B Cells and Antibodies Are Required for Resistance to the Parasitic Gastrointestinal Nematode *Trichurus muris*

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Previous studies using cell transfers and antibody receptor knockout mice have shown that B cells and antibodies are not essential components of the expulsion mechanism in *Trichurus muris* infections. Serum transfer experiments have given mixed results regarding the importance of antibodies in this infection model, and the role of B cells in initiating or maintaining T-cell responses has not been addressed. We used B-cell-deficient μMT mice to determine if B cells play a role in anti-*T. muris* immune responses. In contrast to wild-type C57BL/6 mice, μMT mice were susceptible to infection. Antigen-restimulated mesenteric lymph node cells from infected μMT mice produced only naive levels of Th2-associated cytokines but had increased levels of gamma interferon. However, these mice were capable of mounting a Th2-dependent mucosal mastocytosis, though this was significantly delayed compared to that seen in wild-type mice. Resistance to *T. muris* was restored following reconstitution with naive C57BL/6 splenic B cells, as was in vitro Th2 cytokine production in response to parasite antigen. Treatment of μMT mice with anti-interleukin-12 monoclonal antibody during the first 2 weeks of infection also restored immunity, suggesting that μMT mice can be manipulated to expel worms at the time of T-cell priming. Additionally, treatment of μMT mice with parasite-specific immunoglobulin G1 purified from the serum of resistant NIH mice prevented worm establishment, suggesting an important role for antibodies. Our results as a whole describe the first detailed report of a critical role for B cells in resistance to an intestinal nematode.

The role of T cells in mediating resistance and susceptibility to the parasitic gastrointestinal nematode *Trichurus muris* have been well characterized. This is largely because of the existence of strains of mice resistant and susceptible to the parasite and the early observation of polarized T helper responses in those strains (13). Resistant strains of mouse such as BALB/c, BALB/K, and NIH mount a typical Th2-type response, associated with the production of interleukin-4 (IL-4), IL-5, IL-9, and IL-13 by parasite-antigen-restimulated mesenteric lymph node cells (MLNC). These strains expel their worm burdens by day 18 postinfection (p.i.). Susceptible strains such as AKR mount a dominant Th1 response, associated with low levels of Th2 cytokines and the presence of high levels of gamma interferon (IFN-γ). Here, infections proceed to patentity at around day 35 p.i. (9, 12, 15). Strains such as C57BL/6 and C57BL/10 mounted a mixed Th1/Th2 response, but the majority of infected mice expelled all or most of their worms between days 21 and 28 p.i., through a Th2-mediated response.

The contribution of T cells and the Th1/Th2-associated cytokines have been confirmed by cytokine manipulation studies. Resistant strains deficient in IL-4 or IL-13, or treated with anti-IL-4 receptor or recombinant IL-12, become susceptible, whereas susceptible strains deficient in IFN-γ, or treated with recombinant IL-4, become resistant (3, 4, 11). Furthermore, the importance of CD4+ T cells has been demonstrated by observations that athymic (nude) BALB/c mice (28) and mice depleted of CD4+ T cells by antibody treatment (32) are susceptible to infection with *T. muris*. Finally, transfer of purified immune CD4+ T cells from infected BALB/c mice to SCID mice (which lack T and B cells) confers resistance (14), indicating that B cells and antibodies are not an essential component of the expulsion mechanism in primary infections.

In contrast to T cells, very little is known of the role of B cells in immune responses to *T. muris*. Although the purified T-cell transfer experiments suggest a redundancy for B cells and antibodies in the expulsion mechanism, they do not discount the possibility of B cells playing a role in the priming of T cells and the maintenance of the T-cell response. To determine whether B cells play any role in the immune responses of mice to *T. muris*, we infected B-cell-deficient μMT mice with *T. muris*. The data presented here show that B cells are required for resistance to *T. muris* and that this requirement is associated with the development of a Th2-type response. Furthermore, resistance can be restored by reconstitution with naive B cells or by treatment with anti-IL-12. Finally, prevention of worm establishment can be achieved in μMT mice by treatment with parasite-specific immunoglobulin G1 (IgG1) antibodies purified from the sera of resistant NIH mice. Together, these findings suggest that B cells and antibodies do have important roles in the immune responses of mice to infection with *T. muris*.

