

# Interleukin-4 influences the production of microfilariae in a mouse model of *Brugia* infection

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## SUMMARY

*Sub-cutaneous infection of interleukin (IL)-4<sup>-/-</sup> mice on the BALB/c background with third stage larva (L3) of Brugia pahangi revealed an altered cytokine profile consistent with the absence of the Th2 promoting cytokine IL-4. Splenocytes from IL-4<sup>-/-</sup> mice secreted significantly more antigen (Ag)-specific IL-2 and interferon- $\gamma$  and significantly less Ag-specific IL-5, compared to those from L3-infected wild-type mice. However, levels of Ag-specific IL-13 were similar between groups. Despite the alteration in immune responses, there was no significant difference in recovery of developing worms from the peritoneal cavity of the two strains of mice at any time postinfection. However, at later time points of infection, the IL-4<sup>-/-</sup> mice contained large numbers of microfilariae (Mf) in the peritoneal cavity while the wild-type mice contained comparatively few Mf. The differences in Mf levels appear to relate to differences in worm fecundity in the two strains of mice, with adult female worms from the wild-type mice containing few developing Mf. Moreover, implantation of sexually mature adult female worms into the peritoneal cavity of both strains of mice resulted in equal levels of Mf, confirming that the primary role of IL-4 is to limit fecundity during the maturation phase of infection.*

**Keywords** *Brugia*, IL-4 knock out mice, worm fecundity

## INTRODUCTION

Human filarial infection is characterized by a profound bias in T helper cell responses such that peripheral blood mononuclear cells from actively infected microfilaraemic individuals secrete high levels of interleukin (IL)-4 and little or no interferon (IFN)- $\gamma$  (1,2). This skewing of the immune response is likely to be initiated by infection with the third stage larva (L3) and then maintained by the presence of adult parasites. Studies in BALB/c mice infected with *Brugia pahangi* have shown that exposure to L3 results in a burst of IL-4 transcription within 24 h of infection, which predisposes to a Th2 response in the draining lymph node (3). Adult parasites also contribute to the Th2 bias, as implantation of adult *B. malayi* into the peritoneal cavity of BALB/c mice gives rise to a dominant type 2 response (4). Moreover, two recent studies have shown that adult parasites or their excretory secretory products have the ability to promote Th2 differentiation of naive T cells via their effects on antigen presenting cells (5,6).

An outstanding question in the immunology of filariasis is the relevance of this imbalance in immune response for both parasite and host. In the infected human, the correlation between active infection, the Th2 bias and the absence of overt pathology has led to the proposal that the skewing of the immune response promotes parasite survival while also protecting the host from disease. However, several studies have demonstrated that IL-4 levels are elevated in all exposed individuals, irrespective of clinical status (1,2), while IFN- $\gamma$  is downregulated in actively infected individuals. Therefore, the ratio of IL-4 to IFN- $\gamma$  differs between different clinical groups rather than the absolute amount of IL-4 produced. Interestingly, IL-4 mRNA expression is elevated even in antigen-negative endemic normals (7) who are not infected, suggesting that continued exposure to L3 is the driving force for IL-4 production in this group.

Similarly, the possible role of Th2-mediated responses in protective immunity in lymphatic filariasis remains controversial. Studies in both *Wuchereria bancrofti* and *Onchocerca volvulus* endemic areas have correlated the absence

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of infection (in endemic normals) with elevated production of IL-2 and IFN- $\gamma$  (7,8). In contrast, studies in mouse models support a role for Th2-mediated responses in protective immunity elicited by irradiated L3 (9,10), and a recent study in the *Litomosoides sigmondontis* model has demonstrated that the critical mediator of immunity in secondary infection may be IL-5 (11).

In the experiments reported here, we investigated whether the presence of IL-4 effected the establishment and development of a primary infection of *Brugia pahangi* L3 using IL-4<sup>-/-</sup> mice and their wild-type BALB/c counterparts. While previous studies in IL-4<sup>-/-</sup> mice have addressed the role of this central Th2 cytokine in survival of *B. malayi*, these studies were carried out in IL-4<sup>-/-</sup> mice on the 129  $\times$  C57Bl/6 background (12). BALB/c mice are much more susceptible to L3-induced infections of either *B. pahangi* or *B. malayi* than are C57Bl/6 mice (13) in which strain the survival of L3 is limited. Here, we demonstrate that IL-4 appears to have no role in survival of a primary infection of *B. pahangi* L3 or in the development of the parasite to adulthood despite the fact that IL-4<sup>-/-</sup> mice have an altered cytokine profile (Th1 > Th2) in response to infection. However, the presence of IL-4 does appear to be a critical determinant of microfilariae (Mf) production. Very few Mf were recovered from wild-type mice and adult worms which had developed in these animals were much less fecund than those recovered from the IL-4<sup>-/-</sup> mice, which contained substantial numbers of Mf.

