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Cytokine-dependent inflammatory cell recruitment patterns in the peritoneal cavity of mice exposed to the parasitic nematode *Brugia malayi*

Received: 22 July 2002 / Accepted: 23 August 2002 / Published online: 12 December 2002
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Abstract Mice exposed intraperitoneally to either adult or first larval stage (microfilaria) of the human nematode parasite *Brugia malayi* display polarized cytokine responses. We have used this model to investigate the impact of altered cytokine profiles on inflammatory cell recruitment patterns in vivo. Here we demonstrate that Th2-inducing adult parasites drive the recruitment of eosinophils and macrophages after implant into the murine peritoneal cavity whereas Th1-inducing microfilaria do not. The underlying mechanism of recruitment was further defined by use of mice deficient in the key Th2 cytokines IL-4 or IL5 and mice that lack T cells (nude mice). Recruitment dynamics differed in IL-4 and IL-5 deficient mice, showing reduced or absent eosinophilia. These data emphasize the pivotal role of these cytokines in shaping the cellular profile of inflammatory responses. Surprisingly, the absence of T cells failed to influence inflammatory cell recruitment indicating that recruitment signals are provided by other cell types.

Keywords Eosinophil · Macrophage · Interleukin 4

Introduction

Cytokines and chemokines play a crucial role in inflammatory recruitment processes, and the balance of cytokines produced influences both the phenotype and function of recruited cells [1, 2, 3]. We have used a unique model system to analyse the influence of cytokines

that characterize polarized immune responses upon inflammatory cell recruitment in vivo. Parasitic helminth infection induces a distinctive inflammatory cell infiltrate comprising macrophages and eosinophils and is associated with the development of a pronounced type-2 (Th2) immune response characterized by elevated levels of the cytokines interleukin (IL) 4 and IL-5 [4, 5, 6, 7, 8, 9, 10]. Previous studies in our laboratory have demonstrated that exposure of mice to different life-cycle stages of the nematode parasite *Brugia malayi* leads to recruitment of cells with different biological properties [11]. Implantation of adult parasites into the peritoneal cavity causes the recruitment of peritoneal exudate cells that are profoundly anti-proliferative while the larval microfilariae (Mf) induce the recruitment of cells that are more typically pro-inflammatory [11]. These functional differences require the development of a host immune response and do not become fully apparent until 2–3 weeks post-infection and are consistent with the observation that different life cycle stages of *B. malayi* stimulate contrasting cytokine profiles in infected mice [12, 13]. Adult *B. malayi* parasites drive strong Th2-type responses, with high levels of IL-4, IgG1 and IgE being produced. Conversely, in the early stages of exposure Mf alone induce a Th1-type response, characterized by production of elevated levels of interferon (IFN)- γ accompanied by enhanced IgG2a responses.

We hypothesized that the distinct patterns of cell recruitment and/or activation that occur following implantation of different life-cycle stages of *B. malayi* reflect the contrasting cytokine responses observed, with potentially profound implications for regulation of inflammation under type 1 and type 2 conditions. We therefore carried out an analysis of the cells recruited following implantation of different life cycle stages of *B. malayi* parasites into the peritoneal cavity. Inflammatory cell recruitment following implantation of Th2-inducing adult parasites was characterized by large numbers of eosinophils and macrophages to the site of infection, whereas Th1-inducing Mf did not induce this marked recruitment of inflammatory cells. Mice genetically

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deficient in the production of key Th2 cytokines IL-4 and IL-5 showed attenuated or absent eosinophil/macrophage recruitment, suggesting a pivotal role for these cytokines in shaping the profile of the resultant inflammatory cell recruitment profiles. Further, we found that T cells are not required to provide the appropriate cytokine signals for recruitment of eosinophils and macrophages in response to adult parasites. Together our data provide insight into the dynamics of inflammatory cell recruitment and provide unequivocal evidence that production of type 2 cytokines determines cellular recruitment patterns during chronic nematode infection.

Materials and methods

Parasite isolation

B. malayi adults and Mf were obtained from infected jirds purchased from TRS laboratories (Athens, Ga, USA). Jirds were killed by cardiac puncture under anaesthesia; adult worms were removed from the peritoneal cavity, then washed in RPMI 1640 (Gibco-BRL, UK) supplemented with 50 µg/ml gentamicin. Mf were separated from host material by passage through PD10 Sephadex G-25 M columns (Pharmacia Biotech, Uppsala, Sweden).

