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International Journal for Parasitology 32 (2002) 1277–1284

The Australian Society for Parasitology Inc.
INTERNATIONAL Journal for PARASITOLOGY

www.parasitology-online.com

IL-4 is required to prevent filarial nematode development in resistant but not susceptible strains of mice

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Received 8 May 2002; received in revised form 11 June 2002; accepted 11 June 2002

Abstract

The murine *Litomosoides sigmodontis* model of filarial infection provides the opportunity to elucidate the immunological mechanisms that determine whether these nematode parasites can establish a successful infection or are rejected by the mammalian host. BALB/c mice are fully susceptible to *L. sigmodontis* infection and can develop patent infection, with the microfilarial stage circulating in the bloodstream. In contrast, mice on the C57BL background are largely resistant to the infection and never produce a patent infection. In this study, we used IL-4 deficient mice on the C57BL/6 background to address the role of IL-4 in the development of *L. sigmodontis* parasites in a resistant host. Two months after infection, adult worm recovery and the percentage of microfilaraemic mice in infected IL-4 deficient mice were comparable with those of the susceptible BALB/c mice while, as expected, healthy adults were not recovered from wild type C57BL/6 mice. The cytokine and antibody responses reveal that despite similar parasitology the two susceptible strains (BALB/c and IL-4 deficient C57BL/6) have markedly different immune responses: wild type BALB/c mice exhibit a strong Th2 immune response and the IL-4 deficient C57BL/6 mice exhibit a Th1 response. We also excluded a role for antibodies in resistance through infection of B-cell deficient C57BL/6 mice. Our data suggest that the mechanisms that determine parasite clearance in a resistant/non-permissive host are Th2 dependent but that in a susceptible/permissive host, the parasite can develop in the face of a Th2 dominated response.

Keywords: *Litomosoides*; Th1/Th2; Murine filariasis

1. Introduction

The induction of Th2 cytokine responses is a well-documented feature of infection with helminth parasites. For intestinal nematode infections, the critical importance of these Th2 responses in mediating parasite expulsion from the gut has been established (Finkelman et al., 1997). Remarkably, for tissue helminths such as filarial nematodes that inhabit the skin, lymphatics or body cavities, the role of type 2 cytokines is still not resolved. Recent studies have suggested that, as with intestinal parasites, IL-4 and/or IL-13 may be important for the elimination of filarial parasites (Babu et al., 2000; Devaney et al., 2002; Volkmann et al., 2001). These studies are complicated by the fact that different cytokine pathways may be required to varying degrees at different stages of infection and that immune mechanisms may differ depending on the susceptibility/permissiveness of the murine host.

The rodent filarial nematode *Litomosoides sigmodontis* provides an excellent model system to dissect these pathways as it undergoes the full developmental cycle in inbred laboratory mice. Infection of BALB/c mice with vector-derived larvae leads to patent infection, with the microfilarial stage circulating in the bloodstream (Hoffmann et al., 2000). In contrast, mice on the C57BL background are resistant to infection; larval development rarely occurs past the second moult and mice never develop patent infection (Hoffmann et al., 2000). A striking feature of this model system is that the susceptibility/resistance patterns among mouse genotypes are analogous to infection with the protozoan parasite, *Leishmania major*, in which IL-4 mediates susceptibility, and is in contrast to infection with intestinal nematodes such as *Trichuris muris*, in which IL-4 is critical for parasite elimination (Finkelman et al., 1997; Reiner and Locksley, 1995). These observations suggested the possibility that IL-4 is not the key element in elimination of filarial parasites but may actually facilitate parasite establishment. We thus decided to assess the role of IL-4 in resistance to filarial infection by infecting IL-4 deficient mice on the resistant C57BL/6 background. Our data suggest that in

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0020-7519/02/$20.00
PII: S0020-7519(02)00125-X

fact IL-4 is the critical factor that prevents establishment of infection but that in a susceptible host, the parasite is able to circumvent the destructive Th2 mediated pathways.

2. Materials and methods

2.1. Parasites and mouse infection model

Maintenance of the filaria *L. sigmodontis* and recovery of infective larvae from the infected mites, *Ornithonyssus bacoti*, were carried out as described previously (Le Goff et al., 2000a). IL-4 deficient C57BL/6 breeding pairs were purchased from B&K Universal Ltd with the permission of the Institute of Genetics, University of Cologne, Cologne, Germany. B cell deficient μMT mice (Kitamura et al., 1991) were the kind gift of D. Gray (Edinburgh University, UK). Gene-deficient and wild type (WT) BALB/c and C57BL/6 mice were bred in-house.

All mice used were 6–8 week old males at the start of the experiment. Age-matched groups of deficient and WT mice were injected s.c. (or i.p., as indicated in the text) with 25 infective larvae. Necropsies were performed 20, 40 or 60 days p.i., at which point sera were collected and the spleens were removed for use in cellular assays. For all experiments, the number of recovered worms was counted as described previously (Bain et al., 1994; Le Goff et al., 2000b). Microfilaraemia was determined 60 days p.i. following the method of Petit et al. (1992).

2.2. Histology

Encysted worms were embedded in Cryo-M-Bed embedding compound (Bright Instrument Company Ltd) and frozen at −80°C. Frozen sections (5 μm thick) were fixed with methanol and stained with Giemsa stain, modified (Sigma).

2.3. Antigen preparation

Somatic extracts of mixed sex adult *L. sigmodontis* worms were prepared by homogenisation and centrifugation in phosphate buffered saline (PBS) as described previously (Le Goff et al., 2000a) and the protein concentration determined by the Coomassie Plus protein assay (Pierce). This *L. sigmodontis* antigen was used in both cytokine and antibody assays.

