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Infection of B Cell-Deficient Mice with CDC 1551, a Clinical Isolate of *Mycobacterium tuberculosis*: Delay in Dissemination and Development of Lung Pathology

Catherine M. Bosio,* Donald Gardner, † and Karen L. Elkins2*

Long-term survival of mice infected with *Mycobacterium tuberculosis* is dependent upon IFN-γ and T cells, but events in early phases of the immune response are not well understood. In this study, we describe a role for B cells during early immune responses to infection with a clinical isolate of *M. tuberculosis* (CDC 1551). Following a low-dose infection with *M. tuberculosis* CDC 1551, similar numbers of bacteria were detected in the lungs of both B cell knockout (IgH 6−, BKO) and C57BL/6J (wild-type) mice. However, despite comparable bacterial loads in the lungs, less severe pulmonary granuloma formation and delayed dissemination of bacteria from lungs to peripheral organs were observed in BKO mice. BKO mice reconstituted with naïve B cells, but not those given *M. tuberculosis*-specific Abs, before infection developed pulmonary granulomas and dissemination patterns similar to wild-type animals. Further analysis of lung cell populations revealed greater numbers of lymphocytes, especially CD8+ T cells, macrophages, and neutrophils in wild-type and reconstituted mice than in BKO mice. Thus, less severe lesion formation and delayed dissemination of bacteria found in BKO mice were dependent on B cells, not Abs, and were associated with altered cellular infiltrate to the lungs. These observations demonstrate an important, previously unappreciated, role for B cells during early immune responses to *M. tuberculosis* infections. *The Journal of Immunology*, 2000, 164: 6417–6425.

Cell-mediated immunity (CMI),3 rather than specific Ab responses (humoral immunity), dominates protective immune responses against some bacterial and protozoan organisms, especially intracellular pathogens. During CMI responses, T cells are the primary effector cell rather thanAbs secreted by B cells. T cell-deficient mice, including athymic nu/nu mice, have been used in many ways to elucidate the specific role of T cells. However, no comparable naturally B cell-deficient mice have been identified to date, and thus the role of B cells apart from their role as Ab-producing cells has not been well studied. With the development of B cell knockout mice (1), which are incapable of developing B cells beyond the pre-B cell stage, functions of B cells other than Ab production can readily be studied in infections in which CMI is important.

Interestingly, recent studies analyzing immunity against pathogens controlled by CMI have suggested that B cells may play a role during development of both early and long-term protective immune responses. Jankovic et al. demonstrated that absence of B cells leads to altered dissemination of *Schistosoma mansoni* from the foci of infection, larger granulomas, and suboptimal vaccination against this pathogen (2, 3). B cells have also been implicated in effective T cell priming and secondary immunity following pulmonary infections with *Chlamydia trachomatis* (4). In that study, B cell-deficient mice exhibited suboptimal delayed-type hypersensitivity (DTH) responses and reduced *C. trachomatis*-specific IFN-γ responses. In addition, a role for B cells during respiratory * Bordetella pertussis* infections has been described: following an aerosol infection with *B. pertussis*, B cell-deficient mice developed a persistent infection and, unlike their wild-type counterparts, failed to clear the bacteria (5).

A role for B cells during infections with *Mycobacterium tuberculosis* is less clear. In one report, B cell-deficient mice had slightly greater numbers of bacteria in organs 4–6 wk after i.v. infection with a high dose of the virulent laboratory strain *M. tuberculosis* H37Rv (6). On the other hand, Johnson et al. reported no detectable differences in bacterial burdens, cytokine mRNA, or organ pathology between B cell-deficient and wild-type mice for 45 days following a low-dose aerosol infection with *M. tuberculosis* H37Rv (7). Both of these reports suggested that there was little contribution by B cells in the control or exacerbation of *M. tuberculosis* infections in mice during chronic phases of infection. As described above, reports analyzing other intracellular pathogens suggested that the influence of B cells might lie in acute phases of infection. In addition to phase of infection, the strain of *M. tuberculosis* used could also influence the outcome. In a recent study, Rhoades et al. reported that granuloma development in aerosol-infected mice varied depending on the strain of *M. tuberculosis* used to infect the mice, despite the presence of similar bacterial loads in the lungs (8).