Mouse infection model

For all experiments the mice used were 6- to 8-week old males. Experimental mice were generally purchased from Harlan-UK (Bicester, UK), or obtained from source (Edinburgh University). C57BL/6 IL-4 deficient (IL-4^{-/-}) [14] were purchased from B&K Universal (North Humberside, UK) with the permission of the Institute of Genetics, University of Cologne. C57BL/6 IL-5 deficient (IL-5^{-/-}) [15] mice were the kind gift of Prof. Manfred Kopf (Basel, Switzerland) and were bred in-house. BALB/c nude mice and wild-type controls were obtained from Harlan-UK. Mice were surgically implanted intra-peritoneally under anaesthesia with mixed-sex infections, using six adult female and two adult male *B. malayi* parasites. For Mf infections, mice were injected intra-peritoneally with 2×10⁵ Mf using a 21-gauge needle. Implants were left for 3 weeks unless otherwise stated. At this point, animals were killed by cardiac puncture under anaesthesia, and peritoneal exudate cells (PEC) were harvested by thorough washing of the peritoneal cavity with 15 ml RPMI containing 50 µg/ml gentamicin.

Cyocentrifuge preparation

Harvested PEC were washed and adjusted to 1×10⁶/ml in complete medium. We combined 100 µl cells (1×10⁵) and 100 µl foetal calf serum (Gibco-BRL, UK), and cyocentrifuge preparations were made using a Shandon Cytospin (Shandon, Pa., USA). Cyocentrifuge preparations of PEC were air-dried and fixed for 2 min in methanol prior to staining with Diff-Quik (Dade, Germany). The proportion of macrophages, lymphocytes, mast cells, neutrophils and eosinophils was determined by morphological examination of at least 300 cells in randomly selected fields using a Nikon Microphot-FX microscope.

Flow cytometric analysis of PEC

Harvested PEC were washed and adjusted to a concentration of 1×10⁷/ml in FACS phosphate-buffered solution (PBS; containing 1% bovine serum albumin and 0.1% sodium azide). All antibodies used had previously been titrated to determine the optimum amount required for staining of 1×10⁶ cells. The appropriate

amount of antibody was then added to cell samples to be stained, either in a 15-ml centrifuge tube or in a round-bottomed microtitre plate. Samples were incubated at 4°C for 30 min, washed three times with FACS PBS, then transferred to FACS tubes (Becton Dickinson) and either analysed directly, or fixed with a few drops of 10% formalin and stored in the dark prior to analysis, using a FACScan with CELLQuest software (Becton Dickinson). Tri-colour-conjugated anti-macrophage (clone F4/80) (Caltag) antibodies were used for flow cytometry.

Cytokine assays

The IL-2/IL-4 responsive NK cell line [16] was used to measure levels of IL-4 in splenocyte supernatants, as previously described [13]. Briefly, proliferation of the NK cells at 10⁴ cells/well was measured after the addition of 20 µl culture supernatant in the presence of anti-IL-2 (S4B6) neutralizing Ab (obtained from ascites and used at 2.5 µl/ml, the optimal concentration for neutralization as determined by titration). NK cells and supernatants were incubated for 24 h at 37°C prior to addition of 1 µCi [³H]thymidine in 10 µl complete medium. After a further 12 h of incubation at 37°C plates were harvested and counted using a Top Count microplate scintillation counter (Packard Instrument, Meriden, Conn., USA). IL-5 was measured by capture enzyme-linked immunosorbent assay using purified anti-IL-5 (TRFK5, Pharmingen) paired with biotinylated anti-IL-5 (Pharmingen).

Statistical analysis

Student's *t* test was used to determine the statistical significance of differences between groups. *P* < 0.05 was considered to indicate a significant difference.

