disease of man, the immune response to the parasite and its relationship to clinical disease is relatively well documented (reviewed in [2]). The accepted view is of an infection which strongly polarises the immune response, so that Th2 responses dominate while antigen (Ag)-specific proliferative responses (Th1) are severely impaired. A simplistic view of these data would argue that the imbalance in cytokine profiles (Th2 > Th1) leads to the defective proliferative (Th1) response via the cross-regulatory activity of cytokines such as IL-4 and IL-10. However, there are many additional mechanisms which may be involved, including selective tolerance or deletion of Ag-reactive T cells.

While most studies in the past documented the proliferative defect as being Ag-specific and restricted to those persons with circulating microfilariae, recent studies with larger study populations have suggested a more dynamic picture; e.g., PBMC from a significant proportion (up to 50%) of individuals in all clinical groups, including amicrofilaraemics, were shown to be unresponsive to parasite Ag [3]. These findings probably reflect the presence of subclinical infections in many individuals. Additional studies have now demonstrated that both IFN-γ and IL-5 responses are impaired in chronic infection [4] and have shown that the defect in proliferative responses can extend to other antigens and to polyclonal stimulants, at least in areas of high transmission (C. L. King, personal communication). Furthermore, there appears to be seasonal variation in both mf levels and proliferative responses [5]. These observations, together with recent findings that age has a significant bearing on the cytokine profile of infected individuals [4], argue for a more dynamic view of lymphatic filariasis, in which both the length of exposure to worms and the intensity of transmission are important determinants of the outcome of the immune response.

Defining the role of individual life cycle stages in the human infection is very difficult as lymphatic filariasis is a

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chronic disease (adult worms can live for approximately 5 years) [6] and individuals are simultaneously exposed to multiple life cycle stages. Longitudinal studies in infected populations are necessary to follow through the dynamics of disease and protective immune responses, both of which are thought to develop with age. It is important to acknowledge that immunity in lymphatic filariasis can take several different forms and operates against different life cycle stages: for example, some individuals in endemic areas appear to remain infection-free (the so called endemic normal or putative immune individual). This form of immunity presumably operates against the incoming L3. Other studies support the concept of concomitant immunity in which individuals develop immunity against the invading L3 without clearing their resident adult parasites [7, 8]. Yet another group can clear their circulating microfilariae but retain the adult parasite (as demonstrated by the presence of circulating filarial antigen (CFA) in the absence of mf). The mechanisms underlying these stage-specific immune responses in the human are poorly defined.

While it would be naive to expect a mouse model of lymphatic filariasis to mimic all aspects of the human disease, studies in BALB/c mice infected with either *Brugia malayi* or *B. pahangi* have proved very important for understanding the capacity of different life cycle stages to elicit stage-specific immune responses, for the analysis of mechanisms of immunomodulation and for studies on protective immunity. In the BALB/c mouse, exposure to the L3 and the adult parasite induces IL-4, while the mf elicit Ag-specific IFN-γ at early time points following primary infection [9, 10]. As the L3 is the first life cycle stage to which the host is exposed, the immune response elicited by L3 is likely to have a central role in the establishment of the parasite and in the development of immunity.

2. The L3 – a potent modulator of immune responses

In natural infections, the L3 gain access to the infected host via the wound left by the biting mosquito; thus the parasites enter the body via the skin, and the route of infection and consequently the initiating antigen-presenting cells (APCs) may have an important bearing on the type of immune response elicited. In our studies with the L3 of *B. pahangi*, we have attempted to mimic the natural route of infection by injecting parasites subcutaneously (s.c.); following s.c. infection, splenocytes from infected mice secrete significant amounts of Ag-specific Th2 cytokines (IL-4, IL-5 and IL-10) but no Ag-specific IFN-γ at 12 days postinfection (p.i.). The Th2 dominated response results in the downregulation of polyclonal Th1 responses, so that splenocytes from L3-infected animals proliferate poorly to the polyclonal stimulants concanavalin A (ConA) or anti-CD3, while the Ag-specific response remains intact. This defect in polyclonal proliferative responses can be partially reversed in vitro in the presence of neutralising antibody to IL-4 [10] or to IL-10 [11] or by supplementation of the medium with rIL-2.

Interestingly, blocking IL-4 in vitro does not give rise to an Ag-specific Th1 response, which, together with data from IL-4 KO mice on the 129xC57BL/6 background infected with *B. malayi* L3 [12] suggested that L3 might not elicit Th1 responses in the mouse. However, in vitro treatment with an anti-IL-10 monoclonal antibody of splenocytes from BALB/c mice infected with *B. pahangi* L3 gave the first indication that L3 do indeed prime Th1 cells in vivo [11]. In these experiments, neutralisation of IL-10 in vitro resulted in the secretion of Ag-specific IFN-γ and IL-2 (*figure 1*), without altering levels of Ag-specific IL-4 or IL-5. Moreover, our recent results in IL-4 KO mice on the BALB/c background have shown that infection with L3 does indeed result in the production of Ag-specific IFN-γ and in significant reductions in levels of Ag-specific Th2 cytokines (Devaney et al., unpublished). Thus, when Ag-specific Th2 cells are primed in vivo in the presence of IL-4 (the intact BALB/c mouse), Ag-specific Th1 responses cannot be restored by neutralisation of IL-4 in vitro, but can be rescued by neutralising IL-10. These results suggest that IL-4 and IL-10 operate independently to inhibit Th1 activity in BALB/c mice infected with L3. In contrast, in the absence of IL-4 during T-cell priming in vivo (the IL-4 KO mouse on the BALB/c background), infection with L3 results in Ag-specific Th1 responses.

