Fcγ Receptors Regulate Immune Activation and Susceptibility during *Mycobacterium tuberculosis* Infection[1]

Paul J. Maglione,* Jiayong Xu,† Arturo Casadevall,*† and John Chan[2]*†

The critical role of cellular immunity during tuberculosis (TB) has been extensively studied, but the impact of Abs upon this infection remains poorly defined. Previously, we demonstrated that B cells are required for optimal protection in *Mycobacterium tuberculosis*-infected mice. FcγR modulate immunity by engaging Igs produced by B cells. We report that C57BL/6 mice deficient in inhibitory FcγRIIB (RIIB$^{-/-}$) manifested enhanced mycobacterial containment and diminished immunopathology compared with wild-type controls. These findings corresponded with enhanced pulmonary Th1 responses, evidenced by increased IFN-γ-producing CD4$^+$ T cells, and elevated expression of MHC class II and costimulatory molecules B7-1 and B7-2 in the lungs. Upon *M. tuberculosis* infection and immune complex engagement, RIIB$^{-/-}$ macrophages produced more of the p40 component of the Th1-promoting cytokine IL-12. These data strongly suggest that FcγRIIB engagement can dampen the TB Th1 response by attenuating IL-12p40 production or activation of APCs. Conversely, C57BL/6 mice lacking the γ-chain shared by activating FcγR had enhanced susceptibility and exacerbated immunopathology upon *M. tuberculosis* challenge, associated with increased production of the immunosuppressive cytokine IL-10. Thus, engagement of distinct FcγR can divergently affect cytokine production and susceptibility during *M. tuberculosis* infection. *The Journal of Immunology,* 2008, 180: 3329–3338.

Although protective B cells and Igs are vital to the success of most vaccines currently in use, the significance of humoral immunity against *Mycobacterium tuberculosis* has been questioned for decades (1, 2). Despite this controversy, studies by our group and others have found that B cells can influence tuberculosis (TB)[3] susceptibility and pathologic progression in murine models (3–5). Moreover, the efficacy of specific mAbs (6–8) and arabinomannan conjugate vaccines (9) against *M. tuberculosis* infection indicate that B cell biology may be augmented to enhance protection during TB. B cells influence the maturation of APCs and development of cellular immunity (10), but few studies have focused upon such mechanisms of B cell-mediated immune regulation during TB. In this study, we set out to further address these concepts that link B cell biology with the TB cellular immune response.

Although traditionally separated on the basis of a historical dichotomy, Ab responses can, in fact, be collaborative with cellular immunity, such as during Ab-dependent cell-mediated cytotoxicity or Ag-presentation subsequent to opsonization by Igs (11). In fact, there is considerable evidence that Abs can regulate inflammatory cellular responses (reviewed in Ref. 12). By binding the Fc portion of Igs, FcγR mediate Ag uptake and cellular activation (13, 14), and are a specific means by which Abs can directly affect the cellular immune response (15). Separated into activating and inhibiting types, FcγR mediate immune activation or suppression based upon a threshold determined by the summation of their reciprocal signals (16). Murine models of FcγR deficiency have been useful in assessing the relevance of these receptors against intracellular pathogens, including *Chlamydia trachomatis*, influenza virus, *Leishmania* species, *Plasmodium*, and *Salmonella enterica* (17–22). However, no such study had yet been conducted with *M. tuberculosis*, possibly because of the prevailing view that humoral immunity has little or no role in host defense against this pathogen.

There are two types of FcγR: activation receptors, which share a γ-chain that associates with an intracellular ITAM, and the inhibition receptor FcγRIIB, which contains a cytoplasmic ITIM sequence (15). In the mouse, there are three activating FcγR (FcγRI, FcγRIII, and FcγRIV) and one inhibitory receptor (FcγRIIB) (16). There is an additional activation FcγR in humans, FcγRIIA, not present in mice, with its ITAM intrinsic to the receptor (16). Though dendritic cells and macrophages express all of the putative activation and inhibition FcγR, NK cells only express FcγRIII, and B cells are limited to only FcγRIIB. As both activation and inhibition FcγR may be present in the same tissue microenvironment, they compete for immune complex ligands and the balance of activation and inhibition signals of these receptors determines the threshold of cellular activation and effector responses, most notably of APCs that coexpress both classes of FcγR, dendritic cells and macrophages (15, 16).