**MATERIALS AND METHODS**

**Animals.** Male μMT mice (31) were obtained from breeding pairs maintained at our animal unit (animals originally purchased from Bantin & Kingman, Hull, United Kingdom, and backcrossed six generations on the C57BL/6 background). Male C57BL/6 mice (Harlan-Olac Ltd., Bicester, Oxon, United Kingdom) were used as wild-type controls. Male AKR and NIH mice were purchased from Harlan-Olac Ltd. All mice were infected at 6 weeks of age.

**Parasite and antigens.** The Edinburgh strain of *T. muris* was used throughout. Experimental infections were performed using oral gavage, with levels of infection determined at sacrificial time points by counting the number of worms.
present in the cecum and colon. Briefly, guts were frozen at −20°C for at least 24 h. Worm burden determinations were made by scraping the mucosa to remove early larval stages or by removal of individual worms using fine forceps (adult stages). T. muris excretory/secretory (E/S) antigen was prepared as previously described (1).

Preparation of MLNC for in vitro restimulations. Mesenteric lymph nodes were removed from naive and infected mice and dissociated in Hanks balanced salt solution (supplemented with 2% fetal calf serum, 100 U of penicillin/ml, 100 μg of streptomycin/ml; all purchased from Gibco). MLNC were washed three times and resuspended at 5 × 10⁶ cells/ml in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine (Gibco), 100 U of penicillin/ml, 100 μg of streptomycin/ml, and 7.5 × 10⁻⁵ M monothioglycerol (Sigma-Aldrich). MLNC were stimulated in vitro with T. muris E/S (50 μg/ml) and cultured at 37°C in 5% CO₂. Supernatants were collected after 48 h and stored at −80°C until analyzed.

Cytokine analysis. Sandwich enzyme-linked immunosorbent assays (ELISAs) were used to determine the concentrations of IL-4, IL-5, IL-9, and IFN-γ in the supernatants from in vitro-cultured MLNC. Monoclonal antibodies BV44-1D11 and 2G23.2 (IL-4), TRFK.5 and TRFK.4 (IL-5), and R46A2 and XMG12.1 (IFN-γ) were purchased from Pharmingen (San Diego, Calif.). The anti-IL-9 antibodies used were D9302C12 (Pharmingen) and 229.4 (kindly provided by J. Van Snick, Ludwig Institute for Cancer Research, Brussels, Belgium). The cytokine contents of supernatants were compared to recombinant murine cytokine standards. The detection limit above background for each cytokine was calculated from the optical density values of 16 wells incubated with culture medium, with the cytokine concentration considered positive only if its value exceeded the average plus 3 standard deviations of the background level. Results are presented as means ± standard error (SE).

Serum parasite-specific antibody detection. Blood was collected from sacrificed animals by cardiac puncture and left at room temperature to clot. Serum was then collected from each sample, aliquoted, and stored at −80°C until analysis. Serum levels of parasite-specific IgG1 and IgG2a were determined by ELISA. Briefly, 96-well plates (Dynex, Billingshurst, West Sussex, United Kingdom) were coated overnight with T. muris E/S at 5 μg/ml in carbonate buffer (pH 9.6; 50 μl/well). After blocking with phosphate-buffered saline (PBS) containing 3% bovine serum albumin and 0.05% Tween 20 (Sigma), the plates were incubated with sera serially diluted in PBS-Tween 20 from 1:20 to 1:2,560. Antigen-specific antibodies were detected using biotinylated rat anti-IgG1 (LO-MG1-2; Serotec) or rat anti-IgG2a (R19-15; Pharmingen) antibodies (50 μl/well at predetermined concentrations) and streptavidin-conjugated horseradish peroxidase (0.5 U/ml; 75 μl/well) was used as the substrate, and the plates were read at 405 nm after 20 min with a Dynatech MR-900. Nonsaturating serum dilutions were compared for analysis.

