B cells play a regulatory role in mice infected with the L3 of *Brugia pahangi*

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**Abstract**

Mice infected with the L3 of the filarial nematode *Brugia pahangi* make a strong Th2 response characterized by elevated levels of antigen-specific IL-4, IL-5 and IL-10. Here we show that B cells from these animals are the major proliferating population in vitro with depletion of B cells or infection of µMT mice, resulting in reduced levels of antigen-specific proliferation. B cells also act as antigen-presenting cells (APC) to CD4⁺ cells as demonstrated by the switch in cytokine profiles upon B cell depletion. The efficiency of B cells in antigen presentation is attenuated by IL-10 which down-regulates the expression of B7 ligands on B cells. In support of this hypothesis, blockade of the IL-10R in vivo results in increased proliferation of CD4⁺ cells. We propose that B cells participate in a negative feedback loop: IL-10 elicited by infection with L3 and produced by B cells (and CD4⁺ cells) down-regulates the expression of B7 molecules on the B cell surface, attenuating their efficiency as APC to CD4⁺ T cells and restricting their expansion.

**Introduction**

Lymphatic filariasis is a chronic infection caused by mosquito-borne helminth parasites of the genera *Wuchereria* and *Brugia*. As with other helminth infections, these parasites polarize host immune responses toward a T₄/₂/Treg phenotype with the consequent suppression of T cell proliferative responses (1). This modulation of immune responses is most pronounced in individuals with circulating microfilariae (Mf⁺) and is important for maintaining parasite levels, while also protecting the host from pro-inflammatory pathology. Although the resident adult parasites in the lymphatics and Mf in the bloodstream are the prime candidates for eliciting immunosuppression, the L3, the infective form of the parasite, also has a role in priming the anti-inflammatory phenotype (2, 3). However, defining the role of individual life cycle stages in polarizing the immune response in human infection is difficult as individuals living in an endemic area are exposed to different life cycle stages simultaneously. Thus, the mouse model provides a valuable tool in the analysis of stage-specific responses (4).

Studies in humans and in mouse models of infection have demonstrated that filarial nematodes modulate T cell responses via mechanisms involving IL-10. For example, PBMC from Mf⁺ individuals were found to secrete elevated levels of IL-10 (5), which, when neutralized, resulted in some reversal in proliferative hyporesponsiveness (6). Splenocytes from mice infected with the L3 of *Brugia pahangi* also secrete significant quantities of IL-10 with the resultant suppression of IFN-γ (7) similar to the situation in humans. The principle role of IL-10 is to down-regulate overwhelming and dangerous inflammatory immune reactions. To this end, IL-10 has the ability to suppress virtually all pro-inflammatory cytokines and chemokines, while at the same time inhibiting the expression of B7 ligands on B cells. The inhibitory expression of B7 molecules on the B cell surface, attenuating their efficiency as APC to CD4⁺ T cells and restricting their expansion.

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as APC. B cells are known to be involved in T<sub>H</sub>2 priming, as demonstrated by their ability to preferentially induce T<sub>H</sub>2-type responses in ovalbumin peptide (OVA)-specific clones, compared with adherent cells which induce T<sub>H</sub>1-type responses (9). In various mouse models of infection, B cells, particularly peritoneal B-1 cells, have been implicated in the secretion of IL-10 and in priming T<sub>H</sub>2 responses (10). In addition, several studies have shown that B cells are important for immunity to L3 challenge in filarial-infected mice (11, 12). In this study, we show that B cells are the major proliferating population in splenocyte culture from L3-infected mice and act as APC for T<sub>H</sub>2 cells. B cells from L3-infected mice produce significant amounts of IL-10, in addition to CD4<sup>+</sup> cells, and their efficiency in antigen presentation is attenuated by IL-10 which down-regulates expression of B7 ligands on the B cell surface. Thus, B cells have an important role in filarial infection via their ability to modulate T cell responses.