To further elucidate the role of B cells in tuberculosis, we examined the acute phase of infection with the clinical isolate *M. tuberculosis*, KY/TN 95-031151 (CDC 1551). This isolate was chosen for several reasons. First, it was a recent isolate, cultured from humans 4–4 years ago (9). Second, this isolate had an unusually high rate of transmission and virulence in the human population. Third, this particular isolate...
has been studied in several recent publications (9–11), providing a base of information to which we could compare and contrast our results. In contrast to previous observations, our data suggest a significant role for B cells, not involving Abs, during the initial development of pulmonary granulomas and subsequent dissemination of the *M. tuberculosis* CDC 1551 to other organs.

**Materials and Methods**

**Animals**
Six- to 8-week-old, male, specific pathogen-free C57BL/6J or B cell-deficient mice (Igh6-; Ref. 1) on a C57BL/6J background were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in sterile microisolator cages in a barrier environment at the Center for Biologies Evaluation and Research (Bethesda, MD). Mice were fed autoclaved food and water ad libitum. All experiments were performed under Animal Care and Use Committee guidelines.

**Bacteria and experimental infections**

*M. tuberculosis* KY/TN 95-031151 (CDC 1551; Dr. Frank Collins, Centers for Biologies Evaluation and Research/Food and Drug Administration, Rockville, MD), originally obtained from the Centers for Disease Control (Atlanta, GA), was grown in 7H9 liquid medium + 0.05% Tween-80, aliquoted into 1-mL vials, and frozen at −70°C. Enumeration of stock bacteria before use was performed by thawing several frozen vials and plating dilutions of bacteria onto 7H11 agar. Low-dose aerosol infections were performed as previously described (12). Briefly, mice were placed in a Middlebrook Airborne Infection Apparatus (Glas-Col, Terre Haute, IN). The nebulizer chamber was filled with a 10-mL suspension of *M. tuberculosis* CDC 1551 at 10⁶ bacteria/ml diluted in sterile saline. This concentration was previously demonstrated to reproducibly deliver 50–100 bacilli into the lungs over a 30-min exposure period (12). Delivery dosages were confirmed by sacrificing five randomly selected mice immediately following infection and enumerating bacterial loads.

**Enumeration of bacteria**

Lungs, spleens, and livers were removed aseptically and homogenized in sterile Dulbecco’s PBS (DPBS) using a Tekmar stomacher Model 80 (Tekmar, Cincinnati, OH). Bacterial loads in each organ were determined by plating 10-fold serial dilutions of organ homogenates on Middlebrook 7H11 agar (Difco Laboratories, Detroit, MI). Colonies were counted. −3 wk after plating, Plates that had no visible colonies were considered to be from organ homogenates that had bacterial loads at or below the limit of detection and were therefore assigned a value at this detection limit (10³).

However, if one to nine colonies were visible, the number of bacteria present in that sample was back calculated and all samples averaged. SEM was calculated, and significance of bacterial loads between groups of mice was confirmed by one-way ANOVA, followed by Tukey-Kramer’s multiple comparisons test using GraphPad Instat (GraphPad Software, San Diego, CA).

**Histology**

Intermediate lobes of the right lung of infected mice were harvested at the same time bacterial burdens were determined. Lobes were inflated and fixed with −1 mL 10% buffered paraformaldehyde, and sent to Histopath of America (Millersville, MD) for processing. Lobes were embedded into 5-μm paraffin sections, cut, and stained with hematoxylin and eosin, Zielh-Neelsen, or Masson’s trichrome. Two individuals, one from our laboratory group and one veterinary pathologist, evaluated each slide for size of granulomas and characteristics of infiltrating cells without knowledge of time of infection or treatment group. Spleen and liver samples were also taken from each group of mice, stored in 10% paraformaldehyde, processed, and evaluated, as described above.

**Reconstitution of B cell-deficient mice (BKO) with naive B cells**

Single cell splenocyte suspensions were prepared from naive C57BL/6J mice and treated with ammonium chloride to deplete erythrocytes. Viable cells were enumerated by hemocytometer count and exclusion of trypan blue. In all cases, starting and enriched splenocyte populations were analyzed by flow cytometry using a FACS, as described below. Flow cytometry revealed this method of enrichment for B cells resulted in <5% contaminating T cells in all B cell preparations (data not shown).