IL-10 is known to inhibit Th cell proliferation and IL-2 production directly or indirectly via its effect on APCs (reviewed in [13]). Direct evidence that infection with *Brugia* parasites can influence antigen presentation comes from the work of Allen and colleagues whose elegant studies have demonstrated that a profoundly suppressive APC population is elicited in mice implanted intraperitoneally with L3 or adults of *B. malayi* [14, 15]. In their model system, peritoneal exudate cells from infected mice suppress Ag-specific proliferation of a conalbumin-specific T-cell clone, but not cytokine secretion. In order to investigate the role of splenic APCs in the downregulation of polyclonal Th1 responses in our infection model, T cells from mice infected with L3 of *B. pahangi* were cultured with splenic APCs from noninfected BALB/c mice and cytokine and proliferative responses measured. Replacement of the 'infected' APC population resulted in a dramatic reversal of cytokine and proliferative responses [11]. No defect in polyclonal proliferative responses was observed, and the cytokine profile of L3 infected T cells was exclusively Th1 (i.e. Ag-specific IL-2 and IFN-γ was produced in the absence of Th2 cytokines). The precise phenotype of the Th1-suppressive APC population in our model system has yet to be defined but it was interesting to note that the same reversal of responses could be obtained using splenic APC from L3 infected animals, if these were irradiated prior to culture. Perhaps irradiation preferentially removes the suppressor cell, in which case, B cells are a possible candidate as these cells are more sensitive to γ irradiation than are other APC types [16].

Interestingly some studies in *W. bancrofti*-infected humans have implicated IL-10 in the downregulation of the Ag-specific proliferative response. In these experiments it was observed that PBMC from microfilaraemic individuals secreted high levels of spontaneous IL-10 in vitro and that neutralisation of IL-10 resulted in the production of Ag-specific IFN-γ, but had no effect on IL-5 production [17]. Furthermore, both the proliferative defect

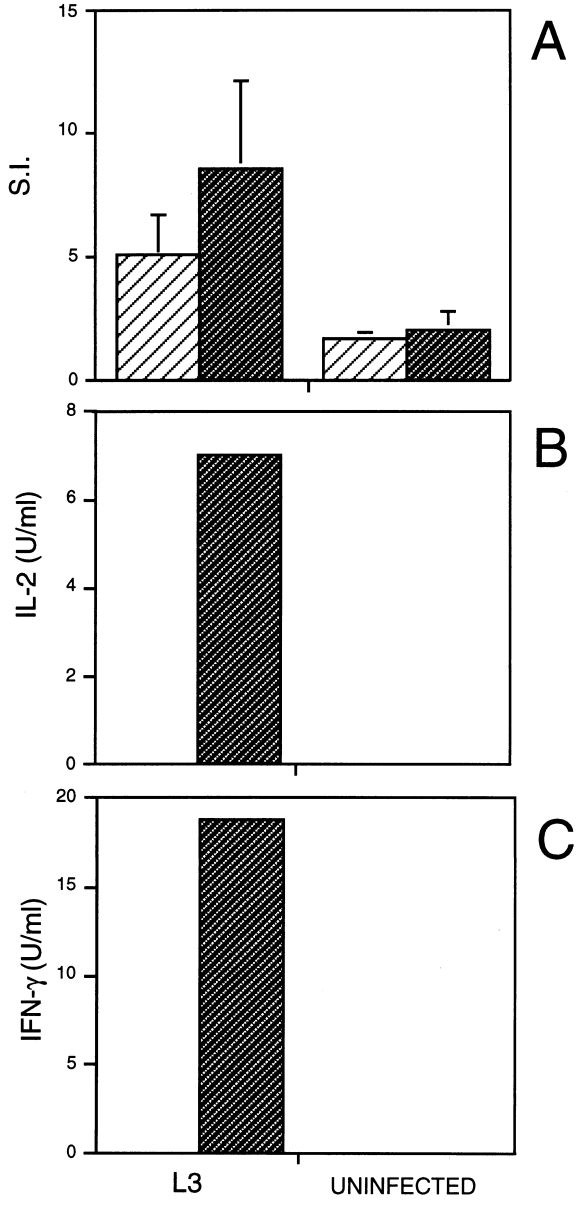

Figure 1. Neutralising IL-10 restores antigen-driven Th1 responses of spleen cells from mice infected with L3. BALB/c mice were injected s.c. with 50 L3 or Hanks balanced salt solution (HBSS) and spleens were removed at day 12 postinfection. Antigen-stimulated cultures were incubated with 10 μg/mL of either an isotype-matched control (R59-40, ▨) or anti-IL-10 (JES5-2A5, ▩) monoclonal antibody. Proliferation (A) and IL-2 (B) and IFN-γ (C) production were measured . **A.** The stimulation indices (SIs) shown are the mean ± SD of five animals per group. **B.** and **C.** Cytokine assays were performed on spleen cells pooled from five animals in each group. Neutralising IL-10 has no significant effect on Ag-specific proliferation (panel A), but does result in the production of Ag-specific IL-2 and IFN-γ, cytokines which are not observed in the absence of neutralising anti-IL-10 antibody. However, neutralisation of IL-10 does affect levels of Ag-specific IL-4 or IL-5. The results presented were comparable in two additional experiments. Data reproduced from Infection and Immunity, 67, 1599–1605.

and IL-10 production appeared to be dependent upon the source of Ags used: Ags from mixed sex adult worms or mf eliciting higher levels of IL-10 and lower levels of proliferation in microfilaraemic individuals compared to patients with pathology, while adult male Ags had no such effect [18]. These results again highlight the importance of stage-specific Ags, despite the fact that many of the Ags in different life cycle stages are shared. Most studies carried out to date in both humans and in the murine models have used crude soluble extracts of adult parasites to restimulate Ag-primed cells in vitro, and thus may overlook the effect of stage-specific Ags. A further complication arises from the realisation that many of these extracts may be contaminated with bacterial products derived from the *Wolbachia* endosymbiont of most filarial worms [19].