**Methods**

**Animals and infection protocols**

*Brugia pahangi* L3 were harvested from infected *Aedes aegypti* mosquitoes at day 9 post-infection (p.i.) as described previously (13). Six-week-old male BALB/c mice were purchased from Harlan-Olac (Bicester, UK). Experiments involving μMT or IL-10<sup>−/−</sup> mice, both on the C57BL6 background, were carried out at the University of Manchester. All animals were housed in filter-topped cages and maintained in accordance with local and Home Office guidelines. Groups of five mice were infected subcutaneously (s.c.) with 50 L3 of *B. pahangi* or an equivalent volume of HBSS in the scruff of the neck. In some experiments, a further group of five mice was infected with 5 × 10<sup>5</sup> Mf by the s.c. route. At day 12 p.i., the mice were killed by CO<sub>2</sub> inhalation and spleens were removed.

**Preparation of spleen cells, proliferation and cytokine assays**

Spleens were removed aseptically and single-cell suspensions were prepared in RPMI by homogenization through Nytex mesh (Cadish and Sons, London, UK) using a syringe barrel. The cells were re-suspended at 1 × 10<sup>7</sup> ml<sup>−1</sup> (for proliferation assays) or 2 × 10<sup>7</sup> ml<sup>−1</sup> (for cytokine assays) in RPMI containing 20% heat-inactivated FCS (Invitrogen, Paisley, UK) to give a final concentration of 10%. Proliferation of splenocytes was measured by the incorporation of [H]<sup>3</sup>thymidine ([H]<sup>3</sup>TdR). Triplicate 100 μl cultures (5 × 10<sup>5</sup> cells per well) in half-area 96-well flat-bottomed plates were incubated in the presence or absence of 10 μg ml<sup>−1</sup> adult *Brugia* antigen prepared exactly as described previously (14). Cells were cultured at 37°C in an atmosphere of 5% CO<sub>2</sub> and pulsed with 0.5 μCi of [H]<sup>3</sup>TdR per well (Amersham Biosciences, Bucks, UK) during the last 16 h of incubation. The cells were harvested and radioactivity was measured in a ‘Topcount’ Microplate Scintillation Counter (Perkin Elmer, Beaconsfield, Bucks, UK).

For cytokine assays, spleen cells were incubated at 1 × 10<sup>7</sup> cells ml<sup>−1</sup> in 1-ml cultures in 24-well flat-bottomed plates in the presence of antigen at 10 μg ml<sup>−1</sup> or medium only. Supernatants were harvested at 48 h, unless otherwise stated, and levels of IL-2, IL-4, IL-5, IL-10 and IFN-γ were determined by two-site ELISA using antibody pairs purchased from Pharmingen (BD, Palo Alto, CA, USA). Results are expressed as picograms per millilitre in reference to commercially available standards (IL-12, IL-4, IL-5 and IL-10 from Pharmingen and IFN-γ from R & D Systems, Minneapolis, MN, USA). The sensitivity of the assay was determined as the mean + 3 SD of the 16 wells containing medium only (RPMI 10% FCS).

**In vivo treatments**

In some experiments mice were injected intraperitoneally with 10 μg neutralizing IL-10R mAb (1B1.3a, Pharmingen) or matched isotype control (GL113) on days 0, 4 and 7 p.i. Proliferation assays, cytokine assays and FACS analysis were carried out on the splenocytes from each group of animals (3–5 animals per group) as described previously.