Results

Contrasting cell recruitment induced by adult or microfilarial parasite stages

We tested the hypothesis that the distinct composition and biological activities of inflammatory cells following peritoneal implantation of mice with either live adult parasites or with Mf reflects the cytokine profiles induced. We therefore examined PEC isolated from unimplanted mice, or mice implanted intra-peritoneally with live *B. malayi* adults (Th2 inducing) or live Mf (Th1 inducing). As a control, dead adult *B. malayi* parasites were implanted to assess the requirement for live parasite products in the recruitment process. The analysis of PEC composition was performed at 3 weeks because at this stage the composition of inflammatory cells in the peritoneal cavity had stabilized [17] and thus likely represents the chronic inflammation typical of nematode infections. Markedly different cell recruitment profiles were seen in response to adult or Mf implantation. Implantation of either adult or Mf parasites induced changes in the PEC composition as evidenced by a decrease in the numbers of mast cells present in comparison to controls (Table 1; *P* < 0.03). However, only implantation of adult parasites resulted in significant increases in the percentage (as well as total numbers) of both macrophages and eosinophils. In addition, a significant number of large, multi-nucleate macrophages

Table 1 Recruited and resident peritoneal cell types. Data represent the mean number ($\times 10^6$) of each cell sub-population recovered in peritoneal washes. The cell composition of PEC from control

| | Control | Dead adult implant | Live adult implant | Live Mf implant |
|-------------|-----------------|--------------------|--------------------|-----------------|
| Macrophages | 1.12 ± 0.5 | 1.47 ± 1.0 | 7.85 ± 2.28 | 0.42 ± 0.16 |
| Eosinophils | 0.01 ± 0.01 | 0.07 ± 0.1 | 1.23 ± 0.53 | 0.04 ± 0.06 |
| Neutrophils | ND | ND | 0.04 ± 0.03 | 0.03 ± 0.04 |
| Lymphocytes | 1.95 ± 0.55 | 1.92 ± 0.44 | 1.84 ± 0.45 | 1.36 ± 0.5 |
| Mast Cells | 0.25 ± 0.09 | 0.19 ± 0.08 | 0.06 ± 0.04 | 0.02 ± 0.02 |
| Total Cells | 3.3 ± 1.1 | 3.7 ± 1.1 | 11.1 ± 3.3 | 1.9 ± 0.6 |

and implanted CBA/Ca mice was determined from cytopins by microscopy. Data shown are mean \pm SD of three to five individual mice separately assayed (ND not detectable)

were present in PEC from adult parasite implanted mice (data not shown), consistent with previously published data [18]. Together these data indicate that the distinct cytokine profiles of Th1 and Th2 responses could contribute to the regulation of recruitment.

Adult parasites induce recruitment of large granular cells and F4/80⁺ cells

We next examined laser scatter properties [forward scatter (size) and side scatter (internal complexity)] of PEC from control, live adult, dead adult, or Mf implanted mice in flow cytometric analysis. We found that adult parasites, but not Mf, caused recruitment of large granular cells to the peritoneal cavity (data not shown). The phenotype of these cells in the various treatment groups was determined using well characterized cell lineage markers including the macrophage marker F4/80 [19]. Consistent with the increased numbers of cells with macrophage morphology recovered from the site of adult parasite implant [20], a marked increase in the proportion of recovered PEC staining positive with F4/80 was evident in adult-, but not Mf-implanted mice (Fig. 1). Studies in our laboratory have demonstrated that these F4/80 positive cells have profound antiproliferative effects on other cells [21] and have the capacity to drive naive T cells toward Th2 cytokine production [22].

Cell recruitment in the absence of host IL-4 or IL-5 production

The striking recruitment of macrophages and eosinophils by adult implanted mice and the ability of adult *B. malayi* parasites to provoke a systemic Th2-type response [13] led us to evaluate the role of type 2 cytokines in the recruitment process. We compared cellular recruitment patterns in wild-type, IL-4 and IL-5 deficient mice. All three groups of implanted mice (C57BL/6, IL-4^{-/-} and IL-5^{-/-}) showed a dramatic expansion of macrophage numbers, but this was somewhat less pronounced in the IL-4^{-/-} mice which had proportionally more lymphocytes than either the wild-type or IL-5^{-/-} mice. Eosinophilia was markedly reduced in IL-4 deficient mice relative to wild-type mice in response to adult parasite implant. However, in comparison to wild-type