Immediately following enrichment of naive B cells, and 4 days before infection, BKO mice were given 1–2.3 × 10⁶ B cells, normal mouse serum (NMS), or immune mouse serum (IMS) i.v. The titer of polyclonal mycobacteria-specific Abs in IMS was determined as described below and was 1/10. All sera were diluted 1/4 in sterile, pyrogen-free DPBS (BioWhittaker, Walkersville, MD) before injection. NMS was obtained from the lateral tail vein of age-matched, naive wild-type mice before the onset of the experiment. IMS was obtained via cardiac puncture from mice that had been infected with *M. tuberculosis* CDC 1551 for 30 days, a time point previously determined to coincide with secretion of Abs against a broad spectrum of mycobacterial Ags (see Results) (13). Control BKO mice were given 200 μL sterile, pyrogen-free DPBS.

**Specificity and quantitation of serum Ig**

Concentrations of serum IgG and IgM were performed by ELISA, as previously described (14). Briefly, Immulon 2 plates (Dynex Technologies, Chantilly, VA) were coated with goat anti-mouse IgG or IgM (Southern Biotechnology Associates, Birmingham, AL) overnight. Plates were then washed and blocked, and dilutions of serum were added. Serial dilutions of purified mouse IgG and IgM (Southern Biotechnology) were used to generate standard curves. Following incubation with serum and standards, plates were washed and goat anti-mouse IgG or IgM Abs conjugated to HRP (Southern Biotechnology) were added. Bound conjugated Abs were visualized using addition of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Samples were read at 405 nm on a Versamax tunable microplate reader with a reference wavelength of 630 nm (Molecular Devices, Sunnyvale, CA), and concentrations of IgG and IgM determined using SoftMax Pro software (Molecular Devices). Titers of mycobacterial specific Abs were determined using plates coated with *M. tuberculosis* Erdman culture filtrate proteins (kindly provided from Dr. J. Bellie, Colorado State University, Fort Collins, CO, National Institutes of Health Contract NO1-A1-75320). Immulon 2 plates (Dynex Technologies) were coated with 2 μg/ml culture filtrate proteins overnight. Plates were then washed and blocked, and serum samples were added in serial dilutions. Following incubation, plates were washed and goat anti-mouse Ig Abs conjugated to HRP (Southern Biotechnology) were added. Bound Abs were visualized, and absorbance of each sample was performed as described above. Titers were determined as the lowest dilution of sample with an absorbance value above the mean of negative control wells by the SE containing serum from uninfected controls (0.111).

**Preparation of pulmonary and splenic lymphocytes**

Pulmonary lymphocytes were prepared as previously described (15). Briefly, mice were killed by CO₂ asphyxiation and lungs were perfused with −10 mL DMEM/10% FCS. Lungs were aseptically removed and finely sliced. Tissues were then incubated at 37°C/5% CO₂ for 1 h in 10 mL collagenase type I (Life Technologies, Grand Island, NY) diluted to 1 mg/mL. Following incubation, tissues were forced through a nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ). Cells were spun at 800 × g for 8 min to pellet cells and washed twice in DPBS/2% FCS. Viable cells were enumerated by exclusion of trypan blue and resuspended in DPBS/2% FCS at the appropriate concentration for flow cytometric analysis and cytospin centrifugation (see below).

Spleens were harvested from control, BKO, and B cell-reconstituted BKO mice at the same time pulmonary cells were retrieved. A single cell suspension was prepared from pooled spleens and erythrocytes lysed with ammonium chloride. Cells were washed, and viability was assessed by exclusion of trypan blue and resuspended in DPBS/2% FCS at appropriate concentrations for flow cytometric analysis and cytospin centrifugation.

**Analysis of lung and spleen cell populations**

To assess different cell types present in lungs and spleens, 5 × 10⁶ cells per cytospin centrifuge chamber (Shandon, Sewickley, PA) were spun through FCS onto slides and stained with modified Wright-Giemsa stain (Hema 3 stain set; Fisher Scientific, Pittsburgh, PA). Approximately 100–200 cells were counted from each slide to assess cell populations. Classification of cells was based on morphological characteristics: neutrophils, multilobed nuclei; lymphocytes, rounded nuclei with little cytoplasm; and monocytes/macrophages, larger cells with kidney-shaped nuclei and an abundance of often foamy cytoplasm.
Pulmonary and spleen cell populations were also analyzed by flow cytometry. Cells were prepared as described above and stained for B220, CD4, or CD8 surface markers. Single cell suspensions were mixed with anti-CD16 (FcBlock) for 10 min on ice. mAb FITC-conjugated rat IgG2a (R35-95) and PE-conjugated rat IgG2b (A95-1) (isotype controls), FITC-conjugated RA3-6B2 (anti-CD45/B220), PE-conjugated RM4-4 (anti-CD4), or PE-conjugated 53-6.7 (anti-CD8a) was added and cells were incubated for an additional 30 min on ice. All Abs were obtained from PharMingen, and optimal concentrations were determined in separate experiments. Cells were washed three times in PBS/2% FCS, fixed in 0.5–2% buffered paraformaldehyde, and analyzed using a Becton Dickinson FACScan flow cytometer (San Jose, CA) with gates set for viable lymphocytes and monocytes according to forward and side scatter profiles.