## MATERIALS AND METHODS

### Mice and infection protocols

Six to 8-week-old male BALB/c mice (Harlan Olac, Bicester, UK) or age-matched IL-4<sup>-/-</sup> mice were used in all experiments. The IL-4<sup>-/-</sup> mice on the BALB/c background (14) were originally obtained from Jackson Laboratories (Bar Harbor, ME, USA) and have since been maintained at the University of Glasgow. All mice were housed in filter top cages.

Mice were infected by the intraperitoneal (i.p.) or the subcutaneous (s.c.) route with 50 L3 of *B. pahangi* harvested from infected *Aedes aegypti* as described previously (15). Syringes were washed out after inoculation of the worms and the precise number of L3 injected was enumerated. At various time points after i.p. infection mice were killed by CO<sub>2</sub> inhalation, the peritoneal cavity lavaged using a fixed volume (10.0 ml) of Hanks' Balanced Salt Solution (HBSS) and the number of larvae in the lavage counted. In some experiments in which mice were infected i.p. with L3, the total number of cells in an aliquot of the peritoneal washings was counted using an automated Coulter counter and then slides prepared by cytopspin for differential white cell counts. Where adult worms were present, the peritoneal washings were processed

for recovery of Mf using a modified Knotts' technique. Once the adult worms had been removed, Mf were pelleted by centrifugation (1000 r.p.m. for 5 min) and then 10.0 ml of 1% formalin added to the tube. Tubes were stored at 4°C until the total numbers of Mf in the pellets were counted. In some experiments, mice were infected by i.p. implantation of adult female worms recovered from infected jirds. Ten females were implanted into each mouse and the numbers of female worms and Mf were counted at 2 weeks p.i.

Larvae at all stages of development were fixed in 10% formalin and measured using a 'Projectina' microscope in which the image of the worm is projected onto a screen. The image was then traced and measured.