Knowing that broadly specific Abs are produced during *M. tuberculosis* infection (23, 24) and that adoptively transferred B cells produce Abs and confer protection to B cell-deficient mice (3), we examined how humoral immunity may influence disease outcome in an endocrine manner via FcγR using gene-deleted mouse strains. C57BL/6 mice deficient in inhibitory FcγRIIB (RIIB$^{-/-}$) manifested improved bacterial control and diminished pathology upon *M. tuberculosis* aerosol challenge, with enhanced stimulation

*Department of Microbiology and Immunology and †Department of Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY 10461

Received for publication December 6, 2007. Accepted for publication December 25, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants R01 HL071241, R01 AI50732, P01 AI063537, and Albert Einstein College of Medicine/Montefiore Medical Center for AIDS Research Grants P30 AI051519 (to J.C.), U54 AI05715805 and R01 AI03377414 (to A.C.), and T32 AI007506, and by a Seed grant from the American Medical Association Foundation (to P.J.M.). This work constitutes partial fulfillment of the thesis requirements for P.J.M. in the Graduate Division of Medical Sciences, Albert Einstein College of Medicine.

[2] Address correspondence and reprint requests to Dr. John Chan, Department of Medicine, Forchheimer Building 406, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail address: jchan@aecom.yu.edu

[3] Abbreviations used in this paper: TB, tuberculosis; PPD, mycobacterial purified protein derivative.

of protective IFN-γ responses that coincide with heightened activation of APCs in the absence of this receptor. In contrast, mice lacking the γ-chain subunit shared by activating FcγR (Fcγ-chain$^{-/-}$) had increased production of immunosuppressive IL-10, worsened TB immunopathology, elevated organ mycobacterial burdens, and heightened susceptibility to *M. tuberculosis* infection, conversely demonstrating that activation FcγR are required for optimum immune activation in this model. Modulation of humoral immunoregulatory pathways may provide novel means of both enhancing TB immunity against acute infection and limiting pathologic sequelae of a chronic host response.

Materials and Methods

Mice

Female C57BL/6 (Charles River Breeding Laboratories), RIIB$^{-/-}$, and Fcγ-chain$^{-/-}$ mice backcrossed to a tenth generation on C57BL/6 (Taconic Laboratories) 8- to 10-wk-old mice were used in experiments. For some experiments, previously established colonies of RIIB$^{-/-}$ and Fcγ-chain$^{-/-}$ on the C57BL/6 background were used (25). All mice were housed in a Biosafety Level III animal laboratory and maintained pathogen-free by routine serological and histopathological examinations. The Institutional Animal Care and Use Committee have approved the animal protocols used in this study.

Mycobacteria and mouse infection

Preparation of mycobacteria and murine aerosol infection were done as previously described (3). Briefly, mice were infected by aerosol using the Lovelace nebulizer (In-Tox Products) with *M. tuberculosis* diluted to a concentration calibrated to deliver ~150 CFU to the lungs. Inoculum dose was confirmed by colony counts on 7H10 agar plates (Difco) of whole lung homogenates at 16–24 h postinfection by aerosol for each experiment.

CFU enumeration

Enumeration of organ mycobacterial burden was as previously performed (3). Bacterial burden was assessed as CFU, determined by the number of colonies on plates after incubation on 7H10 agar plates at 37°C for 21 days. In all experiments, two right lung lobes or approximately one-third of the lung, approximately one-eighth of the liver, and approximately one-half of the spleen were used for enumerations of tissue bacterial burden.

Histopathological and immunohistochemical studies

Tissue samples from lung and spleen were fixed in 10% buffered formalin and subsequently embedded in paraffin. For histopathological and immunohistochemical studies, serial 5- to 6-μm sections were stained with H&E or reserved for immunohistochemical staining. For immunohistochemistry, Ags were exposed using a citrate unmasking solution (Vector Laboratories) and stained as previously described (3). B220 Ab (BD Pharmingen) was detected using the VectaStain ABC kit (Vector Laboratories) and labeled with diaminobenzidine substrate (Sigma-Aldrich). Slides were coverslipped and mounted using VectaMount permanent mounting medium (Vector Laboratories).