2.4. Cytokine assays

Splenocytes were cultured in Dulbecco's Modified Eagle's Medium (Sigma) supplemented with 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% foetal calf serum (complete medium). Spleens were teased to single-cell suspension and erythrocytes were removed by incubation with erythrocyte lysis buffer (Sigma). Whole splenocytes were incubated at 5×10^6 cells/ml with *L. sigmodontis* antigen or with concanavalin A (Sigma) at final concentrations of 10 μg/ml. After 72 h incubation at 37°C, 100 μl of supernatant was taken for cytokine assays. IL-4, IL-10 and IFNγ cytokine concentrations were determined by capture enzyme-linked immunosorbent assay (ELISA) with reference to standard curves of recombinant IL-4 (Sigma), IL-10 (Genzyme) and IFNγ (Sigma). The capture and detection antibodies used were: IFNγ, R46A2 and XMG1.2; IL-10, JES5-2A5 and SXC-1 (Pharmingen, Becton Dickinson, Ltd). IL-4 assays were performed as described by Holland et al. (2000). Cytokine production was assessed after mitogenic (ConA) stimulation at all time points after the infection (20, 40 and 60 days p.i.) and the levels of these cytokines were normal (data not shown).

2.5. Antibody isotype analysis

Measurement of parasite-specific immunoglobulin isotype levels was done as described previously (Le Goff et al., 2000a). Briefly, microtitre plates were coated with 5 μg/ml *L. sigmodontis* antigen. After blocking and incubation with sera (1:200), plates were incubated with isotype-specific anti-mouse immunoglobulin antibodies conjugated with HRP (Southern Biotechnology Associates Inc.). Plates were developed using the ABTS substrate system (KPL Biotechnology). A capture antibody ELISA was used to measure total serum IgE as described previously (Holland et al., 2000).

2.6. Statistical analysis

The two-tailed Kruskal Wallis non-parametric analysis of variance (ANOVA) test was used to assess the statistical significance of differences in parasite recovery, antigen specific cytokine and IgE production among mouse genotypes. Parametric ANOVA was used to assess effects of strain on log-transformed IgG data. Logistic regression analysis was used to test whether the percentage of mice that developed filarial infection or microfilaraemia varied with mouse genotype. To quantify the Th1/Th2 skew of antigen-specific IgG responses to infection, the mean response of naïve mice was subtracted from the optical density for each infected mouse of the same genotype in that experiment. Infection induced IgG2a was divided by induced IgG1 for each infected mouse, and the logarithm (ln) of the ratio was taken prior to parametric ANOVA. Positive ln(IgG2a/IgG1) values denote Th1 skew, while negative values denote Th2 skew.

For most tests, results from several experiments were pooled. Statistical removal of experimental effects is only possible in parametric ANOVA (e.g. in the analyses of log-transformed IgG1, IgG2a, IgG2b, and ln(IgG2a/IgG1) data). When the other data were transformed to meet parametric assumptions, ANOVA indicated significant effects of mouse strain upon all variables, after controlling for experimental effects, and there were no significant experiment-by-strain interactions. However, there were insufficient degrees of

Table 1
Parasite recovery during the course of *Litomosoides sigmodontis* infection in WT and IL-4 deficient mice[a]

Time p.i.	WT BALB/c	WT C57BL/6	IL-4 KO C57BL/6
Day 20	36.00 ± 17.89 ($n = 5$)	14.66 ± 7.21 ($n = 9$)	19.20 ± 13.68 ($n = 5$)
Day 40	17.45 ± 14.56 ($n = 11$)	5.00 ± 6.61 ($n = 16$)[b]	15.20 ± 11.12 ($n = 10$)
Day 60	10.93 ± 6.49 ($n = 15$)	0.73 ± 2.00 ($n = 22$)[b]	9.07 ± 5.34 ($n = 15$)

[a] Parasite recovery = percentage of 25 L3 that had matured and were recovered on days 20, 40 or 60 days p.i. Data shown are combined from different experiments. Several experiments were performed for each time point (Day 20 p.i.: two experiments, Day 40 p.i.: four experiments, Day 60 p.i.: four experiments), and contained at least two different genotypes of mice, one experiment at each time point was performed with all groups of mice. Values are expressed as the mean ± standard deviation (SD) per group for each time point.
[b] Represents statistical significance between the WT C57BL/6 mice and the two other genotypes of mice at the indicated time point.

freedom for pairwise tests on parasite recovery, cytokine secretion, and serum IgE when experimental effects were in the model. Those data thus remained pooled across experiments for the pairwise tests. $P < 0.05$ was considered to represent a statistically significant difference, but P values were corrected for multiple post-ANOVA comparisons.

3. Results

3.1. In the absence of Interleukin 4, resistant C57BL/6 mice are as susceptible as BALB/c mice

Following s.c. L3 infection, *L. sigmodontis* migrates through the lymphatic system and into the coelomic cavities of both resistant and susceptible mice. The larvae then moult to the fourth stage between 20 and 40 days p.i. In resistant C57BL/6 mice, most parasite attrition occurs prior to the final moult to adult worms (Hoffmann et al., 2000). To assess the effect of IL-4 on the development of worms, we analysed parasite recovery rates over the course of infection in WT and IL-4 deficient C57BL/6 mice as well as WT BALB/c mice as a control for full susceptibility. At 20 days p.i., WT C57BL/6 have a lower parasite recovery compared with WT BALB/c. These differences did not achieve statistical significance but similar differences have been observed in other studies (Marechal et al., 1996; Hoffmann et al., 2000) suggesting that in resistant mice there is a small but consistently lower parasite recovery in the first few days. However, a far more significant drop in parasite recovery occurs after the first moult, suggesting that resistance genes act during several stages in the lifecycle. As expected, we observed a significantly lower parasite recovery in WT C57BL/6 mice compared with WT BALB/c 40 days p.i. ($P < 0.05$) and 60 days p.i. ($P < 0.01$). In contrast, parasite recovery in IL-4 deficient C57BL/6 mice did not differ significantly from BALB/c at any time point (Table 1).