Histology. At autopsy, cecal tissue were fixed and removed and fixed in Carney’s solution. Fixed tissues were embedded in wax, sectioned, and stained for mast cells with 0.5% toluidine blue (pH 0.3). Mast cell numbers were counted blindly from 20 randomly selected cecal crypt units.

Serum MMCp-1 detection. Serum levels of mouse mast cell protease 1 (MMCP-1) were determined using a commercially available ELISA kit from Moredun Scientific (Penicuik, Scotland) (27). B-cell purification and adoptive transfer. B cells were obtained from naive C57BL/6 spleens by magnetic negative selection using anti-CD43-coated beads (MidMACS; Miltenyi Biotec). CD43 is expressed on B1 and plasma cells, granulocytes, macrophages, platelets, and NK and T cells but not on mature B-2 cells. μMT recipients were each given 2.5 × 10⁸ B cells intravenously. 93% B-cell purity, <2% CD4⁻ T-cell contamination, determined by flow cytometry, while control mice received Hanks medium. All mice were infected the following day, and worm burdens were determined on day 5 p.i.

Anti-IL-12 treatment. μMT and AKR mice were injected intraperitoneally with rat anti-mouse IL-12 antibody C17.8 (cell line kindly provided by G. Trinchieri, Schering-Plough, Dardilly, France) on days 0, 5, 9, and 14 p.i. (1 mg of antibody per injection). Control mice were injected with rat IgG (Sigma) at the same concentration and time points as those treated with C17.8. As C57BL/6 mice are naturally resistant to T. muris, to ensure that the C17.8 antibody stock was capable of converting an immunocompetent susceptible mouse to a resistant phenotype, male age-matched AKR mice were used. Worm burdens were assessed at days 11, 21, and 35 p.i.

Serum antibody purification and adoptive transfer. For antibody donors we chose the NIH mouse strain. This strain is very resistant to T. muris infection and produces high levels of serum parasite-specific IgG1 from 3 weeks p.i., with levels continuing to rise for many weeks after parasite expulsion (17). NIH mice were given large infections (approximately 250 infective T. muris eggs) and sacrificed on day 50 p.i. Serum was collected and passed over a 5-ml Hi-Trap protein G column (Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom). Bound antibody was eluted with 1.0 M glycine-HCl (pH 2.7) into 1.0 M Tri-HCl (pH 9.0) and dialyzed against PBS for 24 h. After dialysis, the concentration of antibody was determined by measuring the absorbance at 280 nm and adjusted to 5 mg/ml. Antibody solution was then sterile filtered through a 0.8/0.2-μm Acrodisc (Gelman Sciences, Ann Arbor, Mich.) and stored at −80°C.

RESULTS

μMT mice are susceptible to infection with T. muris. Mouse strains are considered to be resistant to T. muris if they can expel all or most of the worms in an infection. Resistant strains typically expel worms between day 11 and 28 p.i., whereas susceptible strains are unable to expel their worms and infections proceed to patency at around day 35 p.i. Hence, worm burdens at day 35 p.i. are typically used to indicate whether a strain is resistant or susceptible. To determine if B cells play a role in the immune responses to T. muris, worm burdens recovered from infected μMT and C57BL/6 mice were analyzed postinfection (Fig. 1). μMT mice were completely susceptible to infection with T. muris, with significantly more worms than C57BL/6 mice at day 35 p.i. (P = 0.0193). Worms recovered from μMT mice at day 35 p.i. were fully developed adults. In contrast, C57BL/6 mice expelled worms from day 21 p.i. and had completely expelled their worms by day 35 p.i.