Preparation of single-cell suspension of lung cells for ex vivo culture and flow cytometry

Single-cell suspensions of lung cells were prepared as previously described (3). In brief, minced lungs were digested in 1 mg/ml collagenase and 30 μg/ml DNase (Sigma-Aldrich) at 37°C for ~60 min, passed through 70-μm nylon cell strainers, and subsequently enriched by RBC lysis. Cells were cultured in RPMI supplemented with L-glutamine, 25 mM HEPES, 10% FBS, and 55 μM 2-ME. For ex vivo cell culture, single-cell suspension lung cells were cultured in supplemented RPMI alone or with 10 μg/ml mycobacterial purified protein derivative (PPD) (Statens Serum Institut, Copenhagen, Denmark) at 1.0×10^7 cells/ml. After 48 h of culture, supernatants were harvested, filter sterilized, and stored at −20°C. Matched Ab pairs for IFN-γ and IL-10 used for ELISA were purchased from R&D Systems and used according to the manufacturer's protocol.

Intracellular cytokine staining and flow cytometry

Monoclonal Abs against the following Ags were used for flow cytometry and were purchased from BD Pharmingen: CD3, CD4, CD8, CD19, Ly6G, IFN-γ, IL-10, B7-1 (CD80), B7-2 (CD86), MHC class II (I-A/I-E), and IL-10R (CD210). The mAb against F4/80 was purchased from Caltag Laboratories. For intracellular cytokine staining, single-cell suspensions of lung cells were cultured for 4 h in the presence of 125 μg/ml brefeldin A (Sigma-Aldrich) and, for designated groups, stimulated with 10 μg/ml of plate-bound anti-CD3 (BD Pharmingen). Subsequently, a Cytofix/Cytoperm kit (BD Pharmingen) was used according to the manufacturer's specifications for intracellular cytokine staining. For in vivo BrdU labeling, 1 mg of BrdU (BD Pharmingen) was injected into mice 16–20 h before sacrificing mice for flow cytometric analysis according to the manufacturer's protocol.

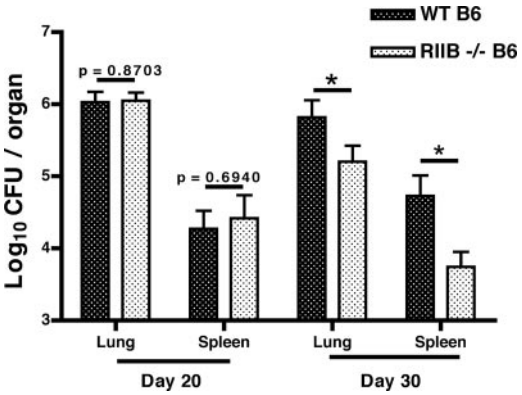

FIGURE 1. FcγRIIB negatively regulates containment of *M. tuberculosis* in C57BL/6 mice. RIIB$^{-/-}$ mice have significantly reduced pulmonary bacterial burden in lungs and spleens 30 days after aerosol challenge with *M. tuberculosis* Erdman. Data shown are representative of three experiments ($n = 4$ or 5 mice per group). *, $p < 0.05$.

In vitro infection of bone marrow-derived macrophages

For preparation of mouse bone marrow-derived macrophages, 4×10^5 bone marrow cells from femurs and tibias were cultured per milliliter of DMEM supplemented with L-glutamine, 10% L929 cell supernatant, and 10% FBS for 5 days, changing medium once on day 3. Macrophages were plated at 3×10^5 cells/ml, allowed to adhere overnight, and infected with *M. tuberculosis* Erdman at a multiplicity of infection of 10:1. Designated samples were also cultured in the presence of immune complex, which was prepared by incubating mouse anti-trinitrophenyl IgG1 (BD Pharmingen) with trinitrophenyl-labeled OVA (Biosearch Technologies) for 1 h at 37°C. After 24 h, macrophage supernatants were collected, filter sterilized, and analyzed by ELISA.

Statistical analysis

Statistical significance was assessed using unpaired Student's t test, calculated using Prism 4 software (GraphPad). Values of $p < 0.05$ were considered significant.