**Results**

**Growth and dissemination of bacteria, and lung histopathology following aerosol infection of BKO mice with M. tuberculosis CDC 1551**

The course of an aerogenic infection of the *M. tuberculosis* clinical isolate CDC 1551 was followed over time. CDC 1551 grew progressively in the lungs of both wild-type and BKO mice over 30 days of infection, and were comparable at all time points (Fig. 1). However, numbers of detectable bacteria in the spleens (and livers, see Fig. 3) varied markedly between the two groups of mice. Wild-type mice had detectable bacteria in their spleens 15 days postinfection, whereas BKO mice did not (Fig. 1). Bacteria were not detected in spleens of BKO mice until 30 days postinfection. In six separate experiments, three of which only compared wild-type and BKO mice (see below), four of five, four of five, and five of five BKO mice had no detectable bacteria in their spleens 15 days postinfection. At 30 days postinfection, the numbers of bacteria in BKO spleens were consistently significantly lower than in the wild-type spleens (*p* > 0.05) (Fig. 1).

To determine pathological consequences of CDC 1551 infection of wild-type and BKO mice, lung samples from each group of mice were evaluated for inflammation and granuloma formation. Histological examination of lung tissues from wild-type mice revealed infiltration of macrophages and lymphocytes to form large granulomatous lesions 30 days postchallenge (Fig. 2A). In contrast to wild-type mice, BKO mice had only small, diffuse granulomas 30 days postinfection (Fig. 2B). The structure of lung tissue also remained intact, exhibiting mild interstitial pneumonia with the majority of cellular infiltrate focused in the perivascular regions (Fig. 2B). Although organized granulomas were visible at this time, they were clearly fewer in number and size compared with wild-type control mice (compare Fig. 2, A and B).
Growth and dissemination of bacteria, and lung histopathology following infection with M. tuberculosis CDC 1551 in BKO mice reconstituted with naive B cells

To further assess the contribution of B cells and Abs in pulmonary pathology and dissemination of mycobacteria, bacterial burdens in target organs, and development of pulmonary inflammation was compared in wild-type, BKO, and BKO mice that received naive B cells before infection (reconstituted BKO). Numbers of CDC 1551 were comparable in the lungs from all three groups of mice (Fig. 3A), but dissemination of mycobacteria to the spleen and liver was substantially delayed in BKO mice compared with wild-type mice (Table I, Fig. 3, B and C). A similar pattern of delayed dissemination was also observed in BKO mice, who had received either IMS or NMS (Table I). In contrast, 15 days postinfection mycobacteria were readily detected in spleens and livers of BKO mice that received B cells (Fig. 3, B and C). In three separate experiments that compared wild-type, BKO, and B cell-reconstituted BKO mice, 3 of 15 BKO mice had no detectable bacteria in their spleens 15 days postinfection (Table I). Reconstituted mice had significantly greater numbers of bacteria in the liver compared with nonreconstituted BKO control mice at this time point (p < 0.05). By day 30, the numbers of bacteria detected in spleens of B cell-reconstituted mice were equivalent to those observed in wild-type animals, and both were significantly greater than those detected in BKO control mice (p < 0.05). In addition to greater numbers of bacteria in reconstituted mice, these animals had marked splenomegaly, as did wild-type mice, compared with nonreconstituted BKO control mice (data not shown). Interestingly, in all three experiments, reconstituted mice had significantly greater numbers of bacteria in their livers and lungs compared with both wild-type and BKO mice 30 days postinfection (p < 0.05).

As seen previously (8) (Fig. 2), histological examination of lung tissues from wild-type mice revealed progressive infiltration of macrophages, followed by lymphocytes and occasionally neutrophils (Fig. 4, A, D, and G). The development of granulomas paralleled increasing bacterial loads in the lungs, and dissemination of bacteria to the spleen in wild-type mice. This pattern of granuloma development is typical of murine tuberculosis (8).