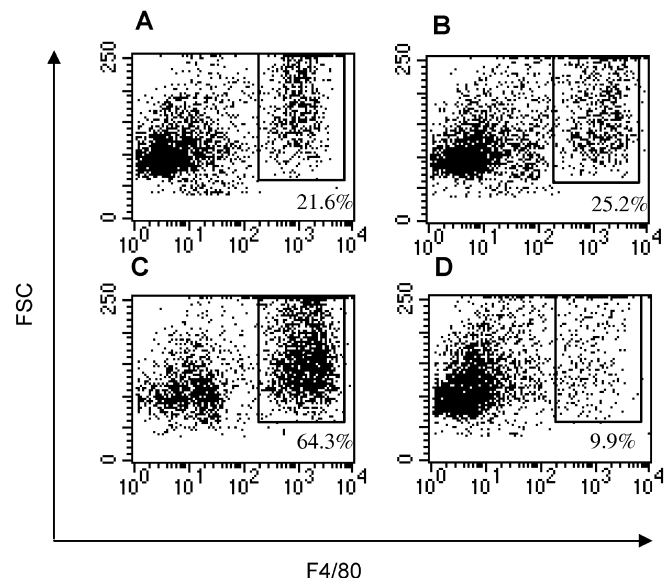


Fig. 1A–D Flow cytometric analysis of PEC from *Brugia*-implanted mice. F4/80⁺ PEC from control (unimplanted, **A**), dead adult (**B**), live adult (**C**), or Mf-implanted (**D**) CBA/Ca mice were gated on the basis of their expression of F4/80. Figures refer to the percentage of total cells from each group staining positive for F4/80. Control PEC were combined cells from four unimplanted mice, dead implant PEC were combined cells from five mice each implanted with ten dead adult parasites, Mf PEC were combined cells from four mice each injected intra-peritoneally with 2×10^5 Mf, and adult-implant-derived PEC were typical results obtained from one implanted mouse. PEC were harvested 3 weeks after implantation

control mice there was a decreased recovery of mast cells (Fig. 2), indicating that inflammatory processes had been initiated. Analysis of PEC composition following intraperitoneal implant of adult *B. malayi* in IL-5 deficient mice revealed a complete absence of eosinophils consistent with previously published data [15, 23] (Fig. 2). Thus, despite normal basal levels of eosinophils [15], IL-5 deficient mice fail to recruit eosinophils to filarial nematode infection. Interestingly, these differences in cell recruitment had no apparent effect on parasite survival as equivalent numbers of live parasites were recovered from the peritoneal cavity of IL-4^{-/-} mice, IL-5^{-/-} mice and similarly implanted wild-type controls (data not shown). These findings indicate that differential cell recruitment patterns were not due to differences in parasite load in infected animals.

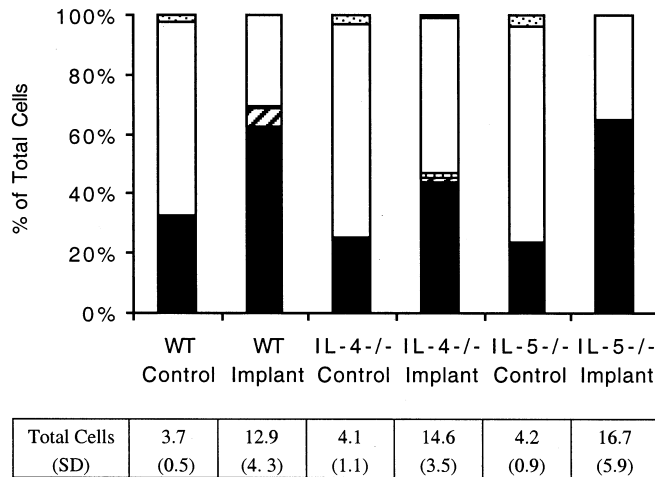


Fig. 2 Peritoneal cell populations in control and adult-implanted wild-type IL-4^{-/-}, and IL-5^{-/-} C57BL/6 mice. Mean percentage of total cells that were identified as mast cells (*spotted bars*), monocytes/blasting cells (*grey bars*), lymphocytes (*open bars*), neutrophils (*vertically hatched bars*), eosinophils (*diagonally hatched bars*), and macrophages (*solid bars*). Figures refer to the mean total number of cells ($\times 10^6$) recovered from the peritoneal cavity of control or implanted mice, with the standard deviation given in parenthesis. PEC were harvested 3 weeks after parasite implant, and cell composition was determined from cytospins by microscopy. Data shown are mean of three to five individual mice separately analysed

Cell recruitment in the absence of host T cells

Since T lymphocytes provide a primary source for cytokines such as IL-4 and IL-5 [24], we next investigated the involvement of host T cells in recruitment of inflammatory cells. Analysis of cell recruitment in athymic (nude) mice implanted with adult parasites in the peritoneal cavity revealed a similar pattern of eosinophil recruitment and loss of mast cells in comparison to wild-type controls (Fig. 3). However, the proportion of macrophages recruited to the peritoneal cavity of infected nude animals was less elevated than was seen in infected wild-type mice. This suggests that T cell amplification of IL-4 or IL-5 is not necessary for appropriate eosinophil recruitment in response to parasite implant but may be more important in recruitment of macrophages.