Results

FcγRIIB inhibits optimal containment of M. tuberculosis *in mice*

It has been demonstrated that Ig engagement of FcγRIIB on APCs can limit optimal activation of T cells in tumor models (26, 27). Knowing that adequate T cell responses are of vital importance to immune control of *M. tuberculosis* (28), we were interested to know whether deficiency in FcγRIIB affected mycobacterial containment in mice. By measuring CFU from lung and spleen homogenates of *M. tuberculosis*-infected mice, we examined how levels of mycobacterial burden compared between wild-type and FcγRIIB-deficient C57BL/6 strains. After aerosol challenge with *M. tuberculosis* Erdman, RIIB$^{-/-}$ mice had significantly reduced bacterial burden in the lungs and spleens 30 days, but not 20 days, after infection (Fig. 1). This finding indicates that the presence of FcγRIIB inhibits optimal containment of *M. tuberculosis* Erdman in mice. The ability of the RIIB$^{-/-}$ mice to control *M. tuberculosis* was evaluated only in the acute phase of infection and not during chronic TB because these mice spontaneously develop autoimmunity as they age (29), the latter a confounding factor that would

render interpretation of results difficult, if not impossible. Similarly, we focused our analysis of the antituberculous immune response of RIIB$^{-/-}$ C57BL/6 mice to the acute phase of infection in subsequent studies.

RIIB$^{-/-}$ mice have increased IFN-γ production and IFN-γ-producing CD4$^+$ T cells in lungs after aerosol challenge with M. tuberculosis *Erdman*

IFN-γ is required for containment of *M. tuberculosis* in mice (30, 31) and polymorphisms in the IL-12/IFN-γ axis are associated with susceptibility to mycobacteria in humans (32). Moreover, aerosol administration of IFN-γ has reported efficacy in patients with multidrug-resistant TB (33). T cell activation is influenced by FcγRIIB regulation of APCs, including the capacity of which T lymphocytes make IFN-γ (26, 27). Consequently, we investigated whether FcγRIIB deficiency affected IFN-γ production in the lungs of C57BL/6 mice infected with *M. tuberculosis*.

IFN-γ produced by lung cells isolated from RIIB$^{-/-}$ mice and stimulated ex vivo with PPD was greater than that of wild-type mice 30 days after aerosol challenge with *M. tuberculosis* Erdman (Fig. 2*A*), but not 20 days after infection (data not shown). No significant difference in IL-10 production measured 30 days after infection when cultured in the presence or absence of PPD was noted (Fig. 2*B*). Intracellular cytokine staining revealed that levels of IFN-γ production by lung cells corresponded with frequencies of CD4$^+$, IFN-γ$^+$ (Th1) T cells both 20 and 30 days after infection, with RIIB$^{-/-}$ mice having increased numbers of Th1 cells 30 days postchallenge (Fig. 2, *C* and *D*). No significant increase in CD8$^+$, IFN-γ$^+$ T cells was noted in RIIB$^{-/-}$ mice relative to wild-type (Fig. 2*E*). Thus, RIIB$^{-/-}$ C57BL/6 mice manifest increased levels of IFN-γ production and CD4$^+$, IFN-γ$^+$ cells in the lungs 30 days after aerosol challenge with *M. tuberculosis* Erdman, suggesting the development of a more robust Th1 response in the absence of FcγRIIB. This heightened Th1 immunity may account for the enhanced ability of RIIB$^{-/-}$ mice to control *M. tuberculosis* relative to wild-type controls.

FcγRIIB regulates levels of immune costimulatory molecules and IL-12p40 during M. tuberculosis *infection*

Heightened T cell responses as a result of FcγRIIB deficiency in autoimmune and tumor models correspond with elevations in costimulatory and MHC molecules as well as increased production of Th1 polarizing cytokines (26, 27, 34). The observed increase in T cell responsiveness in the RIIB$^{-/-}$ mice likely results from greater activation of APCs that have enhanced maturation and increased ability to stimulate T cells in the absence of inhibition by FcγRIIB. Consequently, we examined whether the enhanced Th1 responses in RIIB$^{-/-}$ mice were associated with heightened maturation of APCs as indicated by expression of the immune costimulatory molecules B7-1 (CD80) and B7-2 (CD86) as well as the protein complex responsible for peptide presentation to Th1 cells, MHC class II.