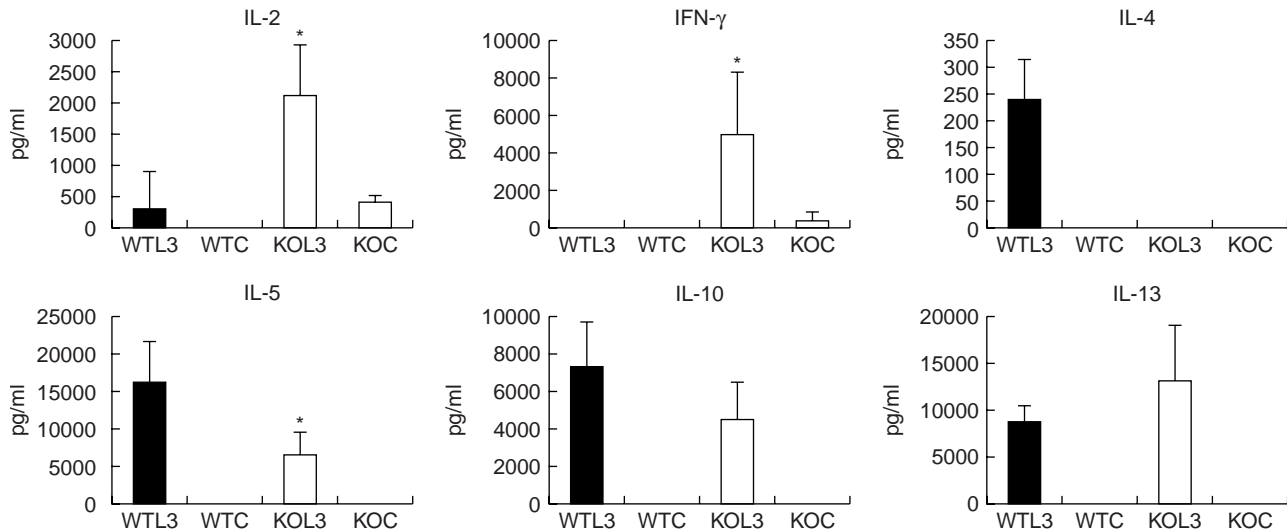
### Immunological assays

Spleens were removed and disrupted by passage through a Nytex membrane into RPMI 1640 containing 5 mM glutamine, 5 mM HEPES buffer, 100 U of penicillin and 100  $\mu$ g of streptomycin per ml (all Gibco BRL, Gaithersburg, MD, USA). Erythrocytes were lysed by treatment with 0.84% NH<sub>4</sub>Cl (pH 7.2), washed twice in RPMI and the number of viable lymphocytes assessed by trypan blue exclusion.  $1 \times 10^7$  cells were cultured in 24-well plates in RPMI containing 10% heat inactivated Myocloned foetal calf serum (Gibco). Cells were stimulated with a soluble extract of *B. pahangi* adult parasites at 10  $\mu$ g/ml or with Concanavalin A at 5  $\mu$ g/ml, as described previously (15). After 48 h of incubation at 37°C and 5% CO<sub>2</sub>, supernatants were removed for analysis of cytokine secretion profiles. For proliferation assays, cells were plated in triplicate in half-area flat bottomed 96-well plates (Costar, Bucks, UK) at a density of  $5 \times 10^5$  cells per well and stimulated with adult antigen (Ag) at 10  $\mu$ g/ml for 72 h or ConA at 1  $\mu$ g/ml for 48 h. During the last 16 h of culture, cells were pulsed with [<sup>3</sup>H]thymidine (0.5  $\mu$ Ci per well) and the incorporation of radioactivity measured using a TopCount Microplate scintillation counter (Canberra Packard, Meriden CT, USA).

Cytokines (IL-2, IL-4, IL-5, IL-10 and IFN- $\gamma$ ) were measured by two-site ELISA using matched antibody pairs (all from Pharmingen, San Diego, CA, USA) exactly as described previously (16). Cytokines were expressed in picograms per millilitre relative to commercially available standards (all Pharmingen, except rIL-10, Research Diagnostics Ltd, Flanders, NJ, USA) IL-13 was measured using a commercially available assay (R&D Systems, Minneapolis, MN, USA).

### Statistical analysis

Differences between groups were analysed using Student's *t*-test or the Mann-Whitney *U*-test. *P* < 0.05 was considered statistically significant.



**Figure 1** Cytokine production is altered in IL-4<sup>-/-</sup> mice infected with L3 of *B. pahangi*. Groups of five BALB/c mice (■) or IL-4<sup>-/-</sup> mice (□) were infected with 50 L3 of *B. pahangi* by the s.c. route. At 12 days p.i., splenocytes from individual animals or from uninfected control mice were cultured in the presence of adult Ag at 10 µg/ml and supernatants collected after 48 h. Cytokines were measured as detailed in Materials and Methods. The results depict the mean and SD of five animals per group. \*Significant difference between IL-4<sup>-/-</sup> mice and wild-type mice. One of three representative experiments is shown.

## RESULTS

### IL-4<sup>-/-</sup> mice have an altered cytokine profile compared to wild-type mice

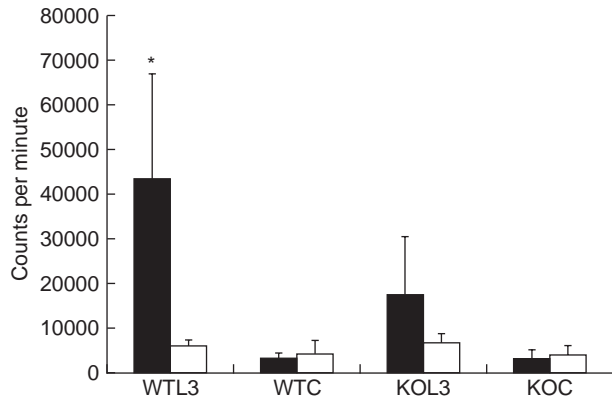
In the first experiments, five IL-4<sup>-/-</sup> mice or five BALB/c wild-type mice were infected by the s.c. route with 50 L3 of *B. pahangi*. This route of infection best mimics the natural situation in which L3 are delivered by the bite of an infected mosquito. At 12 days p.i., there was a significant difference in the profile of cytokines secreted by splenocytes from the IL-4<sup>-/-</sup> mice compared to the BALB/c wild-type mice in response to *in vitro* restimulation with parasite Ag. As shown in Figure 1, splenocytes from wild-type mice secreted an exclusively Th2 cytokine profile (Ag-specific IL-4, IL-5, IL-10) while cells from the knockout mice secreted significantly higher levels of Ag-specific IL-2 and IFN-γ and significantly reduced levels of IL-5 ( $P < 0.05$ ). In all three experiments, levels of IL-10 were reduced in L3-infected IL-4<sup>-/-</sup> mice, but in only one experiment did the difference reach statistical significance. We also measured levels of IL-13 in the IL-4<sup>-/-</sup> mice and their wild-type counterparts. In three separate experiments, high levels of IL-13 were secreted by splenocytes from both groups of infected mice and these were not statistically different between the two groups. Thus, in the IL-4<sup>-/-</sup> mice, s.c. infection with L3 resulted in a mixed T helper cell response at 12 days p.i. Consistent with the reduced Th2 response of the IL-4<sup>-/-</sup> mice, serum from these animals

contained higher levels of IgG2a and lower levels of IgG1 than did L3-infected BALB/c wild-type mice (data not shown).