3. Early cytokine responses elicited by L3 – a role for innate immunity

The experiments reviewed above demonstrated that infection with L3 elicits both Ag-specific Th1 and Th2 cells in the mouse, but that Th1 responses are actively suppressed by IL-4, IL-10 and the APC population. Until recently little was known about the dynamics of the Th cell response following L3 infection. Are both Th1 and Th2 cells elicited from the outset of infection or is there a Th1–Th2 switch? In order to investigate these possibilities, we carried out a series of experiments in which mice were infected with *B. pahangi* L3 or mf via the footpad and cytokine responses in the draining popliteal lymph node were measured at early time points p.i. As the cytokine environment at the time of Ag priming has a profound effect on the T helper subset elicited, it was of interest to determine which cytokines were elicited at early time points p.i. Analysis of cytokine gene expression by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) demonstrated that the L3 elicited a dramatic burst of IL-4 transcription within 24 h of infection (see *figure 2*) [20]. The early IL-4 was specific to infection with L3 as mf did not induce significant amounts of IL-4 during the time course of the experiment (24 h to 7 days). In contrast, infection of BALB/c mice with mf elicited significant levels of IFN-γ mRNA transcription at 4 days p.i. in the popliteal lymph node (*figure 2*) consistent with the results from splenocyte culture following s.c. injection of mf. In these experiments in L3 infected mice, the only cytokine that was observed at early time points p.i. was IL-4, suggesting that there is no Th1–Th2 switch and that Th1 activity is immediately and actively suppressed.

In order to investigate the phenotype of the early IL-4 producer in our model system, a series of cell selection experiments were carried out. These experiments demonstrated that the early burst of IL-4 derived from a T-cell population (*figure 3*) which was shown to be an $\alpha\beta^+$ $CD4^-$ $CD8^-$, i.e. a double-negative T cell [20]. Further characterisation of the DN T-cell population demonstrated expression of TCR Vβ 2,7 and 8 chains (Osborne and Devaney, unpublished observations), suggesting that these cells may belong to the $NK1.1^+$ αβ T-cell family that preferentially uses those particular TCR chains. NK1.1 T cells are known

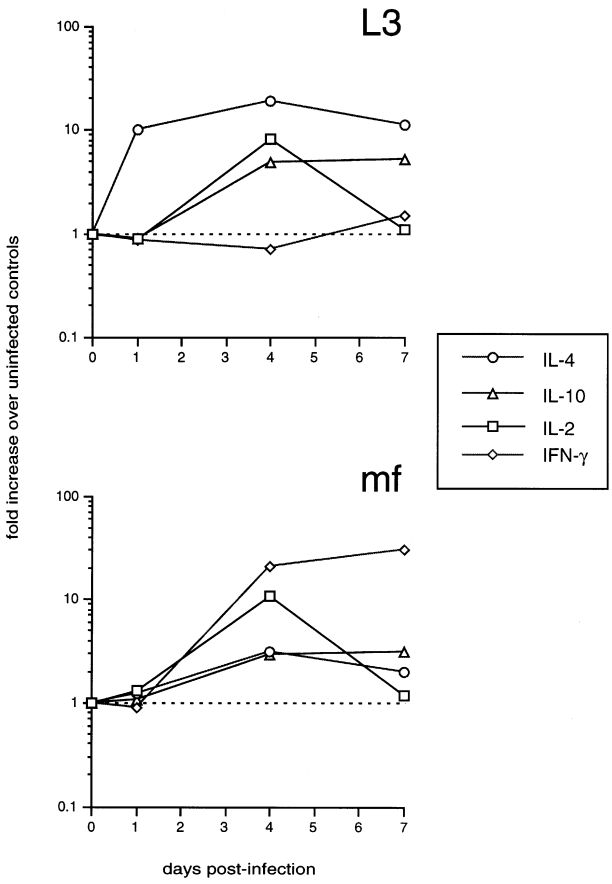

Figure 2. Cytokine mRNA expression pattern of L3 infected mice is dominated by IL-4 at early time points after infection. BALB/c mice were injected into the hind footpads with 30 L3 (top), 6×10^4 mf (bottom) or HBSS and popLN harvested at the indicated time points for RNA extraction. First strand cDNA was synthesised using random primers and PCR carried out with cytokine gene-specific primers or with β-actin. Gels were blotted and blots probed with appropriate cDNA fragments labelled with ^{32}P, subjected to autoradiography at –70 °C and quantified by densitometry. mRNA levels for each designated cytokine were expressed relative to the level in the popLN of uninfected animals, that were assigned a value of one. cDNA concentrations were standardised in individual samples by normalising to the constitutive gene β-actin, that did not show changes greater than 2-fold between compared samples. The results represent one of two comparable time course experiments. Data reproduced from International Immunology, 10, 1583–1590.

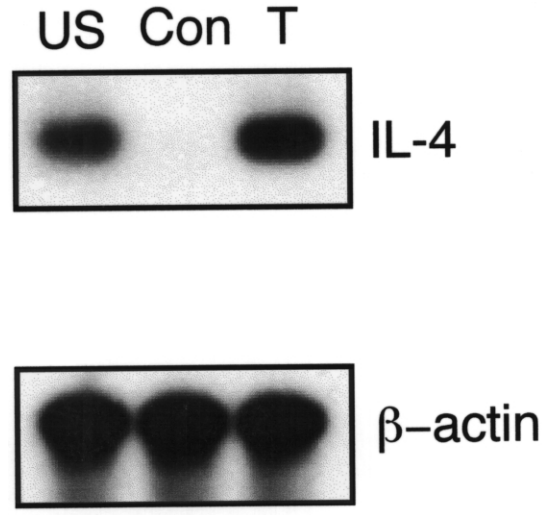

Figure 3. T cells are the source of the IL-4 burst at 24 h p.i. with *B. pahangi* L3. BALB/c mice were injected into the hind footpads with 30 L3 or HBSS and popliteal lymph nodes (popLN) harvested after 24 h. T cells were purified from popLN from infected animals using T-cell enrichment columns (R & D Systems). The T-cell-enriched population was 95.5% $CD3^+$ as assessed by FACS. Total RNA, isolated from unseparated popLN cells from infected (US) and uninfected animals (Con) and from the T-cell-enriched population (T), was analysed for the expression of IL-4 (upper panel) and β-actin (lower panel) mRNA by RT-PCR. Representative autoradiographs are shown.