Influence of background genetics on cell recruitment

It is well established that background genetics have marked effects on cytokine production [25, 26]. Because our experiments necessitated the use of mice on different genetic backgrounds, we chose to determine whether murine strain differences influenced the cell recruitment dynamics that we had observed. Cyto-centrifuge preparations were made of cells recovered from peritoneal washes of BALB/c, C57BL/6 and CBA/Ca mice 3 weeks after implant with either adult or Mf parasites (Fig. 4). All three strains of mice showed significant increases in

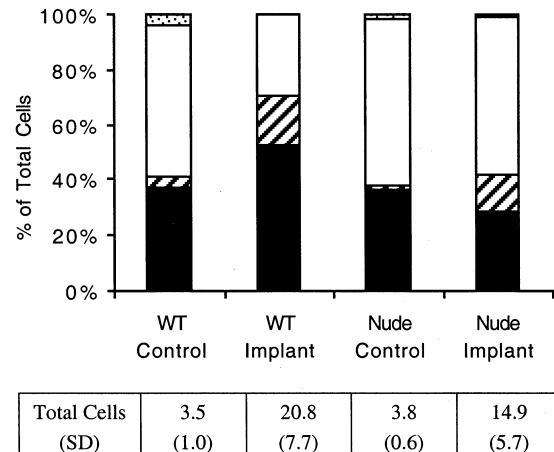


Fig. 3 Peritoneal cell populations in control and adult-implanted wild-type and nude BALB/c mice. Mean percentage of total cells that were identified as mast cells (*spotted bars*), lymphocytes (*open bars*), eosinophils (*diagonally hatched bars*), and macrophages (*solid bars*). Figures refer to the mean total number of cells ($\times 10^6$) recovered from the peritoneal cavity of control or implanted mice, with the standard deviation given in parenthesis. PEC were harvested 3 weeks after parasite implant, and cell composition was determined from cytospins by microscopy. Data shown are mean of three to five individual mice separately analysed

total numbers of cells recovered from the peritoneal cavity in response to adult parasites. However, total cell numbers recovered from both BALB/c and C57BL/6 mice implanted with adult parasites was significantly greater than CBA/Ca mice ($P < 0.01$ and $P < 0.05$; Table 2). None of the strains showed significant increases in total cell numbers recruited in response to implant with Mf stage parasites compared to unimplanted controls (Table 2).

Although similar proportional recruitment of different cell types to the site of adult parasite implant occurred in all three strains of mice, increased numbers of neutrophils were observed in BALB/c and CBA/Ca mice which was not seen in C57BL/6 mice (Fig. 4). This was also reflected in the total number of cells recruited (Table 2). In terms of total numbers of macrophages and eosinophils BALB/c and C57BL/6 mice responded similarly to adult stage parasites. Fewer of these cells were seen in adult-implanted CBA/Ca mice, reflecting the overall lower cell recovery. Among untreated mice BALB/c mice exhibited the lowest proportion of resident mast cells, with CBA/Ca having the highest. BALB/c and CBA/Ca strain mice showed significantly decreased numbers of mast cells recovered from the peritoneal cavity ($P < 0.05$ and $P < 0.02$) of either adult or Mf implanted mice relative to control mice, even given the large increases in total cell numbers recovered from adult implanted mice (Table 2). Although C57BL/6 mice showed a proportional decrease in mast cells in response to both adult and Mf parasite exposure (Fig. 4), when total cell numbers were taken into account (Table 2), a significant decrease in total numbers of mast cells recovered was only seen in Mf implanted C57BL/6 mice