We have previously shown that *in vitro* neutralization of IL-4 in spleen cell culture from L3-infected BALB/c mice results in a significant reduction in Ag-specific proliferation (15), suggesting that the cells which divide in response to Ag are Th2 cells which utilize IL-4 as a growth factor. Cells from the IL-4<sup>-/-</sup> mice infected with L3 showed significantly reduced levels of Ag-specific proliferation compared to the BALB/c wild-type mice ( $P < 0.05$ ) (Figure 2). Comparable results were obtained in a further two experiments.

### The presence or absence of IL-4 does not affect worm recoveries

We next investigated whether the difference in cytokine profiles of L3-infected IL-4<sup>-/-</sup> and wild-type mice would be reflected in differences in worm recovery in the two strains. Because it is not possible to carry out quantitative recoveries from s.c. infected mice, IL-4<sup>-/-</sup> mice or their BALB/c wild-type counterparts were infected by the i.p. route with 50 L3 of *B. pahangi*. Previous studies in IL-4<sup>-/-</sup> mice on the C57Bl6 × 129 background had demonstrated no difference in recovery of *B. malayi* L3 following i.p. infection, but in both the wild-type and the knockout mice, most of the worms had died by day 7 p.i. (12). Groups of mice (Table 1) were infected with 50 L3 and at the following time points



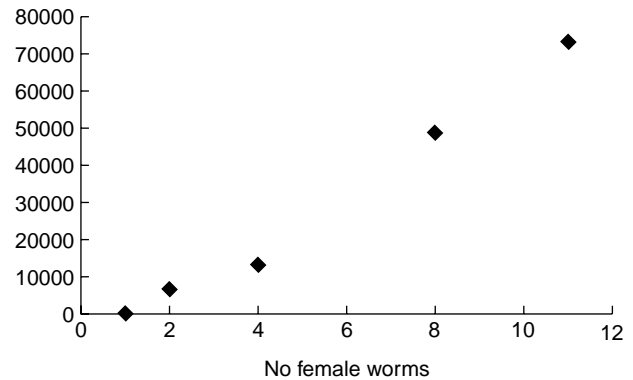
**Figure 2** Ag-specific proliferative responses are reduced in IL-4<sup>-/-</sup> mice. Groups of five BALB/c mice or IL-4<sup>-/-</sup> mice were infected with 50 L3 of *B. pahangi* by the s.c. route. Control animals received HBSS. Proliferative responses were assessed at 12 days p.i. by the uptake of [<sup>3</sup>H]thymidine in spleen cell cultures stimulated for 72 h with 10 µg/ml adult Ag. The results are expressed as c.p.m. incorporated and show the mean plus SD of five animals per group. \*Significant difference between IL-4<sup>-/-</sup> mice and wild-type mice for Ag-specific responses. ■, Cultures with Ag; □, cultures with medium alone.

**Table 1** Worm recoveries from IL-4<sup>-/-</sup> and BALB/c wild-type mice at different time points postinfection

Time p.i.	IL-4 <sup>-/-</sup>	BALB/c wild-type
Day 6	36.8 ± 22.6% (n = 6)	36.2 ± 13.9% (n = 7)
Day 8	28.8 ± 11.1% (n = 5)	47.3 ± 23.9% (n = 4)
Day 15	28.9 ± 21.2% (n = 6)	38.5 ± 14.5% (n = 8)
Day 27	36.7 ± 11.6 (n = 6)	34.2 ± 14.2 (n = 6)
Day 71	14.1 ± 11.4 (n = 13)	7.4 ± 7.7 (n = 12)
Day 77	20.5 ± 19.0 (n = 6)	10.9 ± 7.7 (n = 10)

Figures show the mean percentage recovery of worms ± SD at different time points p.i. Numbers in parenthesis denote the number of animals per group. All infections were administered by the i.p. route. At no time point did the differences in worm recovery reach statistical significance ( $P > 0.05$ ).

the numbers of larvae recovered at were counted: day 6 (moulting L3–L4), day 8 (L4), day 15 (L4), day 27 (L4 to adults) or days 71–77 (patent adults). As shown in Table 1, there was no significant difference in recovery of larvae from the IL-4<sup>-/-</sup> mice at any time point. The recovery of worms from both strains of mice remained very steady at 30–40% until after day 27, at which point most worms have completed the final moult. At day 70 plus, more adult worms were recovered from the IL-4<sup>-/-</sup> mice than from the wild-type mice but this difference did not reach statistical significance ( $P > 0.05$ ).



**Figure 3** Mf levels in IL-4<sup>-/-</sup> mice are correlated with the number of adult female worms. Mf levels in IL-4<sup>-/-</sup> mice were measured as described in Materials and Methods. The graph shows the total number of Mf in the peritoneal lavage of five IL-4<sup>-/-</sup> mice plotted against the number of female worms recovered from individual mice. Mf levels show a positive correlation ( $r = 0.993$ ,  $P = 0.001$ ) with adult female worm burden.