**Cell depletion by magnetic separation**

Splenocytes from L3-infected animals were depleted of B220<sup>+</sup> cells by magnetic separation, prior to *in vitro* culture. In brief, splenocytes pooled from five infected mice were washed in PBS and stained with FITC-conjugated anti-B220 (RM4-5) mAb (Pharmingen) in 500 μl of PBS on ice for 20 min. The antibody was used at 1 μg per 2 × 10<sup>6</sup> cells. Prior to depletion, unseparated cells were plated out and 5 × 10<sup>5</sup> cells were removed for FACS analysis and stained with an isotype control antibody (keyhole limpet haemocyanin (KLH)/G2a-1-1, Pharmingen). The cells were then re-suspended in 90 μl degassed buffer per 10<sup>7</sup> cells (prepared to MACS specifications, Miltenyi Biotec, Bisley, Surrey, UK) with anti-FITC beads (10 μl of beads per 10<sup>7</sup> cells), incubated on ice for 15 min and then ~2.5 × 10<sup>6</sup> cells were loaded onto the column. The flow-through cells were washed in PBS and 5 × 10<sup>6</sup> cells were removed for FACS analysis to determine the efficiency of the depletion. The remaining cells were then re-suspended in RPMI 20% FCS and plated out as described previously. For purification, bound cells were flushed from the column using 5 ml of buffer, following the manufacturer's recommendation. Purified CD4<sup>+</sup> and B220<sup>+</sup> cells were stored in liquid nitrogen prior to analysis.

**RT-PCR detection of IL-10 mRNA by TaqMan**

Total RNA was extracted using Trizol (Invitrogen), according to the manufacturer's protocol. RNA was stored in diethyl pyrocarbonate (DEPC) treated H<sub>2</sub>O at −70°C until use. Levels of IL-10 or HPRT mRNA were quantified from CD4 or B220 populations, purified as described above. Reverse transcription (RT) was carried out in a total of 20 μl using 2 μl of random primers (Promega, Southampton, UK) and 2 μg of total RNA in a volume of 10 μl. The mix was heated to 70°C for 10 min and then cooled on ice for 10 min. Four microlitres of first strand buffer (Invitrogen), 2 μl dithiothreitol (Invitrogen) and 1 μl 10 μM deoxynucleoside triphosphate mix were added and incubated at 42°C. One microlitre of Superscript was added to the mix, which was heated to 42°C for 50 min with a final extension of 70°C. One microlitre of RNase was added and the reaction was incubated at 37°C for 20 min.

TaqMan RT quantitative reverse transcriptase-PCR used gene-specific primers and internal probes for IL-10 (Forward,
5'-ACAACATACTGCTAACCCTTAAAGTCCTG-TAMRA-3'; Reverse, 5'-AGGTAAAACTGGATCATTTCCGATA-3'; Probe 5'-FAM-TGGCAA CCCAAGTAACCCTTAAAGTCCTG-TAMRA-3'; HPRT (Forward, 5'-GCAGTACAGCCCCAAATGG-3'; Reverse, 5'-AACAAAGTCTGGCCTGTA TCCAA-3'; Probe, 5'-FAM-TAAGGTTGCAAGCTTGCTGGTGAAAAGGA-TAMRA-3') (Cruachem, Glasgow, UK) designed to specifically amplify the target cDNA. For the TaqMan protocol, probes were used at a concentration of 5 μM and primers at 10 μM. Each 25 μl reaction contained 12.5 μl TaqMan Universal PCR Master Mix (Applied Biosystems, Warrington, Cheshire, UK), 25 μl probe, 0.75 μl of each primer and 9 μl H2O with 1 μl reaction contained 12.5 l probe, 0.75 l of each primer and 9 l H2O. TaqMan cycling conditions were 95°C for 2 min, 95°C for 10 s and 60°C for 1 min. IL-10 levels are expressed relative to the level of HPRT mRNA.