Serum from infected C57BL/6 mice contained both parasite-specific IgG1 and IgG2a, detectable above naive background levels from day 21 p.i. μMT serum contained no antibody (data not shown).

Parasite antigen-remilitnamated MLNC from infected μMT mice do not produce Th2-associated cytokines and produce low levels of IFN-γ. Resistance and susceptibility to T. muris have been convincingly demonstrated to be associated with the
polarization of immune responses to Th2 and Th1, respectively (3, 4, 9, 11–13, 15). An indication of how a particular mouse has responded to infection can be seen by in vitro antigen-stimulated cytokine production from MLNC. The nature of the Th-type response mounted by infected MT mice was determined by stimulating MLNC from these mice with parasite antigen in vitro. Supernatants recovered from these cultures were analyzed for the presence of Th1-(IFN-γ) and Th2-associated (IL-4, IL-5, and IL-9) cytokines.

Figure 2 shows parasite antigen-stimulated cytokine production from naive and infected μMT and C57BL/6 mice MLNC cultures. MLNC from infected animals were taken at day 21 p.i., which is the peak period of cytokine production in this infection model. Supernatants recovered from these cultures were analyzed for the presence of Th1-(IFN-γ) and Th2-associated (IL-4, IL-5, and IL-9) cytokines.

Figure 2 shows parasite antigen-stimulated cytokine production from naive and infected μMT and C57BL/6 mice MLNC cultures. MLNC from infected μMT mice did not produce levels of IL-4, IL-5, or IL-9 significantly above naive levels (Fig. 2A to C). Only IFN-γ was significantly elevated in these cultures (P = 0.0037) (Fig. 2D), indicating that infected μMT mice were mounting a polarized Th1 response in the absence of a Th2 response. In contrast, levels of all four cytokines were significantly above naive levels in the supernatants of infected C57BL/6 mice (P < 0.05), as is typically observed for this mouse strain. Similar results were obtained from MLNC cultured in the presence of concanavalin A (data not shown).

Mucosal mastocytosis is equivalent in magnitude, but delayed in development, in the absence of B cells. As an indication of the presence of a Th2-type response being mounted by the host, mucosal mastocytosis in the cecum was examined. Figure 3A shows that like wild-type mice, μMT mice are capable of mounting a mucosal mastocytosis in response to infection. In both μMT and C57BL/6 mice, this increase in mast cell numbers was seen from day 11 p.i.; however, peak mastocytosis occurred earlier in C57BL/6 mice (day 21 p.i.) than in μMT mice (day 28 p.i.), with a significant difference in mast cell numbers occurring at day 21 p.i. (P = 0.0209). Mast cell numbers then decreased in both strains, despite the continuing presence of worms in the μMT mice.

Figure 3B shows the changes in serum levels of MMCP-1 during the course of infection. Serum MMCP-1 levels reflected mucosal mastocytosis, indicating that the mast cells were functionally capable of degranulating in both strains. Serum MMCP-1 levels were significantly higher in the μMT mice at day 28 p.i. (P = 0.0339), perhaps reflecting the delayed peak in mast cell numbers seen in the gut compared to wild-type mice and the continued presence of parasites.

Reconstitution with B cells restores resistance to T. muris in μMT mice. To establish if the loss of resistance of the μMT mice was due to the absence of B cells, μMT mice were reconstituted with 2.5 x 10⁷ naive C57BL/6 splenic B cells before infection. Figure 4 shows that μMT mice reconstituted with B cells had restored immunity to T. muris, having worm burdens at day 35 p.i. similar to those in infected C57BL/6 mice at day 35 p.i. (P = 0.1732). μMT mice treated with Hanks solution
harbored worm burdens at day 35 p.i. equivalent to the infective dose of *T. muris* eggs given on day 0 (infective dose indicated by C57BL/6 worm burdens at day 11 p.i.) and significantly different from C57BL/6 worm burdens at day 35 p.i. (\(P < 0.0088\)). Although worm burdens were higher than those observed in Fig. 1, the importance is that \(\mu\)MT mice did not expel worms unless reconstituted with B cells. Both levels of worm burdens (Fig. 1 and 4) were well above threshold levels (approximately 40 worms) below which no expulsion occurs, even in resistant strains.