In contrast to wild-type mice at 15 days postinfection, BKO mice exhibited only slight interstitial pneumonia characterized by a thickening of the alveolar membranes with no visible granulomatous lesions (Fig. 4B). Twenty-two days postinfection, cellular infiltrates included both lymphocytes and macrophage cuffing around many perivascular regions, but only small diffuse granulomatous lesions were visible at this time (Fig. 4E). As described above (Fig. 2), lesions were first observed in BKO mice 30 days postinfection (Fig. 4H). At this time point, macrophages and a few lymphocytes appeared to have extravasated into the infected tissue and established granulomas at foci throughout the lung. Once again, BKO mice had fewer granulomas that were also diffuse and smaller than those observed in wild-type control mice (compare Fig. 4, G and H).

Pathology in the lungs of BKO mice reconstituted with B cells progressed in a manner more similar to that observed in wild-type mice (Fig. 4, C, F, and I). Fifteen days postinfection, reconstituted BKO mice appeared to have extensive interstitial pneumonia similar to that observed in wild-type mice, but with fewer detectable lesions (Fig. 4, A and C). At 22 days postinfection, organized lesions first appeared in the lungs of reconstituted BKO mice (Fig. 4F). These lesions were of similar size and number as those observed in wild-type mice (Fig. 4, D and F). In contrast, BKO mice

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* Number of mice with detectable of M. tuberculosis CDC 1551 in the spleen and liver 15 days following an aerosol challenge.

* Naive B cells.
FIGURE 4. Histopathology in wild-type, BKO, and reconstituted BKO mice following aerosol infection with *M. tuberculosis* CDC 1551. Representative photomicrographs of lung sections from C57BL/6J (wild-type, A, D, and G), BKO (B, E, and H), and BKO mice reconstituted with $2.3 \times 10^7$ naive B cells (C, F, and I) 15 days (A–C), 22 days (D–F), and 30 days (G–I) following aerosol infection with *M. tuberculosis* CDC 1551 are shown. All plates are at $\times 100$ magnification. This experiment is representative of three experiments of similar design.

FIGURE 5. Histopathology in C57BL/6J (WT) wild-type and B cell-deficient (BKO) mice 150 days following an aerosol infection with *M. tuberculosis* CDC 1551. Representative photomicrographs of C57BL/6J (wild-type, A and C) and BKO (B and D) mouse lungs 150 days postinfection. C and D, Magnifications of the boxed areas in A and B: A and B are at $\times 100$, C and D are at $\times 400$. This experiment is representative of two of similar design.
appeared to have intensified interstitial pneumonia 22 days postinfection without developing organized lesions (Fig. 4E). Finally, 30 days postinfection, both wild-type and reconstituted mice had large coalescing lesions in their lungs, while only small lesions were observed in BKO mice at the same time point (Fig. 4, G–I). Reconstituted mice also appeared to have lesions of greater cellularity than those seen in wild-type animals (Fig. 4, G and I). Total numbers of granulomas were also different. For example, at 30 days postinfection, wild-type and reconstituted mice in one representative had significantly greater numbers of granulomas, 10.7 ± 2.3 (n = 3) and 9 ± 1 (n = 3), respectively, compared with BKO mice, 2.7 ± 3.8 (n = 3) (p < 0.05).

To further examine pathological changes in the lungs of wild-type and BKO mice during the chronic phase of *M. tuberculosis* infection, groups of mice were sacrificed 150 days postchallenge and lungs were analyzed for bacteria and histopathology. Although the number of bacteria in the lungs of both groups of mice were not different (data not shown), BKO mice had markedly less severe pulmonary inflammation compared with wild-type animals (Fig. 5). At 150 days postinfection, large granulomatous lesions, consisting primarily of lymphocytes and pockets of macrophages, were observed in wild-type mice (Fig. 5, A and C). The lesions in wild-type mice consolidated the majority of available lung space. In contrast, lesions in BKO mice were small and consisted primarily of macrophages (Fig. 5, B and D). Importantly, BKO mice appeared to have large areas of the lung unaffected by lesion development, compromised only by mild interstitial pneumonia.