Two colour FACS analysis

Cells to be stained were removed from culture, transferred to FACS tubes and washed twice in 200 μl of staining buffer by centrifugation at 1000 r.p.m. for 5 min. Cells were stained with 2 μg per test of fluoroaphore-conjugated antibody in staining buffer or staining buffer only (100 μl per sample) on ice for 20 min. Cells were washed twice in staining buffer before and re-suspended in 300 μl of fixation buffer if not to be analysed immediately. Stained samples were stored in the dark at 4°C. In most experiments, cells from individual animals were analysed, but in some experiments cells from control animals were pooled due to low counts (see Results for details). Samples were gated on lymphocytes, as determined by size and granularity (forward and side scatter). The following mAb were used: FITC-labelled anti-mouse CD80 (B7-1, 16-10A1) or anti-mouse CD86 (B7-2, GL1) and APC-labelled anti-mouse CD55R/B220 mAb (RA3-6B2) (all from Pharmingen). FcR block (Pharmingen) was used at a concentration of 1 μg per test to reduce non-specific binding. Isotype controls were used in all experiments and included FITC-labelled rat IgG2a (KLH/G2a-1-1, Southern Biotechnology Associates, Birmingham, AL, USA) and APC-labelled rat IgG2a (R35-95).

Fig. 1. (A) Depletion of B cells from splenocytes of L3-infected BALB/c mice results in reduced levels of antigen-specific proliferation. Mice were infected s.c. with 50 L3 of *Brugia pahangi* or an equal volume of HBSS only (C). At 12 days p.i. (d.p.i.), splenocytes from L3-infected mice were pooled and B cells were depleted using MACS anti-FITC beads and magnetic columns. The negative fraction was then re-stimulated *in vitro* with 10 μg ml−1 *B. pahangi* antigen. Antigen-specific proliferative responses from whole splenocytes from five animals (L3 mean), from pooled cells prior to depletion (L3 pool + B cells) and from splenocytes depleted of B cells (L3 pool – B cells) were measured at 72 h. Results are expressed as counts per minute. (B) Splenocytes from L3-infected μMT mice display significantly lower antigen-specific proliferation compared with their WT counterparts. The μMT mice on the C57BL/6 background (KO) and WT mice were infected s.c. with 50 L3 of *B. pahangi* or an equal volume of HBSS only (C). At 12 d.p.i., splenocytes from individual mice were re-stimulated *in vitro* with 10 μg ml−1 *B. pahangi* antigen. Antigen-specific proliferative responses from whole splenocytes were measured at 72 h. Results show means ± SD of five mice per group and are expressed as counts per minute. Asterisk indicates the significant difference between L3-infected WT and μMT mice. (C) CFSE labelling of cells from L3-infected mice in antigen-stimulated culture shows that B cells proliferate in response to antigen. Splenocytes from BALB/c mice given 50 L3 of *B. pahangi* or an equal volume of HBSS (C) were labelled with CFSE and cultured with 10 μg ml−1 *B. pahangi* antigen. Cells were harvested at 96 h, surface stained with anti-CD4, anti-CD8 and anti-CD20 mAb and analysed by flow cytometry. The graph depicts the percentage of CD4+, CD8+ or CD20+ cells which have divided in antigen-stimulated culture as a percentage of total lymphocytes. Results are shown as a mean percentage of five mice per group ± SD.
(L3-T7), anti-mouse B220 mAb (RA3-6B2), anti-mouse CD8 (Ly-2) or isotype-matched control (R35-95) (all from Pharmin- gen), as described above, prior to FACS analysis. Samples were gated on total lymphocytes, as determined by forward and side scatter and CD4+, B220+ and CD8+ lymphocytes for analysis of CFSE staining profiles. Controls in each experiment comprised unstained cells, cells stained with anti-CD4, anti-B220 or anti-CD8 or isotype-matched control only and cells stained with CFSE only. Data were analysed using Cellquest software.

Statistical analysis
The Mann–Whitney U-test was used to determine the statistical significance of differences between groups. $P < 0.05$ was considered to be significant.