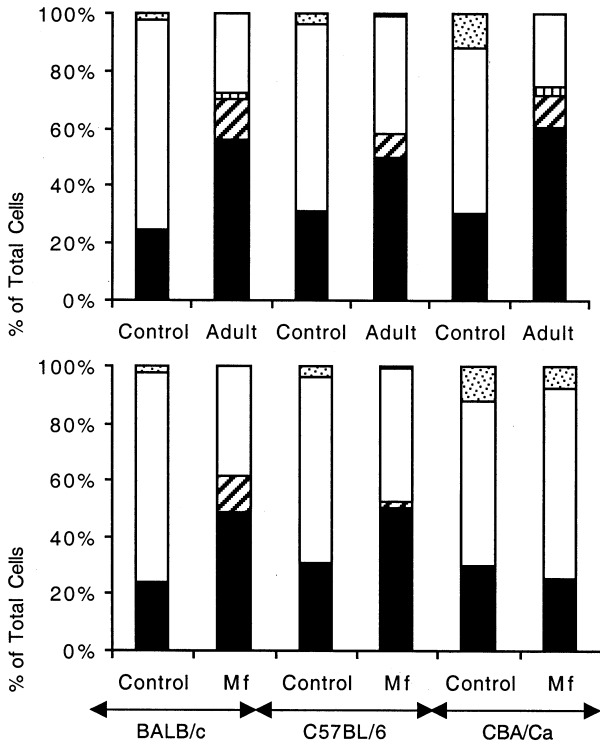


Fig. 4 Peritoneal cell populations in control and parasite implanted BALB/c, C57BL/6 and CBA/Ca mice. Mean percentage of total cells that were identified as mast cells (spotted bars), monocytes/blasting cells (grey bars), lymphocytes (open bars), neutrophils (vertically hatched bars), eosinophils (diagonally hatched bars), and macrophages (solid bars), in control (unimplanted) mice, and mice implanted in the peritoneal cavity with adult (above) or Mf (below) parasites. The cell composition of PEC from control and implanted mice was determined from cytopspins by microscopy. Data shown are mean of three to six individual mice separately analysed

Table 2 Strain comparison of total numbers of recruited peritoneal cells. Data represent the mean total number ($\times 10^6$) of each cell sub-population recovered in peritoneal washes. The cell composition of PEC from control (unimplanted), adult and Mf implanted BALB/c, C57BL/6 and CBA/Ca mice was determined from cytopspins by microscopy. Data shown are mean \pm SD of three to six individual mice separately assessed

| | BALB/c | C57BL/6 | CBA/Ca |
|---------------|------------------|------------------|-----------------|
| Control | 3.25 \pm 0.69 | 2.52 \pm 0.81 | 1.65 \pm 0.60 |
| Adult implant | 13.92 \pm 3.06 | 16.70 \pm 6.59 | 7.40 \pm 1.98 |
| Mf implant | 5.83 \pm 3.17 | 5.58 \pm 3.98 | 1.32 \pm 0.54 |

($P < 0.05$). More marked differences were seen between different mouse strains in recruitment dynamics towards Mf. Notably, BALB/c mice showed a striking eosinophilia in response to Mf that was much less marked in the C57BL/6 and was not observed at all in CBA/Ca mice.

In keeping with published data in BALB/c mice [13], splenocytes from adult parasite implanted mice of all three strains produced elevated levels of type-2 cytokine on stimulation with parasite antigen in vitro. Implanted animals produced high levels of IL-4 and IL-5 and low

levels of IFN- γ with no statistically significant differences between the mouse strains (data not shown). Splenocytes from Mf implanted mice of all three strains tested produced high levels of IFN- γ on stimulation with parasite antigen (BmA) in vitro, also with no significant difference in levels of production being observed between the three strains of mice (data not shown). Surprisingly, BALB/c mice produced high levels of IL-4 and IL-5 in response to Mf stage parasites that were significantly higher than those observed in either C57BL/6 or CBA/Ca mice (Fig. 5), consistent with the elevated eosinophilia in BALB/c mice injected with Mf.

Discussion

Analysis of the inflammatory cell recruitment dynamics following filarial nematode infection has yielded intriguing information about both inflammatory cell recruitment dynamics and the interaction of *B. malayi* with the host immune system. Most notably, recruitment