Flow cytometric analyses of MLNC and spleen cells showed low but detectable percentages of B220<sup>+</sup> cells in the B-cell-reconstituted \(\mu\)MT mice (2.95% of MLNC and 9.46% of spleen cells at day 22 p.i.). However, B-cell reconstitution was clearly sufficient for the successful priming of Th2 cells in vivo and restoration of Th2 cytokine production in vitro. Figure 5A to C shows that whereas antigen-restimulated MLNC from Hanks solution-treated \(\mu\)MT mice failed to produce levels of Th2 cytokines above naive levels (consistent with earlier observations), MLNC from \(\mu\)MT mice reconstituted with B cells produced elevated levels of IL-4, IL-5, and IL-9 and had lower levels of IFN-\(\gamma\) than Hanks solution-treated \(\mu\)MT mice (Fig. 5). Overall levels of cytokines from *T. muris*-infected C57BL/6 and \(\mu\)MT antigen-restimulated MLNC are lower than in Fig. 2 but qualitatively remain the same.

The restoration of a Th2 response in MLNC also resulted in the restoration of parasite-specific IgG1 response in the serum (Fig. 6A); however, no parasite-specific IgG2a was detected in the sera of these mice, perhaps reflecting the low IFN-\(\gamma\) levels detected in vitro compared to that of the infected C57BL/6 mice (Fig. 5D). No antibody was detected in the serum of Hanks solution-treated \(\mu\)MT mice, while both parasite-specific IgG1 and IgG2a were detected in the sera of infected C57BL/6 mice after day 22 p.i. (Fig. 6).

Treatment with anti-IL-12 antibody restores resistance to *T. muris* in \(\mu\)MT mice. IL-12 has been identified as a key cytokine involved in the polarization of Th1 cells during T-cell priming (24, 26, 38). B cells have been shown to downregulate production of IL-12 by dendritic cells in vitro by producing IL-10, thus allowing Th2-priming conditions to develop (50). With the initial observation that infected \(\mu\)MT mice mount a Th1 response (indicated by production of IFN-\(\gamma\) and no Th2 cytokines from MLNC stimulated with parasite antigen in vitro), we treated \(\mu\)MT mice with anti-IL-12 monoclonal antibody to see if it could alter the polarization of the immune response from Th1 to Th2 and hence restore resistance. As C57BL/6 mice are normally resistant to *T. muris*, we included AKR mice (which are naturally susceptible) as a positive control. Anti-IL-12 treatment has been shown to make AKR mice resistant to *T. muris* (A. J. Bancroft, unpublished observations). Figure 7 shows that treatment with anti-IL-12 restores immunity to *T. muris* in \(\mu\)MT mice, resulting in a significant worm expulsion (\(P = 0.0088\)) by day 21 p.i., following kinetics similar to those for C57BL/6 mice and anti-IL-12-treated AKR mice. The ma-
The majority of worms recovered from C57BL/6 and anti-IL-12-treated mice at day 35 p.i. were stunted in development. MT and AKR mice treated with rat IgG maintained their worm burdens throughout the experiment, and worms recovered from these mice were fully developed at day 35 p.i.

Antigen-restimulated MLNC from rat IgG- and anti-IL-12-treated muMT failed to produce elevated levels of Th2 cytokines in vitro, but IFN-γ was lower (though not significantly) in the supernatants harvested from anti-IL-12-treated muMT MLNC compared to rat IgG-treated muMT MLNC. Levels of Th2-associated cytokines were higher in the supernatants of MLNC from anti-IL-12-treated AKR mice compared to the rat IgG-treated group, while IFN-γ was decreased (data not shown).