Results

B cells are the major proliferating population in splenocyte culture from L3-infected mice

B cells were depleted from the spleens of L3-infected mice using magnetic beads and proliferative responses pre- and post-depletion were measured after 72 h of in vitro re-stimulation with 10 $\mu$g ml$^{-1}$ B. pahangi antigen. As shown in Fig. 1(A), depletion of the B cell population resulted in decreased levels of antigen-specific proliferation (62% decrease in the experiment shown). In three separate experiments, the mean percentage reduction was 49 ± 15.2% compared with whole splenocyte culture ($P = 0.0809$). As

in vitro depletion of B cells had a major effect on antigen-specific proliferation, the role of B cells in L3 infection was further examined using $\mu$MT mice. Levels of antigen-specific proliferation were significantly lower (85% reduction) in splenocyte culture from L3-infected $\mu$MT mice compared with wild-type (WT) mice ($P = 0.013$, see Fig. 1B). These data
suggest that either B cells proliferate in response to re-stimulation with parasite antigen, and/or that B cells act as APC for CD4+ T cell proliferation.

To determine the phenotype of cells that proliferate in antigen-stimulated culture, CFSE staining was carried out prior to in vitro re-stimulation of cells. At 96 h, cells were harvested, labelled with anti-CD4, B220 or CD8 mAb and analysed by flow cytometry. Figure 1(C) shows data from a representative experiment (medium only controls not shown) demonstrating that B cells account for the majority of proliferative events in response to antigen. Mean levels of B cell proliferation for the group of five animals (as a percentage of total lymphocytes) was 17.4 ± 5.9% compared with 5 ± 0.6% for CD4+ cells and 2.2 ± 0.8% for CD8+ cells.

Depletion of B cells alters the cytokine profile in splenocyte culture in an IL-10-dependent fashion

As it is well documented that B cells act as APC to generate Th2-like responses with model antigen such as OVA (9), it is possible that these cells act as APC in mice infected with B. pahangi. We therefore examined the production of cytokines in splenocyte cultures following B cell depletion. In four separate experiments, decreased levels of antigen-specific Type 2 cytokines were observed (see Fig. 2). Of all cytokines measured, levels of IL-5 were most reduced (80.5 ± 27.5% reduction over four experiments). IL-10 was reduced by a mean of 53.5 ± 14.9%, while the decrease in IL-4 levels was less pronounced (32 ± 11.3% reduction over four experiments). IL-2 was not detected in the presence or absence of B cells. Levels of IFN-γ were increased in two out of four experiments (3-fold increase in the experiment shown) when B cells were depleted, while in the remaining two experiments there was no increase in IFN-γ following B cell depletion. Differences in IFN-γ production post-B cell depletion showed an interesting correlation with IL-10 levels but not with levels of any other cytokine. In the two experiments in which IFN-γ was produced, levels of IL-10 were reduced to 4000–7000 pg ml−1 following B cell depletion. In contrast, where IFN-γ was not detected following the removal of B cells, IL-10 levels were much higher (~20 000 pg ml−1 post-depletion). The difference in IL-10 levels between experiments may reflect variation in batches of L3 or in the antigen used for re-stimulation. Thus, levels of Type 2 cytokines were reduced following B cell depletion, indicating that B cells act as APC for Type 2 responses, while IFN-γ production was dependent on reduced levels of IL-10.

In order to formally prove that B cells are a source of IL-10 in this model system, B cells or CD4+ cells were purified from the spleens of L3-infected mice by magnetic separation. RNA was prepared from these cells and levels of IL-10 mRNA were assessed directly ex vivo relative to levels of a constitutive gene, HPRT. As shown in Fig. 3, B cells account for ~40% of lymphocyte-derived IL-10 mRNA, while CD4 cells account for ~60%, a result which correlates reasonably well with estimates of IL-10 protein production by B cells in depletion experiments (see Fig. 2).