to be a potent source of early IL-4. The best characterised of the family are $CD4^+$ NK1.1 T cells that secrete IL-4 in response to intravenous (i.v.) injection of anti-CD3 or bacterial superantigen [21]. When BALB/c mice were injected i.v. with L3 of *B. pahangi*, IL-4 was produced in the spleen within 6 h of injection (*figure 4*). As BALB/c mice do not express the NK1.1 marker, it was not possible in our original studies to formally prove that the early source of IL-4 was an NK T cell. Our more recent studies in C57Bl/6 mice suggest that NK T cells are indeed the major producers of IL-4 at 24 h p.i. (Balmer and Devaney, unpublished observations). NK T cells can produce either IFN-γ or IL-4 upon primary stimulation and thus may have an important role in the initial polarisation of immune responses. However, their role in induction of Th2 responses remains controversial, as several studies have shown that NK T cells are not absolutely critical for the evolution of a Th2 response [22, 23]. The variety of early IL-4 sources that have been described following challenge with different antigenic stimuli serves to emphasise the fact that both Ag and route of infection/immunisation will influence the cell type that is activated. Amongst helminth parasites, eosinophils [24], γδ T cells [25] and DN T cells [20] have all been implicated as early IL-4 producers.

Although Th2 responses are a hallmark of many helminth infections, little is known of the parasite Ags responsible for eliciting these responses. We are particularly interested in the possibility that glycolipid antigens released from the L3 may be central to the early IL-4 response. Work in humans first demonstrated that DN T cells recognise the MHC I-related, nonpolymorphic molecule CD1 [26] and that CD1b and CD1c present lipid rather than peptide antigens to T cells. For example, CD1-restricted DN T-cell lines specific for the mycobacterial lipids, mycolic acid and lipoarabinomannan have been described [27, 28]. Moreover, it has been proposed that CD1-restricted DN T cells derived from the skin lesions of leprosy patients are an important factor in determining the Th cell response to *Mycobacterium leprae* via their specific cytokine production [29]. A number of recent

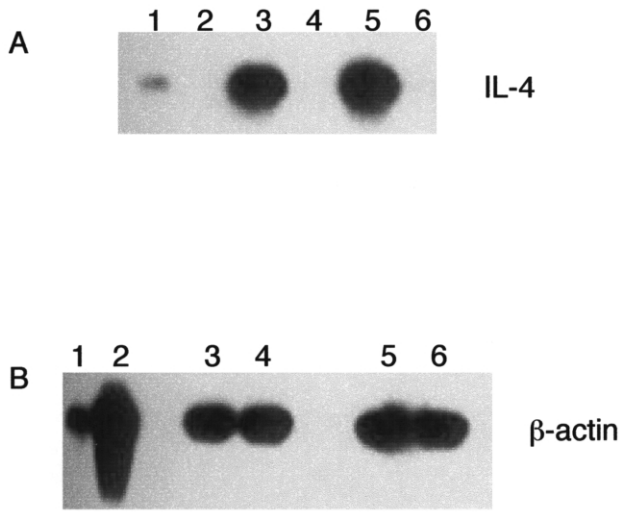

Figure 4. Injection of *B. pahangi* L3 i.v. results in the expression of IL-4 mRNA in the spleen within 6 h of infection. BALB/c mice were injected with 50 L3 of *B. pahangi* or HBSS into the tail vein and spleens removed for RNA extraction at 2 h (lanes 1 and 2), 6 h (lanes 3 and 4) and 24 h (lanes 5 and 6) postinfection. RT-PCR was carried out on cDNA as described in the legend to *figure 2* for IL-4 (upper panel) and β-actin (lower panel). The β-actin signal indicates intact mRNA in all lanes. Lanes 1, 3 and 5 are from L3-infected mice, lanes 2, 4 and 6 are from HBSS-injected mice.

studies have focused on the activation of murine NK T cells by glycosylphosphatidyl inositols (GPIs) [30, 31] and by the synthetic glycolipid, α-galactosylceramide (α-GalCer) presented by CD1d [32]. Injection of α-GalCer results in rapid activation of NK T cells and while both IL-4 and IFN-γ are produced at early time points [33], subsequent exposure to α-GalCer promotes differentiation into an IL-4 producing phenotype [34]. The surface of the L3 contains both lipid and carbohydrate moieties [35] and components of the surface are known to rapidly turnover within 24 h of entry into the mammalian host [36]. Non-protein surface components of the L3 have also been implicated in protective immunity [8]; these Ags, which could not be identified as conventional protein/glycoproteins, were predominantly recognised by IgM antibody and may be glycolipid or carbohydrate. Could the L3 present a lipid antigen that perhaps mimics the conformation of α-GalCer?

What is the significance of the early IL-4 production for parasite and host? While the resulting Th2 response may promote parasite survival and/or influence the development of protective immunity (see below), another alternative is that IL-4 is induced early on in infection as a response to parasite-induced inflammation. Many of the human CD1-restricted DN T-cell lines and also CD1-specific T cells from normal unimmunised mice appear to be autoreactive, in that they recognise $CD1^+$ cells without foreign antigen. It has been suggested that the upregulation of CD1 expression alone or in association with self-peptide induced by the stress of inflammation and/or infection may constitute an internal 'danger' signal that then promotes anti-inflammatory responses through the early secretion of IL-4 [37]. Certainly histological sections taken at the site of injection revealed that the L3 induce an inflammatory response which gradually extends from the palmar surface (the site of injection) to the dorsal surface of the foot. *Figure 5*, panel a, shows a section through an infected footpad at 4 days p.i. demonstrating areas of oedematous and inflamed tissue. The lymphatic vessels are grossly dilated (arrowed), contain proteinaceous material and are surrounded by an inflammatory focus spreading towards the dorsal surface of the footpad. Panel b shows a similar section through the footpad at 7 days p.i. to demonstrate the presence of larvae within a dilated lymphatic vessel (arrowed). While these studies suggest that L3 induce an inflammatory response in the lymphatics, restimulation of popliteal lymph node cells in vitro with parasite Ag failed to elicit detectable levels of pro-inflammatory cytokines. In addition, these experiments demonstrate the tropism of the L3 for the lymphatics even in a host which is not fully permissive to infection.