Although samples of spleens from each group of mice were studied to assess pathological changes, useful comparisons were greatly limited by absence of distinct germinal centers in BKO mice (1). This defect was not changed by reconstitution of these mice with B cells (data not shown), confounding interpretation of pathology in the spleens of the different types of mice.

**Ab responses in wild-type, BKO, and reconstituted mice following infection with *M. tuberculosis* CDC 1551**

To determine the effect of reconstitution of BKO mice with NMS, IMS, or naive B cells on the development of humoral immunity, serum samples from each group of infected mice were analyzed for total polyclonal IgG and IgM, and total mycobacterial specific Abs 30 days postinfection. Wild-type mice had 5-fold greater concentrations of IgG (1.49 μg/ml) and 3000-fold greater concentrations of IgM (240 μg/ml) compared with BKO mice reconstituted with naive B cells (0.299 μg/ml IgG and 0.073 μg/ml IgM). BKO mice reconstituted with NMS or IMS had ~6000-fold less IgG, 227 ng/ml and 272 ng/ml, respectively, than wild-type mice and no detectable IgM (<10 ng/ml). As expected, IgG and IgM were not detected in BKO mice. Only wild-type mice had detectable serum titers, ~1:10 by ELISA, against mycobacterial Ags.

**Comparisons of pulmonary and splenic cell populations during CDC 1551 infection**

To assess the nature of the cellular populations in the lungs and spleens in greater detail, pooled pulmonary and spleen cells were examined by differential staining and flow cytometry. An increase in total cell numbers was observed in each group of mice throughout the course of the experiment (Figs. 6A and 7A). At each time point following infection, both wild-type and reconstituted mice had greater numbers of cells isolated from their lungs and spleens compared with BKO controls (Figs. 6A and 7A).

In the lungs, clear differences in mononuclear cell populations between groups of mice were observed. Fifteen days following infection, all mice had similar numbers of macrophages (Fig. 6A), but by 30 days postinfection, wild-type and reconstituted BKO mice had twice the number of neutrophils and macrophages that were found in nonreconstituted BKO mice. Similarly, by 30 days
postinfection, wild-type and reconstituted BKO mice had at least twice the number of lymphocytes found in nonreconstituted BKO mice (Fig. 6A). Reconstituted BKO mice consistently had even greater numbers of lymphocytes than wild-type mice 30 days postinfection (Fig. 6A).

Analysis of lung cell populations by flow cytometry revealed that these differences were not due to B cells, but to greater numbers of T cells in wild-type and reconstituted mice at each time point following infection compared with BKO mice (Fig. 6B). However, while numbers of CD4+ T cells were about equal in the lungs of wild-type and reconstituted mice 30 days postinfection, reconstituted mice reproducibly had two to three times the number of CD8+ T cells compared with wild-type mice at this time point. Small numbers of B cells were detected in the lungs of wild-type mice throughout the infection, but were not detected in lung of BKO mice (as expected) or reconstituted mice (Fig. 6B). However, we cannot determine whether B cells transferred to reconstituted mice failed to reach lung tissue, or whether numbers that do reach the lung are below the limit of detection (<10^5).

Similarly, examination of splenic lymphocyte populations revealed that reconstituted and wild-type mice had more lymphocytes in their spleens compared with BKO mice at all time points (Fig. 7A). Wild-type and reconstituted mice had fairly stable numbers of lymphocytes in their spleens following infection (Fig. 7A). BKO mice initially had very low numbers of lymphocytes, followed by a steady increase in both CD4+ and CD8+ T cells as the infection progressed (Fig. 7B). Reconstituted mice had notably more cells in their spleens at days 15 and 22 postinfection compared with BKO mice (Fig. 7A), which could not be attributed solely to presence of transferred B cells, although small numbers of B cells were detected in spleens of reconstituted mice (Fig. 7B). Instead, the difference in spleen cell numbers between BKO and reconstituted mice was due to a greater initial increase in CD4+ and CD8+ T cells in spleens of reconstituted mice (Fig. 7B). Over-
two preceding reports, we found clear difference between wild-
type and BKO mice in pulmonary granuloma development and
dissemination following an aerosol infection with *M. tuberculosis*
CDC 1551.