B cells from L3-infected mice express elevated levels of B7 ligands

An important way in which APC modulate T cell responses is by the expression of co-stimulatory ligands such as B7-1 (CD 80) and B7-2 (CD 86). We investigated the role of B cells in antigen presentation by assessing the expression of B7-1 and B7-2 p.i. In these experiments, mice infected with Mf were
B cells in Brugia infection

Fig. 5. Neutralization of B7-2 affects proliferative responses of cells from L3-infected mice. Mice were infected s.c. with 50 L3 of Brugia pahangi or given an equal volume of HBSS only. At 12 days p.i., splenocytes were re-stimulated in vitro with 10 μg ml⁻¹ B. pahangi antigen and proliferative responses were measured at 48 h (A) or 72 h (B) in the presence or absence of 1 μg ml⁻¹ neutralizing B7-1 (AB7-1) or B7-2 (AB7-2) or both mAb together (AB7-1/2). Results are expressed as counts per minute and all values represent the mean ± SD of five mice per group.

included as this life cycle stage elicits a Th1-type response. Samples were gated on the lymphocyte population and the expression of B7-1 and B7-2 was examined on B220⁺ lymphocytes. The percentage of B cells expressing B7-1 increased to a similar extent in both Mf and L3-infected mice (P = 0.0947, L3 versus Mf, see Fig. 4). In contrast, while the numbers of B cells expressing B7-2 increased in both infected groups, B7-2 was up-regulated with approximately twice the efficiency in L3-infected mice compared with Mf-infected mice (P = 0.02, L3 versus Mf), consistent with the Th2-priming activity of the L3. Further analysis of these data demonstrated that the majority of lymphocytes expressing B7 ligands were in fact B cells, consistent with this population functioning as APC: >90% of B7-2⁺ cells were B220⁺, while ~70% of B7-1⁺ cells co-expressed B220 in L3-infected mice. However, not all B cells expressed B7-1 or B7-2 (see Fig. 4).

To investigate whether blocking the interaction of B7-1/2 with CD28 influenced levels of proliferation, mAb to B7-1 or B7-2 was added to splenocyte cultures from L3-infected mice. In keeping with the results described above, neutralizing B7-2 had a more pronounced effect on proliferation than did neutralization of B7-1 (see Fig. 5). Blocking B7-1 affected proliferation at early time points only (48 h), while blocking B7-2 down-regulated antigen-specific proliferative responses at 48 and 72 h. Adding both antibodies together did not further decrease proliferative responses (see Fig. 5). Cytokine levels were also measured in these cultures and as observed previously with B cell depletion, the major effect was on IL-5 production (see Table 1). Blocking B7-1 or B7-2 resulted in reduced levels of antigen-specific IL-5, but only the results with B7-2 reached significance (P = 0.0304, compared with antigen alone). However, simultaneous neutralization of both B7-1 and B7-2 resulted in a significant reduction in antigen-specific IL-4 (P = 0.02), IL-10 (P = 0.0373) and IL-5 (P = 0.0302).

IL-10 suppresses expression of B7-1 and B7-2 on B cells from L3-infected mice

As the results of the experiments in which cytokine profiles were assessed following B cell depletion suggested an interrelationship between B cells, IL-10 and IFN-γ production, we investigated the role of IL-10 on B cell expression of co-stimulatory molecules. As described previously, infection with L3 resulted in a more pronounced up-regulation of B7-2 expression on B cells compared with B7-1. However, as shown in Fig. 6, the addition of a neutralizing mAb to IL-10 resulted in a significant increase in B cell expression of both B7 ligands (antigen plus anti-IL-10 mAb versus antigen alone, P = 0.0122 for both B7-1 and B7-2). To examine the effect of IL-10 on B cell expression of B7 ligands in vivo, IL-10−/− mice were infected with L3 and the percentage of B cells expressing B7-1 and B7-2 was assessed following in vitro re-stimulation with antigen. It was notable that constitutive levels of B7-2 (but not B7-1) were higher on B cells from naive IL-10−/− mice compared with naive WT mice. However, the expression of both B7 ligands was significantly up-regulated in IL-10−/− mice compared with WT mice following infection with L3 (see Fig. 7, P = 0.0122 for both B7-1 and B7-2), confirming the results from in vitro neutralization of IL-10. Thus, IL-10 suppresses the expression of B7 ligands on the B cell surface in L3-infected mice.