4. L3 and protective immunity – possible mechanisms?

The evidence in humans for an immune response directed against L3 comes both from the observation that varying percentages of individuals in endemic areas remain infection-free and from the studies that support the concept of concomitant immunity in lymphatic filariasis. Endemic normals are identified as mf-negative, CFA-negative with no clinical signs of infection. Concomitant immunity is an attractive concept in lymphatic filariasis: in endemic communities, the rate of gain of infection peaks in the 16–20 year age class, supporting the concept of an age-acquired immune response [38]. Experimental data in support of this hypothesis come from the study of Day et al. [7] in Papua New Guinea who demonstrated an age-related gain of infection (as assessed by levels of CFA) up to around 20 years of age. A subsequent study correlated the apparent resistance to new infection with the presence of antibody to the L3 surface [8]. In an important review, Maizels and Lawrence [39] proposed that downregulation of Th1 proliferative responses in the human was a stage-specific phenomenon, restricted to adult or mf Ags, and that the development of pathology was associated with the breakdown of this tolerance. Conversely, as the L3 are the target of protective immune responses, it should be possible to dissociate protective immune responses from those associated with the development of pathology.

Studies in both *Wuchereria bancrofti* [40] and *Onchocerca volvulus* [41] endemic areas have associated the apparent lack of infection in endemic normals with heightened Th1-like responses compared to microfilaraemic individuals. However, whether these individuals are infection-free because of their ability to mount a Th1 response or whether the heightened Th1 response reflects the absence of the Th2-polarising adult parasite remains unclear. While active infection with filarial parasites is clearly associated with Th2 responses, it does not necessarily follow that resistance to infection is associated with

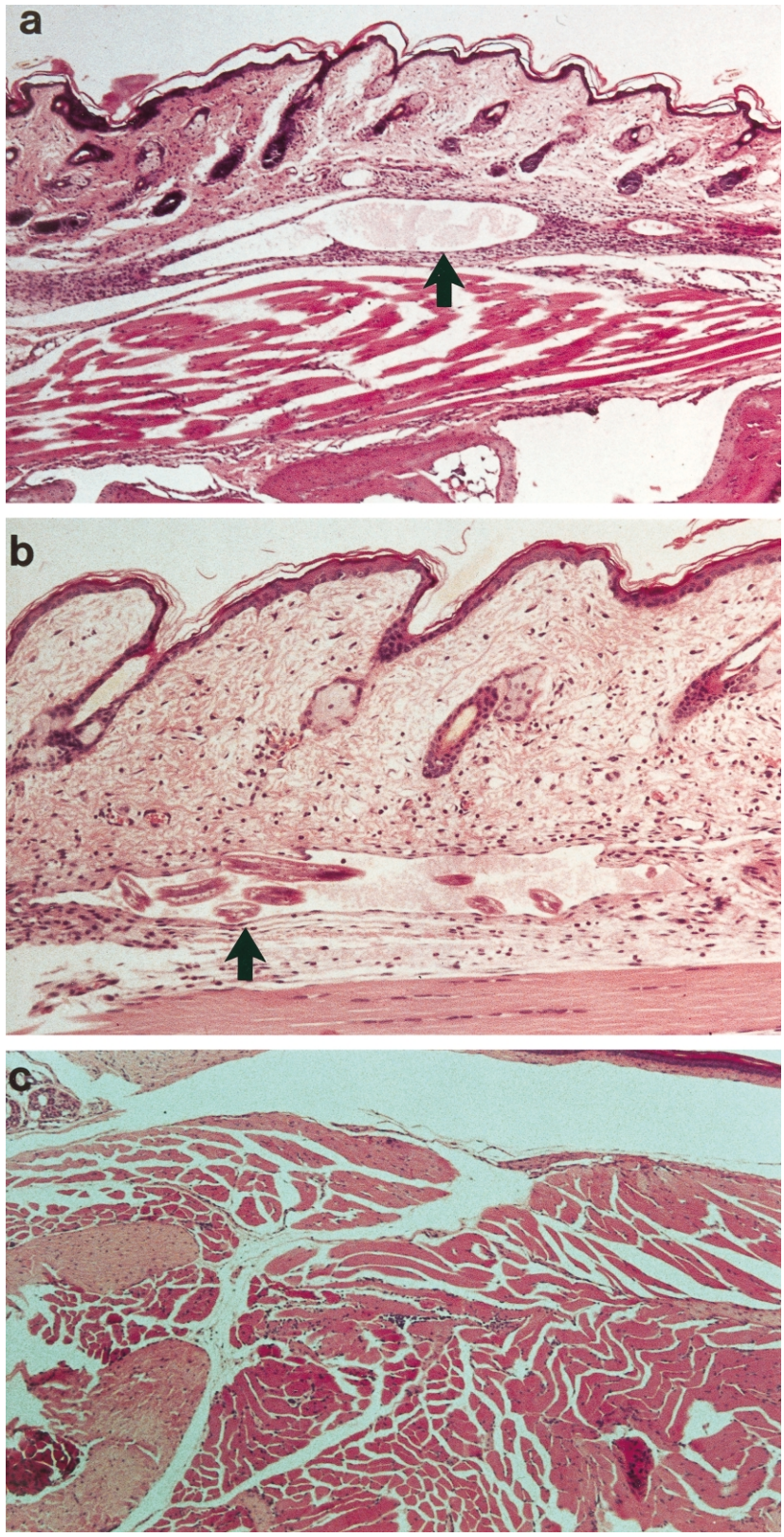

Figure 5. Injection of L3 into the footpad of BALB/c mice elicits an inflammatory response and results in localisation of the larvae in the lymphatics. BALB/c mice were injected into the footpad with 50 L3 of *B. pahangi* or HBSS. At various times postinfection, mice were killed and the footpads removed and processed for histology using standard methods. Panel **a** shows a section through an infected footpad at 4 days p.i., with a dilated lymphatic (arrowed) surrounded by an inflammatory focus. Panel **b** shows a section through a footpad at 7 days p.i. to illustrate a dilated lymphatic vessel containing larvae (arrowed). Panel **c** shows a control footpad at 4 days p.i.