### IL-4<sup>-/-</sup> mice develop high levels of microfilariae

Adult female *B. pahangi* start to produce Mf around day 60 p.i. and two experiments were analysed at time points by which the infection should be patent (day 71 and day 77 p.i.). Five of ten BALB/c mice sacrificed at day 77 p.i. contained microfilariae, while seven of ten contained adult worms, consistent with previous findings from BALB/c mice infected with *B. pahangi* (13). In contrast, 6/6 IL-4<sup>-/-</sup> mice analysed at the same time point contained adult worms and high levels of Mf were recovered from some animals (Table 2). The IL-4<sup>-/-</sup> mice contained a mean number of 23 952 ± 30 485 Mf per infected mouse while the wild-type animals contained a mean of 3023 ± 2139 Mf per infected mouse ( $P = 0.0318$ ). Similar results were obtained in another experiment analysed on day 71 p.i. As shown in Figure 3, there was a wide range in the numbers of Mf recovered from individual animals, and this correlated with the numbers of adult female worms recovered at day 77 p.i. from the IL-4<sup>-/-</sup> mice ( $r = 0.993$ ,  $P = 0.001$ ) (Figure 3). However, the same correlation did not hold for the BALB/c wild-type mice. Even when BALB/c mice contained equivalent numbers of adult worms to the IL-4<sup>-/-</sup> mice, significantly fewer microfilariae were recovered from the wild-types. When Mf output was expressed as Mf per adult female worm recovered, it was apparent that the female worms from the IL-4<sup>-/-</sup> mice were much more fecund than those from the wild-type mice. These results are summarized in Table 2.

Factors such as worm length are known to influence female fecundity, and worms recovered from BALB/c mice tended to be shorter than those from the IL-4<sup>-/-</sup> mice at most time points examined (data not shown, difference in

**Table 2** Adult worm and Mf recoveries at day 77 p.i.

	IL-4 <sup>-/-</sup>	BALB/c
No mice with adult worms	6/6	7/10
Total no male : female	38 : 28	23 : 30
No mice with MF	6/6	5/10*
Mean number MF per mouse with MF	23952 ± 30485	3023 ± 2139†
Mean Mf per adult female with Mf	3329 ± 2794	722 ± 510

Figures show numbers of adult worms recovered at day 77 p.i. from the two groups of mice, the total numbers of male and female worms recovered from the respective group, the numbers of mice with Mf and the mean (± SD) numbers of Mf recovered.

\*One mouse with adult females contained no males worms and hence no Mf.

†Denotes statistical significance between Mf recoveries from IL-4<sup>-/-</sup> mice and wild-type mice ( $P = 0.0318$ ).

lengths not significant at most time points). It was notable that at the later time points (day 70+), the IL-4<sup>-/-</sup> mice contained a greater percentage of male worms than did the wild-type mice (58% males in IL-4<sup>-/-</sup> mice compared to 43% in BALB/c wild-type at day 77 p.i.). Dissection of adult female worms from the wild-type mice revealed that these contained few developing Mf even in animals which contained male worms, while the female worms from the IL-4<sup>-/-</sup> mice contained many mature Mf and larvae at all stages of development. Despite the ability of the IL-4<sup>-/-</sup> mice to support substantial numbers of Mf in the peritoneal cavity, s.c. inoculation of IL-4<sup>-/-</sup> mice with L3 did not result in a microfilaraemia in six out of six animals (data not shown).

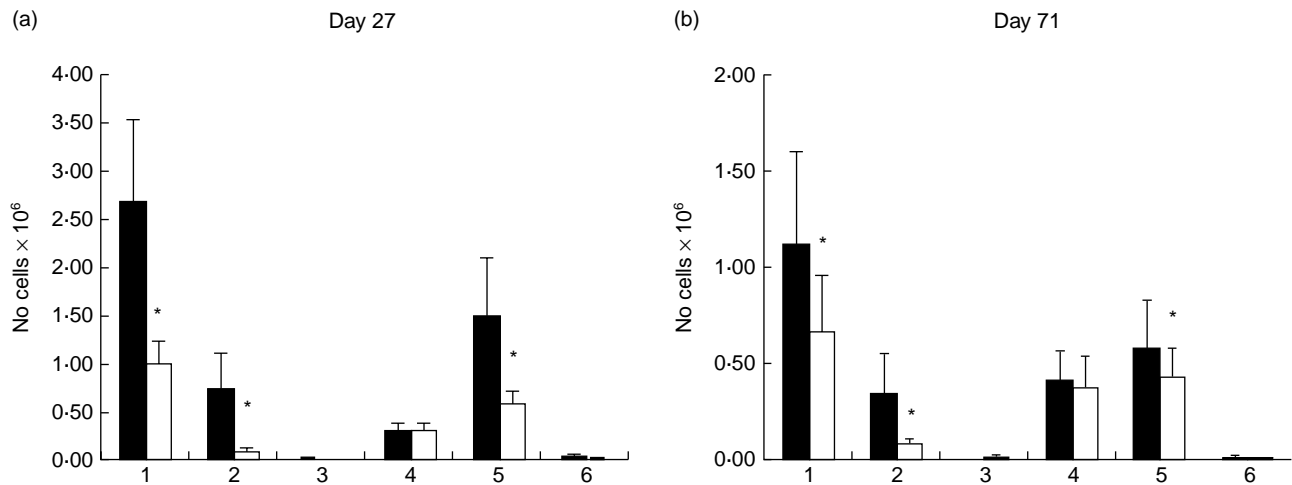
#### Implantation of adult worms into the peritoneal cavity of IL-4<sup>-/-</sup> or wild-type mice results in equivalent production of Mf