By 60 days p.i., the parasites had reached sexual maturity in 93% of both infected BALB/c mice and IL-4 deficient C57BL/6 mice. In contrast, only 13% of infected WT C57BL/6 mice presented with any adult worms ($P < 0.0001$, Fig. 1A). The differences between WT C57BL/6 and IL-4 deficient mice were even more apparent when the condition of the adult worms was investigated. By day 60, the majority of worms from every infected WT C57BL/6 mouse were contained in granulomatous nodules (Fig. 2A, B). These nodules were never found in IL-4 deficient C57BL/6 mice. Parasites found in the IL-4 deficient C57BL/6 mice at this time point were all freely motile and equivalently healthy in appearance to those found in fully susceptible BALB/c mice.

Adult parasite health status was reflected in the presence

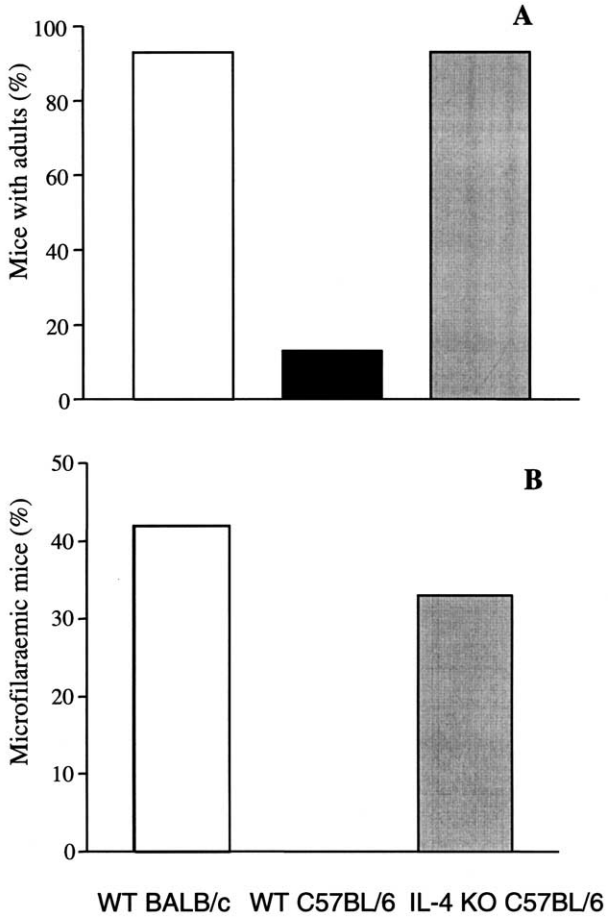

Fig. 1. Percentage of *Litomosoides sigmodontis* infected mice with adult filarial parasites (A) and with circulating microfilaria (B). Filarial and blood microfilarial status were assessed at day 60 post infection in *L. sigmodontis* infected WT C57BL/6 mice ($n = 22$), IL-4 deficient C57BL/6 mice ($n = 15$) and WT BALB/c mice ($n = 15$).

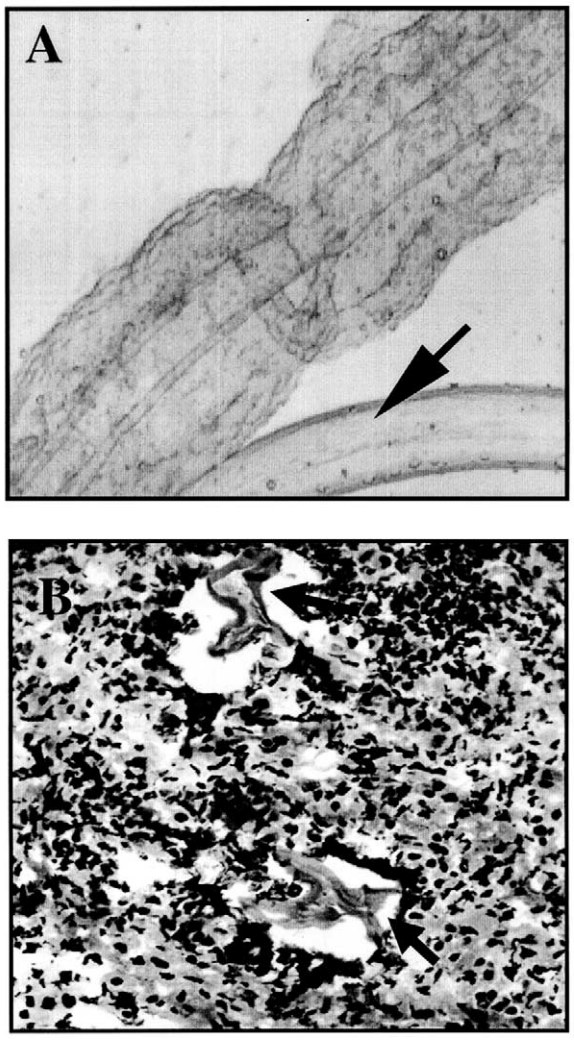

Fig. 2. Formation of granulomatous nodules around adult worms in infected WT C57BL/6 mice 60 days post infection. (A) Damaged worm found in the thoracic cavity of infected WT C57BL/6 mouse. Worms were typically either wholly encapsulated or as shown here encapsulated on one part with the remainder of the worm free (arrow), and still motile. (B) Section of encapsulated adult worm stained with Giemsa, demonstrating the granulomatous nature of the material encapsulating the worm. Arrows show the sections of the worm.

of circulating blood microfilariae in the BALB/c mice (42% positive microfilaraemic mice) and IL-4 deficient C57BL/6 mice (33% positive microfilaraemic mice) but not in the WT C57BL/6 mice, in which microfilariae were never observed ($P < 0.002$, Fig. 1B). In addition, the number of microfilariae found was similar in WT BALB/c mice (11 ± 7.9 microfilariae per 10 μl) and IL-4 deficient C57BL/6 mice (6.2 ± 7.1 microfilariae per 10 μl).