Th1 responses; an L3-specific Th2-associated response could still be important in the development of anti-L3 immunity. Epidemiological studies have demonstrated that in most endemic areas, very few L3 are delivered with each infective bite; thus it may take many infective bites over a prolonged period of time to build up immunity targeted at the L3 [8]. Furthermore, as noted by Allen and Maizels [42], the strict delineation between Th1/Th2 responses may not be helpful in the context of a human infection and it may be more appropriate to define protective immunity in terms of specific effector mechanisms.

Most experiments in mouse models of lymphatic filariasis have supported a role for Th2-associated responses in protective immunity following vaccination. The paradigm for eliciting protective anti-L3 responses in animal models of lymphatic filariasis is the use of irradiated L3 [43]. Irradiated parasites do not develop to sexual maturity [44], irradiation having a lethal effect on male worms with the consequence that the surviving females do not become patent. Immunisation of BALB/c mice with irradiated L3 of *B. pahangi* stimulates high levels of resistance to challenge infection, which is T-cell-dependent [45]. In our studies in which two doses of irradiated L3 were administered s.c., immunity to challenge infection was correlated with a pronounced Th2 response, characterised by high levels of Ag-specific IL-4, IL-5 and IL-9 but no Ag-specific IFN-γ [46]. Depletion of $CD4^+$ cells ablated protection and was correlated with a significant reduction in production of Th2 cytokines [47]. However, the exact mechanisms by which challenge L3 are killed remain unclear. Immunised mice have high levels of circulating eosinophils [46] and make antibody to the L3 surface and studies in other models have suggested that L3 may be killed via an antibody-dependent cell cytoxicity reaction [48]. Similar results have been reported from other mouse models of filariasis, e.g. immunity to *Onchocerca volvulus* L3 appears to be IL-4- and IL-5-dependent [49]. How do we reconcile the data from the mouse models with that from the human studies? Do the contrasting results reflect fundamental differences in the mechanisms of immunity in the different hosts? Are the results in the mouse an artefact of the strong Th2 polarising ability of the L3 in inbred strains of mice which quickly become polarised? The observation that L3 can induce Ag-specific Th1 cells under certain conditions in the mouse (as reviewed above) provides an experimental system for determining whether Th1-mediated responses may have a role in protection in the mouse model.

The mechanisms underlying vaccine-induced immunity to the L3 have been somewhat obscured by studies which have investigated the survival of L3 following a primary infection. Recent studies in a variety of KO mice have yielded conflicting results; some of these studies have suggested that neither CD4, CD8 nor B cells play a role in resistance to primary infection but a more recent analysis of these data has suggested that differences in the susceptibility of the background strain of KO animals used may account for some of these results [50]. When IL-4 KO mice on the 129xC57Bl/6 background were infected with *B. malayi* via the i.p. route, no difference was observed in the recovery of L3 at early time points p.i. demonstrating that the survival of a primary infection with L3 is not IL-4-dependent [12, 51]. However, IL-4 KO mice on this background infected s.c. with *B. pahangi* L3 make very high levels of IL-13 (McAdam and Devaney, unpublished observations), a cytokine which shares some biological activities with IL-4 [52]. Other studies have proposed a role for inducible nitric oxide in resistance to primary i.p. infection with *B. malayi* L3 [53]. However, in our hands we have failed to observe iNOS activity in BALB/c mice infected with L3 by the i.v or s.c. routes, whereas in microfilarial infections, large amounts of NO are generated (O'Connor et al., unpublished). In conclusion, the effector mechanisms by which L3 are killed following a primary infection are far from certain even in the mouse models. It is important to acknowledge that the mechanisms by which L3 are cleared in a primary infection may well be quite different from those in an immunised animal and are likely to be dependent upon variables such as the route of infection, the strain of mouse used and perhaps even the species of parasite.

5. Conclusions and future perspectives

The L3 of filarial nematodes is the critical life cycle stage for infection and is also the target of protective immunity, yet both infection and immunity appear to involve induction of IL-4, at least in mouse models. Infection with L3 induces a potent Th2 response and actively suppresses Th1 responses by mechanisms involving IL-4, IL-10 and APCs, but the significance of this for both the parasite and the host remains unclear. Are Th2-driven responses essential for protective immunity or does the Th2 response in immunised mice merely reflect the early activation of Th2 cells in these animals? Could polarisation in the Th2 direction promote parasite survival by downregulating protective Th1-type responses? These questions are difficult to answer in the *Brugia*-mouse models currently available because these are not fully permissive to infection. In this respect, studies on *Litomosides sigmondontis* in the mouse may prove more illuminating. In the infected human, Th2 responses are likely to be dominated by the presence of adult parasites making it difficult to identify L3-specific protective responses and the Ag which drive these responses.

Likewise, the L3-specific antigens responsible for eliciting the burst of early IL-4 have yet to be characterised. The availability of the *B. malayi* EST data base (http://helios.bto.ed.ac.uk/mbx/fgn/ests.html) represents a significant resource for the identification of cDNAs which are exclusively expressed in the L3 or are highly upregulated in this life cycle stage and ongoing studies in other laboratories have identified likely L3-specific antigens as candidates for the induction of protective immunity (R.M. Maizels, personal communication). It will be of considerable interest to determine the mechanisms by which these Ags stimulate immunity to reinfection in model systems and to investigate whether they are preferentially recognised by endemic normal individuals. The role of carbohydrate or lipid antigens in the induction of Th2 responses is another area of current interest in filarial immunology and

one which should yield significant advances in the next few years. Whether nematode Ags possess equivalents of 'pattern recognition domains' observed in prokaryotes is also an interesting question which is being actively addressed in a number of laboratories.

The host–parasite relationship in filariasis is complex and highly evolved. These parasites have developed a multiplicity of mechanisms for preventing elimination, some of which may also protect the host from disease. Defining the ways by which these parasites modulate immune responses is important for our understanding of both protective immunity and pathology in the infected human but also has the potential to influence thinking in the wider field of basic immunology.

Acknowledgments

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References

[1] Michael E., Bundy D.A., Grenfell B.T., Re-assessing the global prevalence and distribution of lymphatic filariasis, Parasitology 112 (1996) 409–428.