It is often thought that development of granulomatous lesions
and DTH responses following infection with *M. tuberculosis* are
measures of protective immunity in the host (16). For example,
granulomatous lesions might contain pockets of bacteria in in-
fected tissue, thus preventing their dissemination to peripheral or-
gans. Additionally, the cells forming the granuloma, primarily
lymphocytes, and macrophages, are believed to contribute to the
control of bacterial growth throughout the course of the infection.
However, there are several circumstances in which lesion devel-
opment or the generation of cutaneous DTH responses is dissoci-
ated from protective immunity. For example, following infection
with *M. tuberculosis* IFN-γ knockout mice have normal cutaneous
DTH responses (17). Furthermore, CD4 knockout mice develop
normal pulmonary granulomas, but have greater numbers of bact-
teria in their organs and much earlier mean time to deaths com-
pared with wild-type controls (18).

In agreement with those previously published observations, our
data also suggest that lesion development and generation of pro-
tective immunity are dissociative events. This interpretation is
based on two key observations. First, BKO mice had a delay in
dissemination of bacteria to the spleen and liver, not an increase in
dissemination of bacteria in the absence of normal granulomatous
lesions. Second, BKO mice developed very small lesions with lit-
tle cellular infiltrate, instead of the typical large, destructive gran-
ulomatous lesions seen in wild-type mice, despite always having
bacterial burdens in the lung similar to those detected in wild-type
animals. This suggests that development of large granulomas does
not necessarily contribute to prevention of bacterial dissemination
or greater control of bacterial growth in the host. These concepts
gain added importance in light of the interpretation that it is the
increasing pathological changes in the lung, not necessarily in-
creasing numbers of bacteria, that eventually kill the host (8).

Recently, Jankovic et al. (2) described an unexpected contribu-
tion of B cells in the regulation of granuloma development and
excretion of parasites during experimental s.c. *Schistosoma manso-
ni* infections. In their studies, BKO mice failed to control gran-
uloma size, and worm eggs did not disseminate into the gut fol-
lowing infection. However, there was no appreciable difference in
BKO mice in T cell responsiveness or shift in the Th1/Th2 balance
against schistosomal Ags. Similarly, we found that dissemination
was delayed in BKO mice, and that pulmonary granulomas in
these mice failed to reach the number or size of those observed in
wild-type mice up to 120 days postinfection (Fig. 5).

There are a number of hypotheses to explain the unique role of
B cells during *M. tuberculosis* infections described in this study.
First, less severe pulmonary inflammation and delayed dissemi-
nation of bacteria seen in BKO mice could be due to altered re-
responses by T lymphocytes. To address this possibility, cellular
infiltrates into the lungs and spleens of infected wild-type, BKO,
and reconstituted BKO mice were examined (Figs. 6 and 7). BKO
mice had fewer T cells and macrophages in their lungs compared
with wild-type and reconstituted mice following aerosol infection.
This correlated with the reduced inflammation and delayed dis-
ssemination of bacteria from this organ. Similarly, BKO mice had
fewer T cells in their spleens compared with wild-type and recon-
stituted mice, corresponding to lower numbers of bacteria in the
spleens of BKO. These data suggest that BKO mice are capable of
accumulating cells at various sites of infection, but do so in a
slower manner than wild-type or reconstituted mice. Preliminary
experiments assessing various cytokine responses by pulmonary
and splenic lymphocytes stimulated with mycobacterial Ags in
vitro are ongoing. To date, these experiments have indicated that
BKO mice develop T lymphocytes responsive to specific myco-
bacterial proteins (data not shown). This suggests that, although
small numbers of T cells are detectable in the lungs and spleens of
BKO mice, those present are capable of providing protection.
Thus, we believe it is less likely that delayed dissemination and
lesser severe granuloma formation seen in BKO mice are due to
defective responses by T lymphocytes to mycobacterial Ag. Simi-
larly, other investigators have demonstrated that T cells from
BKO mice can be readily primed in vivo (Bosio and Elkins, un-
published data) (19, 20). Rather, the lessened T cell and macro-
phage infiltration appears to be a consequence (rather than a cause)
of the reduced inflammation seen in BKO mice at early time points
postinfection.