3.2. Susceptible BALB/c mice and susceptible C57BL/6 IL-4 deficient mice exhibit opposing cytokine responses

We investigated the cytokine profiles of splenocytes during the time course of *L. sigmodontis* infection in WT and IL-4 deficient mice to determine the immunologic changes resulting from an absence of IL-4. IL-4 and IFNγ were measured as markers of Th2 and Th1 responses, respectively, following in vitro antigenic stimulations. No antigen-specific cytokine production was detected from the splenocytes of infected mice at 20 or 40 days p.i. At 60 days p.i., antigen-specific IL-4 was detected in all infected WT mice but not, as expected, in the gene deficient mice ($P < 0.05$, Fig. 3A). Low levels of antigen specific IFNγ production were detected 60 days p.i. in both infected WT BALB/c and IL-4 deficient C57BL/6 mice, (i.e. in susceptible mice) but none was detected in the resistant WT

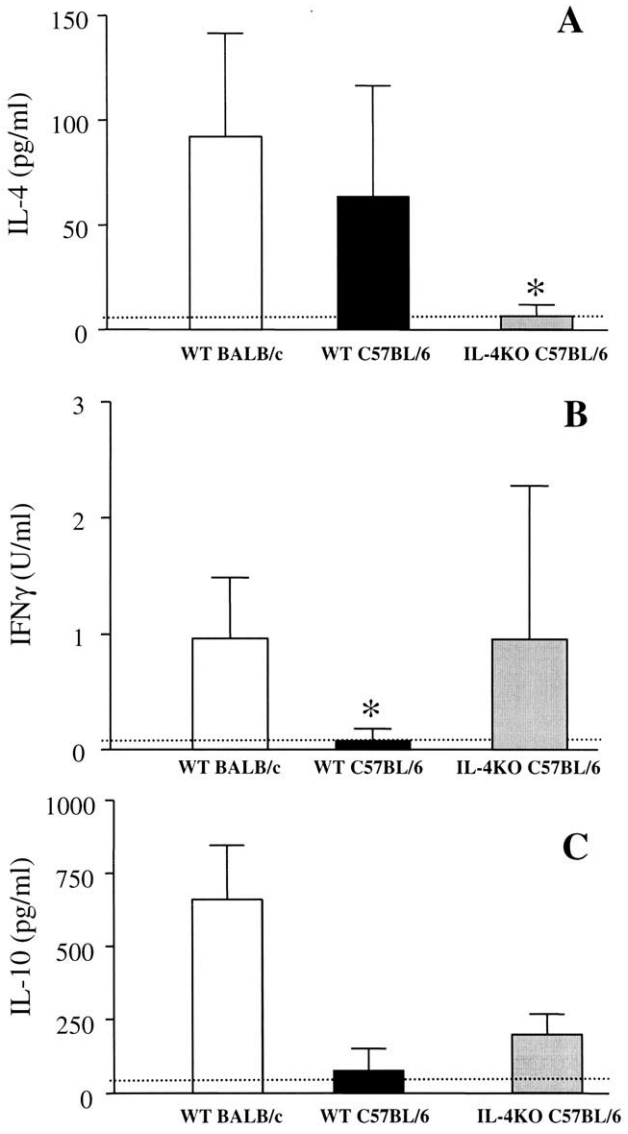

Fig. 3. Antigen-specific cytokine levels secreted by splenocytes in WT and IL-4 deficient mice 60 days post infection. Dotted line represents the cytokine levels in naïve mice. Values are expressed as the mean ± SD. Data of three independent experiments are combined (see Table 1) and shown in (A) and (B). One experiment is shown in (C). (A) *Significant difference between IL-4KO C57BL/6 mice and the two other genotypes of mice. (B) *Significant difference between WT C57BL/6 and the two other genotypes of mice.

C57BL/6 mice ($P < 0.05$, Fig. 3B). Although the possibility exists that IFNγ is promoting susceptibility, this very low-level IFNγ likely reflects the presence of living worms in the two susceptible strains. IFNγ may be evoked by antigens from the endosymbiotic bacteria present in the nematodes (Bandi et al., 2001), or from microfilariae, which in related filarial nematode infections, are reported to induce parasite-specific IFNγ production (Lawrence et al., 1994).

The downregulatory cytokine, IL-10, has been strongly implicated in susceptibility to helminth infection (Schopf et al., 2002) and has a known role in suppression of immune responses in both murine (Osborne and Devaney, 1999) and human filariasis (Cooper et al., 2001; Doetze et al., 2000). We thus investigated the levels of IL-10 in the *L. sigmodontis* infected mice. Susceptible BALB/c mice produced higher levels of IL-10 than IL-4 deficient C57BL/6 mice while resistant WT C57BL/6 mice made no detectable IL-10 in response to infection (Fig. 3C). These differences, however, did not achieve statistical significance ($P < 0.082$).