Passive transfer of parasite-specific IgG1 can prevent worm establishment in muMT mice. Immunocompetent mice that successfully expel T. muris maintain high levels of serum parasite-specific antibody for months (N. M. Blackwell and K. J. Else, unpublished observations). Although expulsion of primary infections can occur in the absence of antibody (14), the high levels of specific antibody present following expulsion in immunocompetent mice may play a role in preventing the establishment of challenge infections. Our results show that parasite-specific IgG1 purified from the serum of resistant mice can prevent establishment of worm burdens if transferred to naive susceptible mice at the time of infection (Fig. 8). Whereas AKR and muMT mice treated with nonspecific IgG had worm burdens at days 11 and 35 p.i. comparable to the infective dose of T. muris eggs given on day 0, parasite-specific IgG1-treated muMT mice at day 11 p.i. (P = 0.0209) and parasite-specific IgG1-treated AKR mice at days 11 and 35 p.i. had significant reductions in worm burdens (P = 0.0209 and 0.0202, respectively). Three of four parasite-specific IgG1-treated muMT mice at day 35 p.i. also had large reductions in worm burdens (9, 48, and 59 worms) compared to the nonspecific IgG-treated group (118, 152, 175, and 279 worms); however, the remaining mouse harbored 174 worms, and hence there was no significant difference between the two muMT groups (P = 0.833). Percentage reductions in worm burdens seen in parasite-specific IgG1-treated mice compared to nonspecific IgG-treated control groups varied: 49.3 and 59.9% reduction for muMT mice at days 11 and 35 p.i., respectively, and 48.9 and 77.9% reduction for AKR mice at days 11 and 35 p.i., respectively. Regardless of these differences, seven out of eight mice receiving parasite-specific IgG1 had large reductions in worm burdens compared to nonspecific IgG-treated mice.

ELISA showed the presence of parasite-specific IgG1 in the
sera of recipient µMT mice and of parasite-specific IgG1 and IgG2a in the sera of both parasite-specific IgG1- and nonspecific IgG-treated AKR mice. No parasite-specific antibody was detected in the serum of nonspecific IgG-treated µMT mice (data not shown).

**DISCUSSION**

Previous studies analyzing the role of B cells in *T. muris* infection have suggested that they are not involved (or are not essential) in protective immunity. Lee et al. (36) showed that transfer of immune B cells to CBA/Ca mice (a resistant strain) failed to accelerate expulsion from days 20 to 16 p.i., whereas transfer of immune T cells did. In addition, transfer of immune CD4+ cells from BALB/c mice to SCID mice confers resistance to recipients in the absence of B cells and antibody (14). Although these studies demonstrated that B cells are not essential for expulsion, the results presented here show for the first time that B cells are required for resistance to *T. muris*, and they suggest that the requirement is involved in the successful priming of a rapid Th2-type response. The B-cell involvement in protective immunity could be through the role as accessory antigen-presenting cell or via antibody production. However, there has been little evidence reported in the literature to support the idea that antibody plays an important role in resistance to a primary intestinal helminth infection (reviewed in reference 10).

In the context of *T. muris* infections, µMT mice were incapable of expelling worms, and in vitro cytokine analyses showed that they mounted a Th1 response in the absence of a Th2 response. Despite the lack of Th2 cytokine production in the MLNC of infected µMT mice, these mice mounted a cecal...

**FIG. 6.** Serum was collected from naive and *T. muris*-infected C57BL/6 (C57), µMT (muMT), and B cell-reconstituted µMT (muMTB) mice and tested for the presence of parasite-specific IgG1 (A) and IgG2a (B) by ELISA. Data shown compare mean optical densities (od) ± SE obtained from serum at a nonsaturating dilution of 1:160 (n = 4 for naive and day 35 p.i. [d35] groups). *, significant increase above naive levels (P < 0.05).