[2] Ottesen E.A., Infection and disease in lymphatic filariasis: an immunological perspective, Parasitology 104 (1992) S71–S79.

[3] Yazdanbakhsh M., Paxton W.A., Kruize Y.C.M., Sartono E., Kurniawan A., Van Het Wout A., Selkirk M.E., Partono F., Maizels R.M., T cell responsiveness correlates differentially with antibody isotype levels in clinical and asymptomatic filariasis, J. Infect. Dis. 167 (1993) 925–931.

[4] Sartono E., Kruize Y.C.M., Kurniawan A., Maizels R.M., Yazdanbakhsh M., Depression of antigen-specific Interleukin-5 and Interferon-γ responses in human lymphatic filariasis as a function of clinical status and age, J. Infect. Dis. 175 (1997) 1276–1280.

[5] Sartono E., Lopriore C., Kruize Y.C.M., Kurniawan-Atmadja A., Maizels R.M., Yazdanbakhsh M., Reversal in microfilarial density and T cell responses in human lymphatic filariasis, Parasite Immunol. 21 (1999) 565–571.

[6] Vanamail P., Ramaiah K.D., Pani S.P., Das P.K., Grenfell B.T., Bundy D.A., Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area, Trans. Roy. Soc. Trop. Med. Hyg. 90 (1996) 119–121.

[7] Day K.P., Grenfell B.T., Spark R., Kazura J.W., Alpers M.P., Age-specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea, Am. J. Trop. Med. Hyg. 44 (1991) 518–527.

[8] Day K.P., Gregory W.F., Maizels R.M., Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea, Parasite Immunol. 13 (1991) 277–290.

[9] Lawrence R.A., Allen J.E., Osborne J., Maizels R.M., Adult and microfilarial stages of the filarial parasite *Brugia malayi*, stimulate contrasting cytokine and Ig responses in BALB/c mice, J. Immunol. 154 (1995) 1216–1224.

[10] Osborne J., Hunter S.J., Devaney E., Anti-interleukin-4 modulation of the Th2 polarized response to the parasitic nematode *Brugia pahangi*, Infect. Immun. 64 (1996) 3461–3466.

[11] Osborne J., Devaney E., Interleukin-10 and antigen-presenting cells actively suppress Th1 cells in BALB/c mice infected with the filarial parasite *Brugia pahangi*, Infect. Immun. 67 (1999) 1599–1605.

[12] Lawrence R.A., Allen J.E., Gregory W.F., Kopf M., Maizels R.M., Infection of IL-4-deficient mice with the parasitic nematode *Brugia malayi* demonstrates that host resistance is not dependent on a T helper 2-dominated immune response, J. Immunol. 154 (1995) 5995–6001.

[13] Moore K.W., O'Garra A., De Waal Malefyt R., Vieira P., Mosmann T.R., Interleukin-10, Ann. Rev. Immunol. 11 (1993) 165–190.

[14] Allen J.E., Lawrence R.A., Maizels R.M., APC from mice harbouring the filarial nematode, *Brugia malayi*, prevent cellular proliferation but not cytokine production, Int. Immunol. 8 (1996) 143–151.

[15] Macdonald A.S., Maizels R.M., Lawrence R.A., Dransfield I., Allen J.E., Requirement for in vivo production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites, J. Immunol. 160 (1998) 1304–1312.

[16] Sopori M.L., Cohen D.A., Cherian S., Perrone R.S., Kaplan A.M., Antigen presentation in the rat. II. An Ia^+ radiosensitive T cell can present antigen to primed Ia- T cells, J. Immunol. 134 (1985) 1369–1373.

[17] Mahanty S., Ravichandran M., Raman U., Jayaraman K., Kumaraswami V., Nutman T.B., Regulation of parasite antigen-driven immune responses by Interleukin-10 (IL-10) and IL-12 in lymphatic filariasis, Infect. Immun. 65 (1997) 1742–1747.

[18] Mahanty S., Luke H.E., Kumaraswami V., Narayanan P.R., Vijayshekaran V., Nutman T.B., Stage-specific induction of cytokines regulates the immune response in lymphatic filariasis, Exp. Parasitol. 84 (1996) 282–290.

[19] Taylor M.J., Hoerauf A., *Wolbachia* bacteria of filarial nematodes, Parasitol. Today 15 (1999) 437–442.

[20] Osborne J., Devaney E., The L3 of *Brugia* induces a Th2-polarized response following activation of an IL-4-producing CD4-CD8-αβ T cell population, Int. Immunol. 10 (1998) 1583–1590.

[21] Yoshimoto T.O., Paul W.E., $CD4^+$, $NK1.1^+$ T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3, J. Exp. Med. 179 (1994) 1285–1295.

[22] Brown D.R., Fowell D.J., Corry D.B., Wynn T.A., Moskowitz N.H., Cheever A.W., Locksley R.M., Reiner S.L., β2-Microglobulin-dependent $NK\ 1.1^+$ T cells are not essential for T helper cell 2 immune responses, J. Exp. Med. 184 (1997) 1295–1304.

[23] Chen Y.H., Chiu N.M., Mandal M., Wang N., Wang C.R., Impaired $NK1^+$ T cell development in and early IL-4 responses in CD1-deficient mice, Immunity 6 (1997) 459–467.

[24] Sabin E.A., Pearce E.J., Early IL-4 production by non-$CD4^+$ cells at the site of antigen deposition predicts the development of a T helper 2 cell response to *Schistosoma mansoni* eggs, J. Immunol. 155 (1995) 4844–4853.

[25] Ferrick D.A., Schrenzel M.D., Mulvania T., Hsieh B., Ferlin W.G., Lepper H., Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens in vivo, Nature 373 (1995) 255–257.

[26] Porcelli S., Morita C.T., Brenner M.B., CD1b restricts the response of human CD4-8-T lymphocytes to a microbial antigen, Nature 360 (1992) 593–597.

[27] Beckman E.M., Porcelli S.A., Morita C.T., Behar S.M., Furlong S.T., Brenner M.B., Recognition of a lipid antigen by CD1-restricted $\alpha\beta^+$ T cells, Nature 372 (1994) 691–694.