3.3. The two susceptible mouse genotypes mount antibody isotype responses at opposing ends of the Th1/Th2 axis

Although cytokine measurements can provide an important snapshot of the immune response at a particular point in time, the assessment of antibody isotypes reflects the cumulative response to infection and thus may provide a broader picture of the overall response. We therefore evaluated the levels of parasite-specific immunoglobulins IgG1, IgG2a and IgG2b in serum samples, as well as the IgG2a/IgG1 ratio, as a measure of the overall shift towards a Th1 or Th2 dominated response.

Parasite-specific antibody production was not observed until 40 days p.i. Consistent with the cytokine pattern at day 60, high levels of specific IgG1 were detected at day 40 (Fig. 4A) and day 60 (data not shown) in the infected WT mice (BALB/c and C57BL/6) but not in the infected IL-4 deficient mice. In infected IL-4 deficient C57BL/6 mice, the reduction of the IgG1 level was accompanied by a shift toward parasite-specific IgG2a and IgG2b. In order to evaluate the overall bias of the immune response towards a Th1 or Th2 type response, the IgG2a/IgG1 ratio for each animal was calculated. This analysis revealed that, as expected, BALB/c mice were strongly Th2 biased, IL-4 deficient C57BL/6 mice were Th1 biased while the resistant C57BL/6 mice were intermediate in their response (Fig. 4B).

Polyclonal IgE is another reliable measure of Th2 cell activation (Finkelman et al., 1988) and by 40 days p.i., WT BALB/c mice made higher levels of polyclonal IgE relative to C57BL/6 (Fig. 4C). In the absence of IL-4, IgE responses in C57BL/6 mice were only detectable at very low levels (Fig. 4C). The polyclonal IgE data support the antigen-specific IgG isotype data and demonstrate that the two susceptible mouse strains (WT BALB/c and IL-4 deficient C57BL/6) exhibited high and low Th2 biased immune responses, respectively.

3.4. Antibodies are not required for resistance in C57BL/6 mice

At this stage in the investigation, there did not appear to be any correlation between antibody status and susceptibility or resistance. However, previous studies by Babu et al.

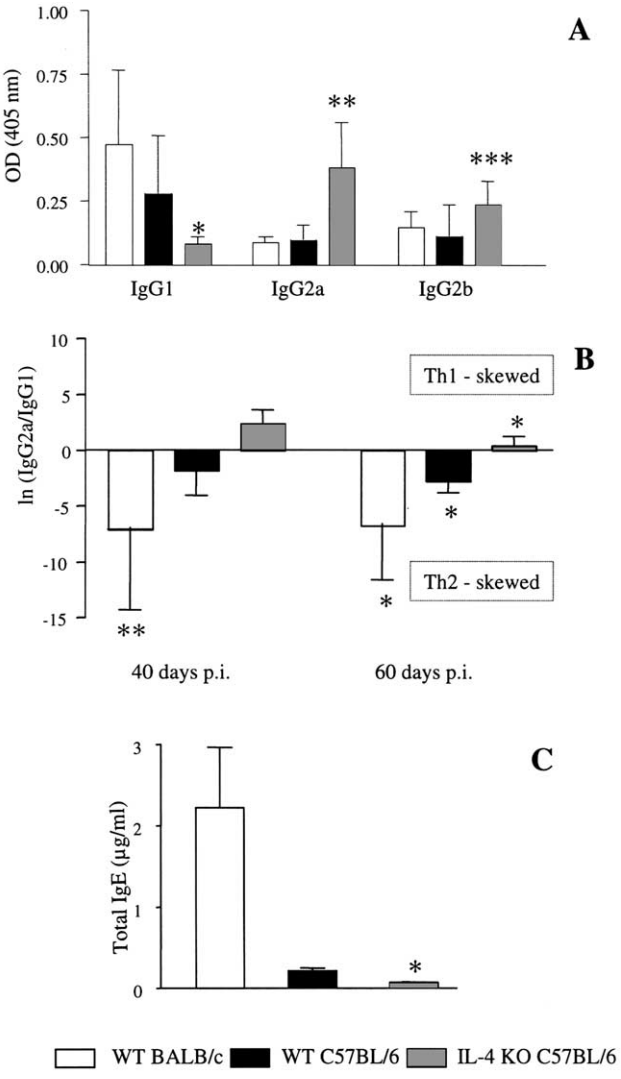

Fig. 4. Antibody isotype levels in *Litomosoides sigmodontis* infected mice. (A) *L. sigmodontis* antigen-specific IgG isotype levels. (B) *L. sigmodontis* antigen-specific IgG2a/IgG1 ratios. (C) Total IgE levels. Data in (A) and (C) are shown in WT and IL-4 deficient mice 40 days post infection. Values are expressed as the mean ± SD. Data of three independent experiments are combined (see Table 1) and shown. OD, optical density. (A) *Significant difference between the IL-4 deficient C57BL/6 mice and the WT BALB/c mice ($P < 0.01$). **Significant difference between the IL-4 deficient C57BL/6 mice and the two other genotypes of mice ($P < 0.01$). ***Significant difference between the IL-4 deficient C57BL/6 mice and the WT C57BL/6 mice ($P < 0.01$). (B) **Significant difference between WT BALB/c and the two other C57BL/6 genotypes ($P < 0.05$). *Significant difference between WT BALB/c and WT C57BL/6 ($P < 0.0002$) and IL-4 deficient C57BL/6 mice ($P < 0.0001$). The two C57BL/6 genotypes also mounted responses of significant difference ($P < 0.03$). (C) *Significant difference between the WT BALB/c mice and the two other genotypes of mice ($P < 0.05$).