**FIG. 7.** *T. muris* worm burdens (mean ± SE) for µMT and AKR mice treated with either anti-IL-12 monoclonal antibody C17.8 or rat IgG (rIg) on days 0, 5, 9, and 14 p.i. (1 mg of antibody per intraperitoneal injection). Infected C57BL/6 mice were included as controls. Mice were infected with approximately 150 infective embryonated *T. muris* eggs on day 0, and worm burdens were counted in groups of four mice per strain at the time points shown. *, significant difference in worm burdens between rat IgG- and C17.8-treated groups of the same mouse strain (P < 0.05).

**FIG. 8.** *T. muris* worm burdens (mean ± SE) for µMT and AKR mice treated with either parasite-specific IgG1 (pIgG1) or nonspecific IgG (nIgG) on days 0, 1, and 3 p.i. (1 mg of antibody per intraperitoneal injection). Mice were infected with approximately 175 infective embryonated *T. muris* eggs on day 0 (n = 4 per group). *, significant difference between parasite-specific and nonspecific IgG-treated groups of the same mouse strain (P < 0.05). d11, day 11 p.i. group; d35, day 35 p.i. group.
mastocytosis, albeit delayed, which is dependent on the Th2 cytokines IL-4, IL-9, and IL-10. Thus, the local cytokine environment in μMT mice must be conducive to the development of a mast cell response. Mucosal mastocytes feature in the immune responses of both resistant and susceptible animals (16, 35) and can be depleted from C57BL/6 mice with anti-c-Kit monoclonal antibody treatment without altering the resistance phenotype (5). Additionally, the observation that serum MMCP-1 levels (an indication of mucosal mast cell degranulation) were elevated in infected μMT mice suggests that antibodies are not required for mucosal mast cell degranulation. This is also in agreement with the observation that serum MMCP-1 levels increase in FcγR−/− mice infected with T. muris (5). Antibody-independent degranulation has also been demonstrated in vitro, using bone marrow-derived mast cells stimulated with transforming growth factor β (41). Hence, cytokine action or possibly parasite antigen may induce mast cell degranulation in T. muris-infected mice.

A role for B cells in the priming of naive T cells has been both supported (7, 8, 29) and discounted (18, 19, 22, 34) in the literature. More recent studies have suggested that dendritic cells are the main cell type responsible for presenting antigens to naive T cells, while the presence of B cells is required for successful proliferation of primed T cells (23, 37, 45, 46) or in restimulating antigen-experienced T cells (21, 47). Our results suggest that naive T cells can be primed for Th1 responses in the absence of B cells, as evident from the IFN-γ produced from infected μMT mice compared to wild-type mice during the anti-egg response in Schistosoma mansoni infections (20, 30). Reconstitution of μMT mice with B cells restored the Th2 response to P. chabaudi chabaudi infections (33), while production of Th2 cytokines is significantly reduced in μMT mice compared to wild-type mice during the anti-egg response in Schistosoma mansoni infections (20, 30). Reconstitution of μMT mice with B cells restored the Th2 response to P. chabaudi chabaudi infection (33), as was observed in this study, suggesting that B cells are required for the successful development of a Th2 response in T. muris-infected mice. However, studies by Brown and Reiner (6) have highlighted the importance of genetic background in studies of immune responses to infection in μMT mice. C57BL/6 μMT mice are resistant to Leishmania major infection and mount a Th1-type response, while BALB/c μMT mice are susceptible and mount a Th2-type response, as indicated by in vitro cytokine production and ex vivo reverse transcription-PCR analysis of local lymph nodes. Thus, the ability of B cells to influence Th1 and Th2 development in infection models may be dependent on genetic background. As BALB/c and C57BL/6 mice differ in the ability to mount Th2 responses to T. muris infection (2), we are currently investigating if the importance of the B cell varies according to genetic background.