In order to investigate whether there might be differences in Mf levels in the two strains of mice when fecund females were implanted into the peritoneal cavity, 10 female *B. pahangi* were implanted i.p. into five BALB/c mice or five IL-4 knockout mice. Adult worms and Mf were recovered 2 weeks p.i. IL-4<sup>-/-</sup> mice contained significantly greater numbers of females at 2 weeks p.i. than did BALB/c wild-type mice ( $6.2 \pm 0.83$  from wild-type mice compared to  $7.4 \pm 0.89$  from IL-4 knockout mice,  $P < 0.05$ ). However, levels of Mf were not significantly different between the two strains (mean number Mf recovered from wild-type mice,  $1.41 \pm 0.18 \times 10^5$  compared to  $1.61 \pm 0.233 \times 10^5$  from knockout mice,  $P > 0.05$ ). When these results are presented as Mf per female recovered, the results are identical at  $2.2 \times 10^4$  Mf per female worm, confirming that the reduced numbers of Mf in the peritoneal cavity of wild-type mice following L3 infection likely relates to defects in worm fecundity rather than increased killing of Mf in the peritoneal cavity.

#### Cell recruitment into the peritoneal cavity varies significantly in IL-4<sup>-/-</sup> mice versus wild-type

Although there was no significant difference in worm recovery between the two strains of mice, there were significant differences in the recruitment of cells into the peritoneal cavity of BALB/c mice versus the IL-4<sup>-/-</sup> mice. By day 27 p.i., the peritoneal washings from BALB/c mice contained many more cells than the IL-4<sup>-/-</sup> mice ( $P = 0.007$ ). Figure 4(a) shows absolute numbers of cells recovered from both groups of mice at 27 days p.i. When the contribution of individual cell types was analysed by staining of cytospin preparations, it was clear that the wild-type mice contained elevated numbers of eosinophils ( $P = 0.007$ ) and macrophages ( $P = 0.011$ ) compared to IL-4<sup>-/-</sup> mice. There were no significant differences in the absolute numbers of lymphocytes while neutrophils and mast cells accounted for a very small proportion of the total cells in both strains of mouse. At day 71 p.i., the total number of cells in the peritoneal cavity of both strains of mice was much reduced compared to day 27 p.i. (Figure 4b) but the wild-type mice still contained significantly greater numbers of cells than did the IL-4<sup>-/-</sup> mice ( $P = 0.004$ ). The general make-up of the cellular infiltrate was very similar with wild-type mice containing significantly greater numbers of eosinophils ( $P = 0.0001$ ) and macrophages ( $P = 0.02$ ) than did the IL-4<sup>-/-</sup> mice.

In some experiments in which mice were infected i.p., spleens were removed and cultures initiated for cytokine analysis. In general, the pattern of cytokine secretion seen in the i.p. infected mice followed that observed in the s.c. infected animals (i.e. reduced levels of Th2 cytokines in IL-4<sup>-/-</sup> mice and increased levels of IL-2 and IFN- $\gamma$ ). However, in agreement with previous studies on BALB/c mice infected i.p. with L3 of *B. pahangi* (17), the level of Th2 cytokines secreted by splenocytes was reduced compared to s.c. infected animals at the same time point. No Ag-specific IL-10 was observed in i.p. infected wild-type animals at day 15



**Figure 4** Cell recruitment into the peritoneal cavity is reduced in IL-4<sup>-/-</sup> mice. The graph shows the number of cells per ml in the peritoneal lavage of BALB/c wild-type (■) mice or IL-4<sup>-/-</sup> mice (□) at (a) 27 days p.i. or (b) 71 days p.i. Total cells (1) were enumerated by Coulter counting while numbers of eosinophils (2), neutrophils (3), lymphocytes (4), macrophages (5) or mast cells (6) were counted by differential staining of slides prepared by cytopsin. The results are presented as means plus SD of six mice per group. \*Significant difference between IL-4<sup>-/-</sup> mice and wild-type mice.