Table 2
Parasite recovery of *Litomosoides sigmodontis* from infected WT and B deficient C57BL/6 mice 60 days post infection[a]

	Mouse strain	Genotype	Inoculation	Recovery rate (%)	%Mf
Exp. 1					
	BALB/c	+/+	s.c.	9.6 ± 2.7	ND
	C57BL/6	+/+	s.c.	0.0 ± 0.0	0
	–	μMT − /μMT −	s.c.	0.0 ± 0.0	0
Exp. 2					
	BALB/c	+/+	i.p.	4.0 ± 0.0	40
	C57BL/6	μMT − /μMT −	i.p.	0.0 ± 0.0	0
	C57BL/6	μMT − /μMT −	s.c.	0.0 ± 0.0	0

[a] In both experiments, one group of infected WT BALB/c were added as a control of good infection. Values are expressed as the mean ± SD of five mice in each group. ND: circulating microfilariae were not examined in the BALB/c mice in this experiment.

(1999) had found that in the absence of B cells, C57BL/6 mice were susceptible to infection with *Brugia malayi*. We therefore decided to resolve this discrepancy by testing the role of antibodies in resistance to *L. sigmodontis* infection. We infected WT and B cell deficient C57BL/6 mice with 25 infective larvae by s.c. inoculation and assessed parasite survival and microfilaraemia 60 days p.i. No worms were found in any infected WT or B cell deficient C57BL/6 mice and no mice were microfilaraemic. In the control infected WT BALB/c mice, all the mice were infected (Table 2). Because our results with s.c. inoculation of *L. sigmodontis* differed from those of Babu et al. (1999), we chose to assess whether the route of inoculation could explain the difference. We thus inoculated mice i.p. with 25 infective *L. sigmodontis* larvae and again assessed parasite survival. The route of inoculation did not alter the outcome; no worms were recovered from infected WT or B cell deficient C57BL/6 mice (Table 2) demonstrating that the resistant phenotype is not dependent on antibody.

4. Discussion

Immune responses to helminth infection are Th2 dominated and the importance of these responses in the expulsion of gastrointestinal nematodes is firmly established (Finkelman et al., 1997). Similar studies of tissue nematode infection have failed to achieve a clear consensus on the role of Th2 cytokines. We believe that the rodent filarial parasite, *L. sigmodontis*, which can develop to adulthood and patency in susceptible BALB/c mice but fails to develop in resistant C57BL/6 mice may help us to make sense of the diverse findings regarding the role of IL-4 in filarial infection. The most striking finding in our current study is that both BALB/c mice and IL-4 deficient C57BL/6 mice are able to support full infection to patency and thus, the parasite is able to survive to adulthood in the face of diametrically opposed immune responses. The possibility therefore exists that resistance, as seen in WT C57BL/6 mice, requires the establishment of a response that is intermediate between the highly polarised responses observed in the BALB/c and IL-4 deficient C57BL/6 mice.

We have attempted to put our findings into the context of previous studies that have investigated the role of IL-4 in the establishment of infection with L3 stage larvae of various filarial nematodes (Table 3). Rajan and colleagues (Babu et al., 2000; Spencer et al., 2001) have observed a significantly higher recovery of *B. malayi* parasites in mice defective in Th2 responses on both the BALB/c and C57BL/6 backgrounds. This is consistent with our data on infection of

Table 3
Summary of studies targeting the impact of IL-4 on filarial infections in mice[a]

Authors	Species	Mice-route of inoculation of L3	Host	Worm recovery		
				Adults	Blood microfilariae	Cavity microfilariae
Babu et al. (2000)	*Brugia malayi*	BALB/c-L3 i.p.	R	+		+
Devaney et al. (2002)	*Brugia pahangi*	BALB/c-L3 s.c.	S	=		+
Volkmann et al. (2001)	*Litomosoides sigmodontis*	BALB/c-L3 s.c.	S	=	+	+
Lawrence et al (1995)	*Brugia malayi*	129 × C57BL/6-L3 i.p.	VR	0		
Spencer et al. (2001)*	*Brugia malayi*	C57BL/6-L3 i.p.	R	+		+
Le Goff et al. (this study)	*Litomosoides sigmodontis*	C57BL/6-L3 s.c.	R	+	+	

[a] Symbols indicate that infection increase (+) or is not modified (=) in IL-4 deficient or IL-4 receptor deficient (*) mice compared with WT mice. 0, No parasite recovered; R, resistant/non-permissive host to the infection; VR, very resistant/non-permissive host to the infection; S, susceptible/permissive host to the infection; L3, infective larvae; i.p., intraperitoneal inoculation; s.c., subcutaneous inoculation; blood microfilariae, presence of microfilaraemia in the blood; cavity microfilariae, presence of microfilaraemia in the body cavity (in the thoracic or peritoneal cavities).

C57BL/6 mice with *L. sigmodontis*. In contrast, Devaney et al. (2002) and Volkmann et al. (2001) using BALB/c mice found no effect of IL-4 on the development of infective larvae to adulthood using *Brugia pahangi* and *L. sigmodontis*, respectively. Interestingly, we also found no significant difference in adult recovery between WT and IL-4 deficient mice on the BALB/c background (unpublished observation). These diverse findings together support the hypothesis that IL-4 is the critical or limiting factor in the development from infective larvae to adulthood when the parasite infects a resistant/non-permissive host (Table 3). In contrast, when the parasite infects a susceptible/permissive host it is apparently able to avoid the IL-4 driven effector mechanisms. One consistent finding among all these studies is that once infection is established and the parasite has succeeded in achieving sexual maturity, IL-4 is the key determinant in the level of microfilariae. However, the data point to an impact on worm fecundity rather that to a direct effect on microfilaria survival (Devaney et al., 2002; Volkmann et al., 2001).