[28] Sieling P.A., Chatterjee D., Porcelli S.A., Prigozy T.I., Mazzaccaro R.J., Soriano T., Bloom B.R., Brenner M.B., Kronenberg M., Brennan P.J., Modin R.L., CD1-restricted T cell recognition of microbial lipoglycan antigens, Science 269 (1995) 227–230.

[29] Porcelli S.A., Morita C.T., Modlin R.L., T cell recognition of non-peptide antigens, Curr. Opin. Immunol. 8 (1996) 510–516.

[30] Joyce S., Woods A.S., Yewdell J.W., Bennink J.R., De Silva A.D., Boesteanu A., Balk S.P., Cotter R.J., Brutkiewicz R.R., Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol, Science 279 (1998) 1541–1544.

[31] Schofield L., McConville M.J., Hansen D., Campbell A.S., Fraser-Reid B., Grusby M.J., Tachado S.D., CD1d-restricted immunoglobulin G formation to GP1-anchored antigens mediated by NKT cells, Science 283 (1999) 225–229.

[32] Kawano T., Cui J., Koezuka Y., Toura I., Kaneko Y., Motoki K., Ueno H., Nakagawa R., Sato H., Kondo E., Koseki H., Taniguchi M., CD1d-restricted and TCR-mediated activation of V α 14 NKT cells by glycosylceramides, Science 278 (1997) 1626–1629.

[33] Singh N., Hong S., Scherer D.C., Zerizawa I., Burdin N., Kronenberg M., Koezuka Y., Vann Kaer L., Activation of NK T cells by CD1d and α-galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype, J. Immunol. 163 (1999) 2373–2377.

[34] Burdin N., Brossay L., Kronenberg M., Immunization with α-galactosylceramide polarizes CD1-reactive NK T cells towards Th2 cytokine synthesis, Eur. J. Immunol. 29 (1999) 2014–2025.

[35] Proudfoot L., Kusel J.R., Smith H.V., Kennedy M.W., Biophysical properties of nematode surface, in: Kennedy M.W. (Ed.), Parasite Nematodes-Antigens, Membranes and Genes, Taylor & Francis Ltd, London, 1991, pp. 1–26.

[36] Carlow C.K.S., Perrone J., Spielman A., Phillip M., A developmentally regulated surface epitope expressed by the infective larva of *Brugia malayi* which is rapidly lost after infection, Molecular paradigms for eradicating helminthic parasites, Alan R. Liss Inc., New York, 1987, pp. 301–310.

[37] Bendelac A., CD1: presenting unusual antigens to unusual T lymphocytes, Science 269 (1995) 185–186.

[38] Vanamail P., Subramanian S., Das P.K., Pani S.P., Rajagopalan P.K., Bundy D.A., Grenfell B.T., Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection, Trans. Roy. Soc. Trop. Med. Hyg. 83 (1989) 689–693.

[39] Maizels R.M., Lawrence R.A., Immunological Tolerance: The key feature in human filariasis? Parasitol. Today 7 (1991) 271–276.

[40] Dimock K.A., Eberhard M.L., Lammie P.J., Th1-like antifilarial immune responses predominate in antigen-negative persons, Infect. Immun. 641 (1996) 2962–2967.

[41] Elson L.H., Calvopina H.M., Paredes Y.W., Araujo N.E., Bradley J.E., Guderian R.H., Nutman T.B., Immunity to onchocerciasis: Putative immune persons produce a Th1-like response to *Onchocerca volvulus*, J. Infect. Dis. 171 (1995) 652–658.

[42] Allen J.E., Maizels R.M., Th1-Th2: reliable paradigm or dangerous dogma, Immunol. Today 8 (1997) 387–392.

[43] Oothuman P., Denham D.A., McGreevy P.B., Nelson G.S., Rogers R., Successful vaccination of cats against *Brugia pahangi* with larvae attenuated with 10 kRad cobalt 60, Parasite Immunol. 1 (1979) 209–216.

[44] Devaney E., Bancroft A.J., Egan A., The effect of irradiation on the third stage larvae of *Brugia pahangi*, Parasite Immunol. 15 (1993) 423–427.

[45] Hayashi Y., Nogami S., Nakamura M., Shirasaka A., Noda K., Passive transfer of protective immunity against *Brugia malayi* in BALB/c mice, Jpn. J. Exp. Med. 54 (1984) 183–187.

[46] Bancroft A.J., Grencis R.K., Else K.J., Devaney E., Cytokine production in BALB/c mice immunized with radiation attenuated third stage larvae of the filarial nematode, *Brugia pahangi*, J. Immunol. 150 (1993) 1395–1402.

[47] Bancroft A.J., Grencis R.K., Else K.J., Devaney E., The role of $CD4^+$ cells in protective immunity to *Brugia pahangi*, Parasite Immunol. 16 (1994) 385–387.

[48] Yates J.A., Higashi G.I., Ultrastructural observations on the fate of *Brugia* malayi in jirds previously vaccinated with irradiated infective stage larvae, Am. J. Trop. Med. Hyg. 35 (1986) 982–987.

[49] Lange A.M., Yutanawiboonchai W., Scott P., Abraham D., IL-4 and IL-5-dependent protective immunity to *Onchocerca volvulus* infective larvae in BALB/cBYJ mice, J. Immunol. 153 (1994) 205–211.

[50] Rajan T.V., Babu S., Sardinha D., Smith H., Ganley L., Paciorkowski N., Porte P., Life and death of *Brugia malayi* in the mammalian most: Passive death vs active killing, Exp. Parasitol. 93 (1999) 120–122.

[51] Lawrence R.A., Lymphatic filariasis: What mice can tell us, Parasitol. Today 12 (1996) 267–271.

[52] Zurawski G., De Vries J.E., Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells, Immunol. Today 15 (1994) 19–26.

[53] Rajan T.V., Porte P., Yates J.A., Keefer L., Shultz L.D., Role of nitric oxide in host defence against an extracellular metazoan parasite, *Brugia malayi*, Infect. Immun. 64 (1996) 3351–3353.