**Table 3** Ag-driven cytokine levels in IL-4<sup>-/-</sup> and BALB/c mice at day 71 postinfection i.p.

Cytokine (pg/ml)	BALB/c	IL-4 <sup>-/-</sup>
IL-2	382 ± 333	1448 ± 1390*
IFN-γ	1365 ± 1184	7680 ± 960*
IL-4	135 ± 71	0
IL-5	1850 ± 866	264 ± 288*
IL-10	0	0

Splenocytes from individual IL-4<sup>-/-</sup> or BALB/c wild-type mice were cultured in the presence of adult Ag at 10 µg/ml and supernatants collected after 48 h. Cytokines were measured as described in Materials and Methods. Figures show mean Ag-specific cytokine levels (± SD) in 12 BALB/c mice and 13 IL-4<sup>-/-</sup> mice infected by the i.p. route at day 71 p.i. Cytokines are expressed in pg/ml. \**P* < 0.05.

p.i. or day 71 p.i. or at any time point in IL-4<sup>-/-</sup> mice (day 15, 35 or 71). IL-5 levels were significantly elevated in wild-type mice compared to IL-4<sup>-/-</sup> mice at all time points examined, while IFN-γ levels were elevated in the knockout mice. These data are summarized for a single time point in Table 3.

## DISCUSSION

The results of this study demonstrate that IL-4 or IL-4 mediated responses have little effect upon the ability of the L3 of *B. pahangi* to establish in the peritoneal cavity of the

BALB/c mouse. At all time points, equivalent numbers of developing worms were recovered from the wild-type mice and the IL-4 knockout mice. These observations suggest that, at least in a primary infection, IL-4 does not influence worm recovery, despite the fact that the cytokine profiles of the two strains of mice were markedly different. As reported previously, splenocytes from BALB/c mice infected with L3 secrete exclusively Ag-specific Th2 cytokines at 12 days p.i. (4,15). In contrast, splenocytes from the IL-4<sup>-/-</sup> mice showed a mixed T helper cell profile with elevated levels of IFN-γ and IL-2, reduced levels of IL-5 and IL-10 and high levels of IL-13, which is usually considered to be a Th2 associated cytokine (18). As both IL-5 and IL-10 can be expressed by a variety of cell types (19,20), it is possible that the residual levels of secretion observed in the knockout mice derive from sources other than Th2 cells. However, production of Th2 cytokines in IL-4<sup>-/-</sup> mice has been reported previously (21), and was suggested to reflect Th2 priming in the absence of IL-4 as judged by the expression of the Th2 marker ST2L in these mice. In the present study, splenocytes from both mouse strains secreted high levels of IL-13 in response to *in vitro* restimulation with Ag suggesting that, in this model system, IL-13 does not compensate for IL-4 in terms of effects on Mf production, polarization of the cytokine response or cell recruitment. These results contrast with those from the *Trichuris muris* model (22): in that system MLN cells from IL-4<sup>-/-</sup> mice on the C57Bl6 background secreted significantly lower levels of IL-13 than did wild-type mice. Other studies have shown little difference in IL-13 levels in IL-4<sup>-/-</sup> mice infected with *Leishmania major*

(21) or in mice infected with *T. muris* when IL-4 is inhibited *in vivo* by B7 blockade (23). These results may in part reflect differences in the background strain of the animals used, the propensity of the infectious agent to elicit IL-13 and/or the cellular source of IL-13.

Our previous studies in BALB/c wild-type mice demonstrated that a combination of IL-4, IL-10 and the antigen-presenting cell population actively suppressed Th1 responses elicited by s.c. infection with the L3 of *B. pahangi* (15,17). Analysis of cytokine production in the IL-4<sup>-/-</sup> mice confirmed that infection with L3 does indeed result in Th1 priming with production of Ag-specific IL-2 and IFN- $\gamma$ . Levels of Ag-specific proliferation of splenocytes from L3 infected IL-4<sup>-/-</sup> mice were also consistently lower than wild-type mice. This may reflect a limited expansion of Th2 cells in the knockout mice or, alternatively, may be due to the increased expression of IFN- $\gamma$  in these cultures which can downregulate T cell proliferation via the induction of NO (16). Interestingly, high levels of NO have been observed in cultures of splenocytes from L3-infected IL-4<sup>-/-</sup> mice (data not shown), but further studies will be required to formally prove that NO suppresses proliferation in this system.