Another explanation for the disparity in inflammation and dis-
semination between BKO and wild-type mice might be suboptimal
responsiveness of host macrophages in BKO mice to infection,
including C3 receptor-mediated uptake and altered Fc receptor-
mediated functions (21–23). We believe this scenario is unlikely,
since BKO and wild-type mice had similar bacterial loads in the
lungs throughout the course of infection. This suggests that bac-
terial uptake and control capabilities of host macrophages were not
significantly altered in BKO animals compared with wild-type
controls. To date, physiological differences between macrophages
from BKO and wild-type mice have not been described. Others
have suggested a role for Abs, including opsonizing Abs, during
*M. tuberculosis* infections (6, 22). Since nonspecific Ig was de-
tected in mice reconstituted with B cells, a role for natural Abs
cannot be ruled out. However, no differences were observed be-
tween mice that received NMS or IMS compared with nonrecon-
stituted BKO mice. This suggests that opsonizing mycobacterial-
specific Abs have minimal, if any, effect on the development of
protective immunity during early stages of mycobacterial infections.

B cells, which secrete IL-8, macrophage-inflammatory protein-
1α, and monocyte chemoattractant protein-1 (24, 25), could be
an important source of chemokines involved in granuloma devel-
opment and subsequent dissemination of bacteria, resulting in re-
cruitment of appropriate cells to infected foci during the first few
weeks of infection. Increased expression and secretion of chemo-
kines (including IL-8, macrophage-inflammatory protein-1α, and
RANTES) have been demonstrated in bronchoalveolar lavage flu-
ids from humans with active pulmonary tuberculosis (24, 25), mu-
rine pulmonary granulomas (26), and dermal DTH responses in
*Mycobacterium bovis* bacillus Calmette-Guerin-infected rabbits
(27). Interestingly, Rhoades et al. found significant differences in
chemokine mRNA levels from mice infected with either CSU22 or
CSU46, two clinical isolates of *M. tuberculosis*, compared with
mice infected with *M. tuberculosis* Erdman (26). Since it is also
likely that dissemination of *M. tuberculosis* to peripheral organs
occurs via a host cell, an increase in the influx of these cells to the
primary site of infection in response to chemokines would increase
the opportunity for the bacteria to escape the site of initial infection
and colonize other organs. In the absence of B cells, the concen-
tration of chemokines may be reduced, resulting in delayed pul-
monary granuloma development and delayed dissemination of
bacteria to peripheral organs despite equivalent bacterial loads.

Another explanation for the role of B cells in delayed pathology
and dissemination concerns control of cell trafficking. Mechanisms
that control granuloma formation during mycobacterial infections
are not well understood. Formation of granulomatous lesions ap-
pears to depend on a complex network of cellular responses, cell
trafficking, and secretion of appropriate chemokines and cytokines.
However, at this time, it is difficult to determine whether the effect
of B cells in reconstituted mice was due to direct localization of B cells in the lung, or due to an indirect and systemic mechanism. In this study, we were unable to detect B cells in the lungs 15 days or later following reconstitution. In previous studies, adoptively transferred B cells localized primarily in the spleen and liver, but were detected systemically throughout the host 24 h after transfer (28). Other studies demonstrated that the numbers of adoptively transferred B cells in the spleen and lymph node were maximal at 1–3 days after transfer, but declined dramatically by day 9 (29). Furthermore, it is likely that only Ag-specific B cells stimulated by infection engraft successfully, but numbers of such cells would be very small. These small numbers of transferred B cells in reconstituted mice may exert an effect on trafficking of other cells into the lung either locally or systemically.

The response we observed in BKO mice in this study could also be, in part, attributed to unique Ags associated with different strains of Mycobacterium. One of the major constituents of the mycobacterial cell wall is lipoarabinomannan (LAM). This structure has been shown to be an immunomodulatory molecule capable of participating in a variety of immune responses, including chemokine secretion (30). The structure of LAM varies among species and strains of mycobacteria (30). Therefore, it is possible that LAM from CDC 1551 has a structure different from that found in M. tuberculosis Erdman, resulting in differential stimulation of B cells or other cells influencing the immune response. In support of this possibility, a recent manuscript demonstrated that lipids isolated from CDC 1551 were more effective at inducing lymphocytes to secrete TNF-α and IL-12 when compared with lipids from other strains of virulent mycobacteria (10).

To our knowledge, this is the first documentation of a clear role for B cells in the progression of murine infection with virulent M. tuberculosis. Further elucidation of the specific contributions of B cells during infections of M. tuberculosis will increase our understanding of early immunological events that regulate bacterial dissemination, pathological consequences of infection, and disease progression.

Acknowledgments

We thank Dr. Frank Collins for assistance in administration of aerosol infections, and Dr. Collins, Dr. Michael Brennan, and Dr. Scott Stibitz for critical reviews of the manuscript.

References