Blocking the IL-10R results in increased levels of antigen-specific proliferation

If IL-10 is a critical factor in determining B7 expression, blockade of the IL-10R might be expected to affect cellular responses in vivo. To examine the effect of IL-10 on B7 expression in vivo, L3-infected BALB/c mice were treated with a mAb to the IL-10R and B7 expression was assessed on the B cell surface following re-stimulation with antigen for 72 h. Figure 8(A and B) show that blockade of IL-10 signalling resulted in an increase in the percentage of B cells expressing B7-1 and B7-2. However, in three separate experiments, these differences failed to reach statistical significance (P = 0.0662 for B7-1; P = 0.403 for B7-2, isotype control versus anti-IL-10R in the experiment shown), although in each experiment the trend for increased expression of B7-1 was observed.

As the engagement of B7 on the APC surface with CD28 on the T cell is critical for proliferation, we determined whether blockade of the IL-10R affected the proliferation of CD4⁺ cells. As shown in Fig. 8(C), levels of antigen-specific proliferation were significantly increased in splenocyte culture from mice treated with the IL-10R-neutralizing antibody compared with mice given the isotype control (P = 0.0122). To determine the
phenotype of cells that proliferate when the IL-10R is blocked, CFSE staining was carried out prior to in vitro stimulation with \(10 \text{ pg ml}^{-1} \) \text{Brugia pahangi} antigen. At 96 h, cells were harvested and labelled with anti-CD4 mAb or B220 mAb, and analysed by flow cytometry. The results presented in Fig. 8(D) demonstrate that levels of B cell proliferation were unaffected by blockade of the IL-10R, with a mean of 15.2% of B cells proliferating in mice given the blocking antibody, compared with 14.9% in mice given the isotype control. In contrast, there was a difference in levels of CD4+ proliferation between groups, with a mean of 5.5% of CD4+ cells proliferating in splenocyte culture from isotype control treated mice, compared with 12.4% in cultures from mice given the blocking mAb. However, using CFSE labelling, the difference in proliferation of CD4+ cells between the groups fell just short of statistical significance \((P = 0.0662)\).

**Discussion**

In recent years, the ability of IL-10 secreted by T cells (T\(_{Th2}\) and particularly T regulatory cells) to modulate the immune response has been the focus of much interest. In this paper, we show that B cells may also have an important role in regulating immune responses in mice infected with the L3 of \text{Brugia pahangi} and that their functional capacity is regulated at least in part by IL-10. B cells were shown to be the major proliferating population in antigen-stimulated splenocyte culture from L3-infected mice, while the decrease in Type 2 cytokine secretion following B cell depletion also suggests a role in antigen presentation. In all experiments, depletion of B cells from cultures of L3-infected mice resulted in decreased levels of IL-5 and IL-10, presumably reflecting their capacity to act as APC for Th2 cells. However, B cells themselves contribute substantially to IL-10 production in L3 infection, as shown by quantitative RT-PCR and by depletion experiments. In contrast, the decrease in IL-4 levels was more modest when B cells were removed. This may relate to the fact that IL-4 transcription is initiated very early p.i. with L3 (15) and is very stable. Alternatively, it may reflect the multiple possible sources of IL-4 in addition to Th2 cells, such as basophils and mast cells (16–19). Perhaps, the most significant effects of B cell depletion were on IFN-\( \gamma \), where in two out of four experiments, levels were greatly increased following B cell depletion.
of IL-10 and the capacity to produce IFN-γ was directly correlated with levels of IL-10 in the cultures following B cell depletion. Thus, the magnitude of the IL-10 response appears to determine whether IFN-γ is produced or not. Additional studies will be required to determine whether the effect of B cell depletion on IFN-γ secretion derives indirectly from a reduction in antigen presentation to CD4+ cells or more directly from reduced B cell-derived IL-10. Previous studies support both possibilities as neutralization of IL-10 in vitro or replacement of the APC population in L3-infected mice both restore IFN-γ production (7).