Although higher numbers of adult worms were recovered at day 70 plus from the IL-4<sup>-/-</sup> mice than from the BALB/c wild-type mice, the differences in recovery did not reach statistical significance at any time point. Major differences were observed in the quantity and the make-up of the cellular infiltrate in the peritoneal cavity of infected IL-4<sup>-/-</sup> mice and the BALB/c controls. The recruitment of cells into the peritoneal cavity presumably reflects the strong Th2 response in wild-type mice, as demonstrated by high levels of IL-5 and increased numbers of eosinophils in these animals. Despite the profuse cellular infiltrate containing many eosinophils in wild-type mice, the worms appeared to survive and develop normally. Eosinophils have been implicated in worm killing in many previous studies, although usually in the context of a secondary infection (10,11).

The most significant difference between the two strains of mice related to the recovery of microfilariae. IL-4<sup>-/-</sup> mice contained large numbers of Mf in the peritoneal cavity, a finding that appears to relate to the greater fecundity of adult female worms in these mice rather than to increased killing of Mf in the wild-type mice. When the results were expressed as Mf per adult female worm, the female worms recovered from the IL-4<sup>-/-</sup> mice produced almost five-fold more Mf than those recovered from the wild-type mice (Table 2). Dissection of individual female worms from the IL-4<sup>-/-</sup> mice confirmed that these worms contained many more Mf at all stages of development than did adult female worms from the wild-type mice. Even in BALB/c mice that had Mf in the peritoneal cavity, the adult female worms contained very few developing stages or mature Mf. These

results were confirmed by implanting fecund female worms into the peritoneal cavity of both strains of mice. In that situation in which adult females were already producing Mf, there was no significant difference in Mf recoveries from either strain of mouse, demonstrating that the differences in Mf recovery cannot be explained by an increased rate of killing of Mf in the wild-type mice.

These results indicate that IL-4 influences Mf levels via effects on worm fecundity. IL-4 or IL-4-mediated responses may have a direct effect upon the female worm or may influence male fertility or embryo development. In the experiments reported here, there was a tendency for IL-4<sup>-/-</sup> mice to contain greater numbers of male worms than the wild-type mice. In a previous study, male *B. pahangi* were shown to be much more susceptible to the effects of ionizing radiation than were female worms (24) suggesting that male worms may be less resistant to oxidative or immunological stresses than female worms. In *Schistosoma mansoni* infection, the concept of anti-fecundity immunity is well documented in response to vaccination (25). Within the nematodes, it has previously been observed that fecundity of *Strongyloides ratti* worms is reduced in the presence of IL-5: infection of IL-5<sup>-/-</sup> mice results in increased worm fecundity compared to worms from wild-type mice (26). As *S. ratti* reproduces parthenogenetically, effects on male worms could be excluded in that study. In the present study, IL-5 levels were significantly higher in wild-type mice than in IL-4<sup>-/-</sup> mice but it is not possible to make a direct association between Mf production and IL-5 levels on the basis of the data presented here. It would be interesting to implant adult *Brugia* into the peritoneum of IL-5<sup>-/-</sup> mice and investigate whether IL-5 may directly affect Mf production. Studies in a mouse model of *Onchocerca* infection implicated IL-5, but not IL-4, in Mf killing following primary and secondary infection (27).

The previous studies of Rajan and coworkers have highlighted the importance of background strain when comparing the relative susceptibility of various inbred strains of mice to infection with L3 of *Brugia* species (28,29). The results of this study are in contrast to those reported recently by Babu *et al.* (28), in which IL-4<sup>-/-</sup> mice on the BALB/c background were infected with L3 of *B. malayi*. In that study, no living worms were recovered from wild-type BALB/c mice by 6 weeks of infection, whereas in this study with *B. pahangi*, equivalent numbers of worms were recovered from wild-type and IL-4<sup>-/-</sup> mice at all time points. The differences in our respective results underscore the importance of the species of parasite in use (*B. malayi* versus *B. pahangi*), as well as the background strain of mouse used. There are clearly fundamental differences in the susceptibility of BALB/c mice to infection with *B. pahangi* and *B. malayi* as BALB/c mice are susceptible to infection with L3 of

*B. pahangi*, although only low numbers of adult worms are recovered. However, our results do agree on one important point: the ability of the IL-4<sup>-/-</sup> mice to support a significant level of Mf production. Similar results were reported recently by Volkmann *et al.* (30) using *L. sigmondontis* in IL-4<sup>-/-</sup> mice on the BALB/c background.

In human filarial infection, a significant percentage of infected individuals may be amicrofilaraemic but actively infected, as demonstrated by the presence of circulating antigen in the absence of microfilariae (31). Indeed as many as 70% of infected individuals are amicrofilaraemic in some *Loa loa* endemic areas (32). It is interesting to speculate whether the absence of Mf is indicative of an active killing of Mf in the circulation (as is usually assumed) or could reflect alterations in worm fecundity. Seasonal variations in Mf levels have been recorded (33) which may indicate that worm fecundity is a variable parameter, although the basis by which this is mediated remains to be determined.

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