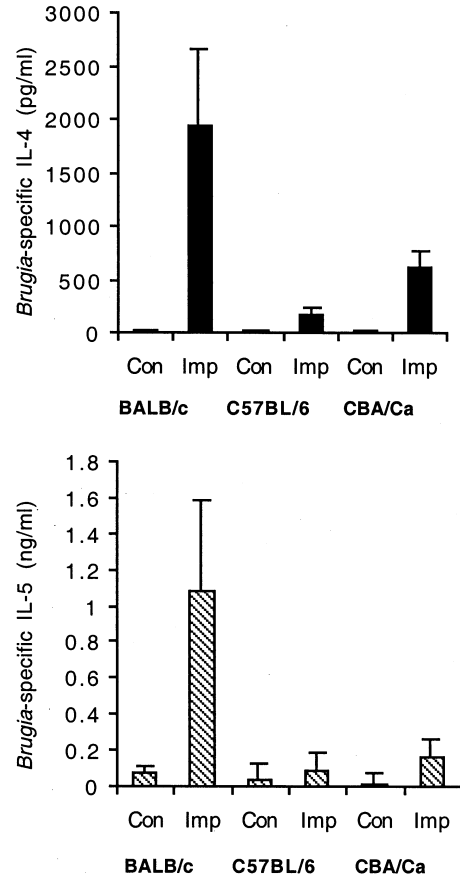


Fig. 5 Mf-induced splenocyte cytokine production. IL-4 (solid bars), or IL-5 (diagonally striped bars) production by splenocytes from control (unimplanted) or implanted (200,000 Mf) BALB/c, C57BL/6 or CBA/Ca mice. Cultures were stimulated with 5 μ g/ml BmA, and levels of cytokine produced were measured by bioassay (IL-4), or by enzyme-linked immunosorbent assay (IL-5). Data shown are mean \pm SD of results obtained from supernatants of five individual mice in each group separately assayed

of cells to the adult stage of *B. malayi* differed markedly from that generated by the Mf stage of the parasite. Cells recovered from mice implanted in the peritoneal cavity with adult parasites show a marked reduction in mast cells, and an increase in both macrophages and eosinophils, in comparison to unimplanted control mice [20]. During the course of these investigations, eosinophils consistently comprised 10–20% of total cells recovered from the peritoneal cavity of adult parasite implanted mice. Mf-implanted mice showed a minor increase in the proportion of lymphocytes, a decrease in the proportion of macrophages, and a reduced eosinophilia in comparison to adult implants. Additionally, the large increase in total cell numbers that was apparent in adult-implanted mice was not seen with Mf-injected animals.

A reduction in the proportion and numbers of mast cells recovered from the peritoneal cavity of *B. malayi* implanted mice suggests that the presence of the parasite has activated these cells, causing either degranulation or adherence to the wall of the peritoneal cavity. The activation of mast cells could have many implications for recruited inflammatory cell function and the outcome of infection. Human and murine mast cells play a key role in the recruitment of inflammatory cells through the release of mediators such as histamine and tryptase [27] and are capable of producing IL-4 on stimulation [28]. Indeed, IL-4 itself has been shown to induce the release of the eosinophil chemokine eotaxin by epithelial cells [29]. It is possible that mast cells contribute to IL-4 production in response to the parasite to drive the subsequent Th2 phenotype that is typical of helminth infection. However, since mast cell populations are depleted following Mf implantation (which is not characterized by early IL-4 production), other factors must also be involved.

IL-4 has also been shown to induce cultured monocytes/macrophages to form giant multinucleated cells [30]. Although the high levels of IL-4 present in filarial-infected animals may promote the emergence of the multinucleate macrophages seen in cytopspins from adult implanted mice, multinucleate macrophages were also recovered from IL-4 deficient mice, suggesting that other cytokines, such as IL-13, may also play a role. Multinucleate macrophages isolated from filarial-infected animals have previously been shown to express similar receptor levels and have equivalent phagocytic capacity to the mononuclear cells [18].

The importance of both IL-4 and IL-5 in driving appropriate cellular recruitment to *B. malayi* infection was highlighted in IL-4 and IL-5 deficient mice. Both strains mounted significantly reduced eosinophilias towards the parasite, in comparison to wild-type mice. However, there was a significant increase in total cell numbers recovered from infected IL-4^{-/-} and IL-5^{-/-} mice in comparison to controls and a significant decrease in mast cell numbers, indicating that these inflammatory processes remained intact in these animals. The numbers of macrophages recruited to parasite exposure was reduced relative to wild type in IL-4 deficient

mice, with an increase in the number of lymphocytes present, suggesting that IL-4 has additional roles in determining the recruitment and/or expansion of particular cell types. While there was no eosinophil recruitment in IL-5^{-/-} mice, some eosinophils were recruited in IL-4 deficient mice, indicating an IL-4 independent pathway of eosinophil recruitment. This is consistent with models of eosinophil recruitment in the lung in which reduced but not absent eosinophilia is seen in both IL-4 deficient and IL-13 deficient mice, but double deficient mice completely fail to recruit eosinophils [31]. Devaney et al. [32] also recently found significantly reduced eosinophils in IL-4^{-/-} BALB/c mice infected with *B. pahangi*. However, these results differed somewhat from our study as they found significantly reduced total cell recruitment in the absence of IL-4 on the BALB/c background.