The ability of B cells to act as APC to T cells is dependent on their expression of co-stimulatory molecules interacting with CD28 on the T cell surface. Several previous studies have demonstrated that B7-1 and B7-2 do not deliver identical co-stimulatory signals. Essentially, B7-1 appears to be a more neutral differentiation signal, while signalling through B7-2 favours Th2 expansion (20–22). The results presented here showed a good correlation between the up-regulation of B7-2 on B cells and T cell induction. While the percentage of B cells expressing both B7 ligands was increased following L3 infection, levels of B7-2 were most elevated, consistent with the T cell priming activity of the L3. In contrast, infection with Mf resulted in a similar up-regulation of both B7 ligands. In addition, blocking B7-2 in vitro had a more pronounced effect on antigen-specific proliferation of splenocytes from L3-infected animals than did blocking B7-1. The significant decrease in levels of proliferation upon blockade of B7-2 may reflect reduced T cell proliferation and a reduction in T-dependent B cell responses.

Our results show that blockade of IL-10, either in vitro using a mAb or in vivo using IL-10−/− mice, enhances the expression of B7-1 and B7-2 on B cells. We also attempted to demonstrate the effect of signalling via the IL-10R on B7 expression in vivo. B cells from mice treated with an mAb to the IL-10R showed a trend for increased expression of B7-1, but this fell short of significance. However, blockade of the IL-10R did affect levels of antigen-specific proliferation. CFSE-labelling experiments showed no difference in B cell expansion in these animals, while CD4+ cells showed an increased capacity for proliferation. Thus, B cells may modulate the expansion of CD4+ cells in L3-infected mice in a negative feedback loop: IL-10 elicited by infection with L3 and produced by B cells (and/or CD4+ cells) down-regulates the expression of B7 molecules on the B cell surface, attenuating their efficiency as APC to CD4+ T cells and restricting their expansion.

A growing body of work implicates B cell-produced IL-10 in immune regulation. For example, in mouse models of Schistosoma mansoni infection, splenic B cells have been shown to proliferate in response to the oligosaccharide, lacto-N-fucopentaose (LNFPIII), which contains the trisaccharide Lewis antigen and is found in soluble egg antigen. LNFPIII also stimulated splenic B cells to produce IL-10 (23). Whether the L3 of B. pahangi contain similar molecules that drive B cell proliferation and cytokine production is currently unknown. B cell-derived IL-10 also regulates pro-inflammatory CD4 responses in various models of autoimmune disease (24–26). While a number of laboratories have identified a novel population of dendritic cells (DC) that are B220+ CD11c+ and have a regulatory function in mice via their ability to secrete IL-10 (27–29), these low-density cells are unlikely to be significantly represented in the cellular populations analysed in this study as their efficient isolation requires collagenase digestion and density gradient centrifugation.

In conclusion, our studies show that B cells may have an important function in modulating immune responses in filarial-infected mice via their capacity for proliferation in response to parasite antigen and their ability to act as APC. A recent study demonstrated a novel population of ‘DC-like’ B cells in...
the low-density fraction of mouse splenocytes that express B220 but are CD11c−/CD255, distinguishing them from B220+ CD11+ DC described above. These cells have the capacity to produce large amounts of IL-10 and to stimulate naive T cells, and may act to regulate immune responses in vivo (30). Thus, further studies will be required to precisely phenotype the IL-10-producing B cells described in the present study and to determine whether these cells constitute a specific subset which, by their production of IL-10, has a regulatory function in L3-infected mice.

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Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>APC</td>
<td>antigen-presenting cells</td>
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<td>CFSE</td>
<td>carboxyfluorescein diacetate succinimidyl</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cells</td>
</tr>
<tr>
<td>DEPC</td>
<td>diethyl pyrocarbonate</td>
</tr>
</tbody>
</table>

References

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