We would argue that it is essential to understand the IL-4 mediated mechanisms by which the resistant/non-permissive host is able to prevent the establishment of infection. It is these mechanisms that must be overcome or downregulated in the susceptible/permissive host–parasite combinations. Eosinophils are obvious candidates for the Th2 driven effector cell and substantial previous data support a role for eosinophils in the destruction of nematode larvae, particularly in resistant/non-permissive hosts (Meeusen and Balic, 2000). However, we found that IL-5 deficient C57BL/6 mice are just as resistant as WT mice to primary infection with *L. sigmodontis*, suggesting that eosinophils are not the critical factor in innate resistance.

Recent studies have implicated neutrophils as potentially important in the killing of filarial parasites (Brattig et al., 2001; Saeftel et al., 2001) and one study has shown that neutrophils are essential for nodule formation around *Litomosoides* adult worms in WT BALB/c mice (Al-Qaoud et al., 2000). Neutrophils are more typically associated with type 1 responses such as IFNγ than type 2 responses but the Al-Qaoud et al. (2000) study demonstrated that neutrophil accumulation can be dependent on IL-5. Further, IFNγ mediated killing seems unlikely in this system, as IFNγ levels were higher in both susceptible strains in the present study (Fig. 3B). The possibility that macrophages activated under Th2 conditions have a role in parasite killing also exists. We have previously shown that macrophages recruited by filarial nematodes produce very high levels of Ym1 (Falcone et al., 2001), a molecule involved in crystal deposition, a phenomenon with no known function but which may have the potential to directly damage parasites.

Numerous studies have addressed the role of antibodies in immunity to filarial nematodes and contradictory data exist concerning B cells in filarial infection. Our own work using B cell deficient mice on the C57BL/6 background shows that these mice are still resistant 60 days after s.c. inoculation suggesting that antibodies do not mediate resistance. Previous studies done with *Brugia* infection in B cell deficient mice on the same background suggest that B cell deficient mice are more susceptible to i.p. infection (Babu et al., 1999). We have ruled out the possibility that these contradictory findings were due to different sites of inoculation. It is possible that there are distinct protective immune mechanisms unique to each filarial nematode species. However, B cells play important roles in regulating the Th2 response (Skok et al., 1999) and thus it may be that the ability of B cells to regulate T cell responsiveness differed in these systems due to differences in antigen load or animal house status.

Identifying the factors that enable the parasite to avoid Th2 mediated destruction in susceptible mice will provide essential clues to how filarial parasites survive in the face of a profound Th2 response, the normal situation in many human helminthic infections (Devaney and Osborne, 2000; Lawrence, 1996). One obvious candidate for such a factor is IL-10, which has the capacity not only to downregulate Th1 and Th2 responses (Moore et al., 2001), but also has a direct downregulatory effect on the Th2 driven effector cells such as mast cells and eosinophils (Moore et al., 2001). In *B. pahangi* infected BALB/c mice, IL-10 production suppresses T cell proliferative responses and the expression of antigen-specific Th1 responses (IL-2 and IFNγ) (Osborne and Devaney, 1999). Our preliminary findings (Fig. 3), though not achieving statistical significance, suggest that infected BALB/c mice produce higher levels of antigen-specific IL-10. The ability of the parasite to induce IL-10 may be the critical factor in determining susceptibility. Sufficiently high levels of IL-10 could contribute to suppression of T cell proliferation and help the parasite to avoid the IL-4 driven effector mechanisms, which mediate destruction in the resistant/non-permissive host.

Acknowledgements

The authors would like to thank Eva Malone and Marisa Magennis for invaluable assistance and Rick Maizels, Matthew Taylor and Salah Tawill for helpful discussion and critical comments on the manuscript.

This work was supported by the EC (contract: ICA4-CT-1999-10002), the Edna McConnell Clark Foundation and the Wellcome Trust.

References

Al-Qaoud, K.M., Pearlman, E., Hartung, T., Klukowski, J., Fleischer, B., Hoerauf, A., 2000. A new mechanism for IL-5-dependent helminth control: neutrophil accumulation and neutrophil-mediated worm encapsulation in murine filariasis are abolished in the absence of IL-5. Int. Immunol. 12, 899–908.

Babu, S., Shultz, L.D., Klei, T.R., Rajan, T.V., 1999. Immunity in experimental murine filariasis: roles of T and B cells revisited. Infect. Immun. 67, 3166–7.

Babu, S., Ganley, L.M., Klei, T.R., Shultz, L.D., Rajan, T.V., 2000. Role of gamma interferon and interleukin-4 in host defense against the human filarial parasite *Brugia malayi*. Infect. Immun. 68, 3034–5.

Bain, O., Wanji, S., Vuong, P.N., Marechal, P., Le Goff, L., Petit, G., 1994. Larval biology of six filariae of the sub-family Onchocercinae in a vertebrate host. Parasite 1, 241–54.

Bandi, C., Trees, A.J., Brattig, N.W., 2001. *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. Vet. Parasitol. 98, 215–38.

Brattig, N.W., Buttner, D.W., Hoerauf, A., 2001. Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. Microbes Infect. 3, 439–46.

Cooper, P.J., Mancero, T., Espinel, M., Sandoval, C., Lovato, R., Guderian, R.H., Nutman, T.B., 2001. Early human infection with *Onchocerca volvulus* is associated with an enhanced parasite-specific cellular immune response. J. Infect. Dis. 183, 1662–8.

Devaney, E., Osborne, J., 2000. The third-stage larva (L3) of *Brugia*: its role in immune modulation and protective immunity. Microbes Infect. 2, 1363–71.