Recent papers have identified mechanisms whereby B cells preferentially induce Th2 differentiation at the time of T-cell priming. B cells can downregulate IL-12 production by dendritic cells during T-cell priming by producing IL-10, thus allowing T-cell-derived IL-4 to have an autocrine effect on Th2 development (50). Anti-IL-12 antibodies can convert Th1-inducing conditions to Th2-inducing conditions during in vitro CD4+ T-cell priming by dendritic cells (24). We have shown that treatment of μMT mice with anti-IL-12 antibody confers resistance to T. muris. We hypothesize that this treatment mimics the action of B-cell-derived IL-10 during T-cell priming, by reducing in vivo levels of IL-12 and allowing a dominant type 2 response to develop, as was observed in anti-IL-12-treated AKR mice. The lack of in vitro Th2 cytokine production from anti-IL-12-treated μMT mice may have been due to less efficient in vitro antigen presentation in the absence of the B cell, as has been previously suggested (6). The use of anti-IL-12 antibody to alter mouse strain resistance to parasitic infection has been shown in L. major infection (25), where treatment of C3H mice with anti-IL-12 during the first 3 weeks of infection resulted in a switch from a normal resistant Th1 response to a susceptible Th2 response. However, a reversion to a Th1 response occurred when treatment stopped at 3 weeks p.i. In our study, treatment only lasted for the first 2 weeks of infection. Had treatment lasted longer, more complete worm expulsion may have occurred in both μMT and AKR strains. Th2 cytokine production might also have been observed in the in vitro-cultured μMT MLNC.

Finally, we have identified a possible role for antibodies in anti-T. muris immune responses. It has been difficult to assign any role or importance to antibodies in resistance to T. muris, as despite being part of a Th2 response, expulsion of T. muris can occur in the absence of a detectable IgG1 and IgE response. Experiments involving serum transfers from resistant to susceptible animals have given mixed results, ranging from protection or enhanced expulsion to an absence of any effect (17, 49, 51). Serum would, however, also contain cytokines, chemokines, and possibly parasite antigens, making it difficult to attribute any effects seen to antibodies. Passive transfer of monoclonal T. muris-specific IgA can give 30 to 50% protection to CBA mice, however (44). Here we have shown that parasite-specific IgG1 purified from the serum of resistant NIH mice reduced worm burdens in recipient μMT and AKR mice. Previously, the only convincing demonstration of an association between the passive transfer of a particular antibody isotype and resistance to a gastrointestinal nematode has been with Heligmosomoides polygyrus infections (43), where parasite-specific IgG1 limits worm establishment in recipient mice and causes developmental stunting of any worms that do infect the host.

From our study, we hypothesize that the parasite-specific antibody prevents initial worm establishment, possibly by blocking enzymes or other antigens important to the parasite in successfully penetrating the hosts’ mucosal epithelial cells. Prevention of cell invasion by antigen-specific antibody has been demonstrated in vitro with other intracellular parasites, such as Toxoplasma gondii (48), Neospora caninum (42), and a nematode closely related to T. muris, Trichinella spiralis (39, 40). The development of similar assays for T. muris larvae would allow the effects of anti-T. muris antibody responses to be assessed directly in vitro. As high levels of parasite-specific antibodies are not usually generated early after infection, it is doubtful that antibody has a role in the expulsion of primary infections. However, it is possible that antibody plays an important role in the prevention of establishment of challenge infections. We are currently investigating whether parasite-specific IgG2a is protective.

In summary, our results have identified a clear role for the B cell in protective immunity to a gastrointestinal nematode. During a primary T. muris infection, the B cell appears to be
important for the development of a type 2 response (by cytokine action or costimulation). Parasite-specific antibodies associated with type 2 responses appear to be capable of limiting worm establishment and thus may play roles in limiting challenge infections. Future studies will address the mechanisms by which the B cell provides conditions conducive for the generation and maintenance of Th2 cells and the mechanisms by which parasite-specific antibodies prevent worm establishment.

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