Eosinophilia in response to *B. malayi* implant persists for many weeks and can represent a large proportion of the total cells recovered from the peritoneal cavity during this time. However, even given this eosinophil-rich environment, adult filarial parasites have been shown to be able to survive for several months, and Mf for at least 1 month in the peritoneal cavity of mice [33]. We have recovered live adult parasites as long as 6 months after implant into the peritoneal cavity (unpublished data). These findings do not support a role for eosinophils as the effector cell type in filarial killing. Consistent with this suggestion, we could see no evidence for increased parasite survival in IL-5 deficient mice that completely failed to develop eosinophilia.

The results obtained from the implant of athymic mice indicate that T cells are not critical for appropriate cell recruitment to the site of filarial infection. Although the absence of T lymphocytes resulted in reduced total numbers of recruited cells in comparison to wild-type implants, nude mice showed a marked increase in macrophage recruitment, a prominent feature in wild-type implants. In addition to this nude implants showed characteristic eosinophilia in response to adult stage parasites. These observations require further investigation as they conflict with studies that suggest a requirement for T cells in eosinophil recruitment [34]. However, they are consistent with studies demonstrating lung eosinophilia in *Toxocara canis* infected nude mice [35]. Differences between these studies may reflect the genetic background of the mice used, the site of cellular recruitment and/or the nature of the stimulating agent. Regardless, our results suggest that in the absence of host T cells, the development of appropriate cytokine/chemokine recruitment signals can occur, perhaps provided by an alternative cellular source. As athymic mice displayed an equivalent reduction in mast cells recovered from the site of adult parasite implant to wild type mice, it is possible that mast cells provide an alternative source of cytokine.

Little is known about inherent differences that might exist in the immune response of different inbred strains of mice to filarial parasites. Comparative studies in this field have primarily dealt with the survival of parasites within various mouse strains and have not examined the immune

response elicited in any great detail [36]. We have examined cytokine responses and cellular recruitment profiles of BALB/c, C57BL/6 and CBA/Ca mice in response to adult and Mf stage *B. malayi*. Adult parasites stimulated recruitment of large numbers of inflammatory cells to the peritoneal cavity of BALB/c, C57BL/6 and CBA/Ca mice, whereas Mf did not. All three strains of mice provoked a marked eosinophilia coupled with loss of mast cells in response to adult parasite implant. Conversely, there were notable strain differences in the response to Mf, the most striking of which were the Mf-driven eosinophilia and macrophage recruitment seen in BALB/c and C57BL/6 mice that was not present in CBA/Ca mice. It is possible that the differences in eosinophilia reflect the cytokine environment that was stimulated by Mf in each strain. BALB/c mice displayed the most Th2-like cytokine production against Mf. As eosinophils may themselves provide an important source of IL-4 during helminth infection [37], it is possible that the eosinophilia evident in BALB/c mice implanted with Mf contribute to, as well as being a consequence of, the resultant Th2-type cytokine response that is seen in these mice. These data illustrate that, as might have been expected, the genetic background of the murine host can have a distinct influence on the phenotype of the elicited response to *B. malayi*. Differences were not as great as those seen in other experimental infections in which opposing, highly polarized responses can be seen in different mouse strains, which can have an impact on parasite survival [38, 39]. This could be due to the fact that mice are not a permissive host for *B. malayi*. However, the strain differences in response to filarial infection detailed here should be borne in mind when comparing studies on nematode parasites that have been carried out using different mouse strains.

Data presented in this manuscript demonstrate that the IL-4 inducing adult stage of *B. malayi* generates a markedly different population of cells after implant into the murine peritoneal cavity in comparison to IFN- γ inducing Mf. This striking difference in PEC populations is likely to reflect the alternate cytokine environments induced by the two life cycle stages. The differential response to the two stages of the filarial life cycle and to adult parasites in IL-4 deficient mice helps to define chronic inflammatory processes dependent on IL-4. The mechanisms underlying control of inflammatory cell recruitment and subsequent activation are central to our understanding of host-pathogen interactions and ultimately the way in which disease progresses.

Acknowledgements This work was supported by the Medical Research Council and the Wellcome Trust. We thank Rick Maizels for helpful advice and discussion.

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