Devaney, E., Gillan, V., Wheatley, I., Jenson, J., O'Connor, R., Balmer, P., 2002. Interleukin-4 influences the production of microfilariae in a mouse model of *Brugia* infection. Parasite Immunol. 24, 29–37.

Doetze, A., Satoguina, J., Burchard, G., Rau, T., Loliger, C., Fleischer, B., Hoerauf, A., 2000. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by Th3/Tr1-type cytokines IL-10 and transforming growth factor-beta but not by a Th1 to Th2 shift. Int. Immunol. 12, 623–30.

Falcone, F.H., Loke, P., Zang, X., MacDonald, A.S., Maizels, R.M., Allen, J.E., 2001. A *Brugia malayi* homolog of macrophage migration inhibitory factor reveals an important link between macrophages and eosinophil recruitment during nematode infection. J. Immunol. 167, 5348–54.

Finkelman, F.D., Katona, I.M., Urban Jr, J.F., Holmes, J., Ohara, J., Tung, A.S., Sample, J.V., Paul, W.E., 1988. IL-4 is required to generate and sustain in vivo IgE responses. J. Immunol. 141, 2335–41.

Finkelman, F.D., Shea-Donohue, T., Goldhill, J., Sullivan, C.A., Morris, S.C., Madden, K.B., Gause, W.C., Urban Jr, J.F., 1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annu. Rev. Immunol. 15, 505–33.

Hoffmann, W., Petit, G., Schulz-Key, H., Taylor, D., Bain, O., Le Goff, L., 2000. *Litomosoides sigmodontis* in mice: reappraisal of an old model for filarial research. Parasitol. Today 16, 387–9.

Holland, M.J., Harcus, Y.M., Riches, P.L., Maizels, R.M., 2000. Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. Eur. J. Immunol. 30, 1977–87.

Kitamura, D., Roes, J., Kuhn, R., Rajewsky, K., 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature 350, 423–6.

Lawrence, R.A., 1996. Lymphatic filariasis: what mice can tell us. Parasitol. Today 12, 267–71.

Lawrence, R.A., Allen, J.E., Osborne, J., Maizels, R.M., 1994. Adult and microfilarial stages of the filarial parasite *Brugia malayi* stimulate contrasting cytokine and Ig isotype responses in BALB/c mice. J. Immunol. 153, 1216–24.

Lawrence, R.A., Allen, J.E., Gregory, W.F., Kopf, M., Maizels, R.M., 1995. Infection of IL-4 deficient mice with the parasitic nematode *Brugia malayai* demonstrates that host resistance is not dependent on a Th2 dominated immune response. J. Immunol. 154, 5995–6001.

Le Goff, L., Loke, P., Ali, H.F., Taylor, D.W., Allen, J.E., 2000a. Interleukin-5 is essential for vaccine-mediated immunity but not innate resistance to a filarial parasite. Infect. Immun. 68, 2513–7.

Le Goff, L., Martin, C., Oswald, I.P., Vuong, P.N., Petit, G., Ungeheuer, M.N., Bain, O., 2000b. Parasitology and immunology of mice vaccinated with irradiated *Litomosoides sigmodontis* larvae. Parasitology 120, 271–80.

Marechal, P., Le Goff, L., Petit, G., Diagne, M., Taylor, D.W., Bain, O., 1996. The fate of the filaria *Litomosoides sigmodontis* in susceptible and naturally resistant mice. Parasite 3, 25–31.

Meeusen, E.N., Balic, A., 2000. Do eosinophils have a role in the killing of helminth parasites? Parasitol. Today 16, 95–101.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., O'Garra, A., 2001. Interleukin-10 and the Interleukin-10 receptor. Annu. Rev. Immunol. 19, 683–765.

Osborne, J., Devaney, E., 1999. Interleukin-10 and antigen-presenting cells actively suppress Th1 cells in BALB/c mice infected with the filarial parasite *Brugia pahangi*. Infect. Immun. 67, 1599–605.

Petit, G., Diagne, M., Marechal, P., Owen, D., Taylor, D., Bain, O., 1992. Maturation of the filarial *Litomosoides sigmodontis* in BALB/c mice; comparative susceptibility of nine other inbred strains. Ann. Parasitol. Hum. Comp. 67, 144–50.

Reiner, S.L., Locksley, R.M., 1995. The regulation of immunity to *Leishmania major*. Annu. Rev. Immunol. 13, 151–77.

Saeftel, M., Volkmann, L., Korten, S., Brattig, N., Al-Qaoud, K., Fleischer, B., Hoerauf, A., 2001. Lack of interferon-gamma confers impaired neutrophil granulocyte function and imparts prolonged survival of adult filarial worms in murine filariasis. Microbes Infect. 3, 203–13.

Schopf, L.R., Hoffmann, K.F., Cheever, A.W., Urban Jr, J.F., Wynn, T.A., 2002. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. J. Immunol. 168, 2383–92.

Skok, J., Poudrier, J., Gray, D., 1999. Dendritic cell-derived IL-12 promotes B cell induction of Th2 differentiation: a feedback regulation of Th1 development. J. Immunol. 163, 4284–91.

Spencer, L., Shultz, L., Rajan, T.V., 2001. Interleukin-4 receptor-Stat6 signaling in murine infections with a tissue-dwelling nematode parasite. Infect. Immun. 69, 7743–52.

Volkmann, L., Saeftel, M., Bain, O., Fischer, K., Fleischer, B., Hoerauf, A., 2001. Interleukin-4 is essential for the control of microfilariae in murine infection with the filaria *Litomosoides sigmodontis*. Infect. Immun. 69, 2950–61.