Indicating that APCs do achieve a greater state of activation during TB in the absence of FcγRIIB, RIIB$^{-/-}$ mice expressed elevated levels of B7-1, B7-2, and MHC class II molecules on the surface of lung cells by flow cytometry 30 days, but not 20 days, after *M. tuberculosis* Erdman challenge (Fig. 3*A*). Because FcγRIIB receptors are also known to influence the production of T cell-polarizing cytokines by APCs (26, 27, 34), we examined whether the absence of FcγRIIB affected levels of the Th1-polarizing molecule IL-12p40 produced by macrophages infected with *M. tuberculosis*. RIIB$^{-/-}$ macrophages produced significantly greater levels of IL-12p40 24 h after mycobacterial infection in the presence of IgG-OVA immune complex, but not in the absence of immune complex (Fig. 3*B*). Thus, the absence of FcγRIIB enhanced the expression of B7-1, B7-2, and MHC class II in the lungs of mice with TB as well as amplified the IL-12p40 response of *M. tuberculosis*-infected macrophages. This enhanced level of immune activation temporally corresponded with the heightened Th1 responses and enhanced mycobacterial containment noted 30 days after infection.

As another means of assessing the enhanced activity of APCs in RIIB$^{-/-}$ mice, we examined maturation and expansion of CD4$^+$ T cells locally within the lungs of mice with TB. CD45RB is downregulated on effector and memory CD4$^+$ T cells (35), and we used this marker as a measure of immune activation state. Fewer CD45RB$^+$CD4$^+$ T cells were detected in the lungs of RIIB$^{-/-}$ mice compared with wild-type mice (Fig. 3*C*), indicating a greater extent of immune activation in the FcγRIIB-deficient mice. To examine whether there was an increase in the general expansion of CD4$^+$CD45RB$^-$ T cells in RIIB$^{-/-}$ mice, we measured division of these cells using in vivo BrdU incorporation. Interestingly, there was no increase in the frequency of BrdU-incorporating CD4$^+$CD45RB$^-$ T cells between RIIB$^{-/-}$ mice and controls 30 days after *M. tuberculosis* challenge (Fig. 3*D*). These results suggest that FcγRIIB limits immune activation and Th1 polarization, but not the general expansion of CD4$^+$ T cells, during *M. tuberculosis* infection in C57BL/6 mice.

RIIB$^{-/-}$ mice have reduced pulmonary immunopathology 30 days after infection with M. tuberculosis

RIIB$^{-/-}$ mice have a diminished inductive threshold for immune activity and exacerbated inflammatory pathology in autoimmune models (36, 37). FcγRIIB inhibits excessive inflammation by limiting activity of leukocytes, including B cells, dendritic cells, macrophages, and NK cells (15, 16). Similar to autoimmunity, host pathology during TB is the product an excessive host response resulting from continuous inflammatory stimulation by persistent Ag. It is, in fact, the immunopathologic damage of an excessive host response during TB that is responsible for much of this disease's morbidity (38). These considerations, coupled with our observations that FcγRIIB-deficient mice have heightened Th1 responses and markers of immune activation led us to examine how FcγRIIB deficiency affected TB immunopathologic progression.

We challenged RIIB$^{-/-}$ and wild-type mice with *M. tuberculosis* Erdman by aerosol and analyzed lungs 30 days after infection, examining the extent of pulmonary inflammation in tissue sections and cell counts at a time in which the IFN-γ productive capacity of RIIB$^{-/-}$ mice was greater than that of wild-type mice (Fig. 2). Inspection of H&E stained lung sections taken 30 days after infection suggested that RIIB$^{-/-}$ mice had reduced pulmonary infiltrate compared with wild-type (Fig. 4*A*). Similarly, quantification of total lung cells 30 days after infection revealed that RIIB$^{-/-}$ mice had significantly fewer cells in the lungs at this time than wild-type (Fig. 4*B*). There were approximately one-third fewer cells in the left lung of infected RIIB$^{-/-}$ mice ($4.16 \times 10^7 \pm 4.65 \times 10^6$) compared with that detected in wild-type ($6.10 \times 10^7 \pm 4.90 \times 10^6$) ($p < 0.05$). Thus, corresponding with enhanced Th1 responses, RIIB$^{-/-}$ mice exhibited reduced pulmonary infiltrate during the acute phase of *M. tuberculosis* infection.

Increased pulmonary neutrophilia is associated with enhanced susceptibility and exacerbated inflammatory pathology in mouse models of TB (3, 39), and reduction in neutrophils correlates with TB treatment success in humans (40). Accordingly, we examined the levels of Ly6G$^+$, F4/80$^-$ neutrophils in the lungs of RIIB$^{-/-}$ and wild-type C57BL/6 mice upon airborne challenge with *M. tuberculosis*. Although no difference was noted between groups at days 11 or 20 postinfection, by 30 days after inoculation, RIIB$^{-/-}$

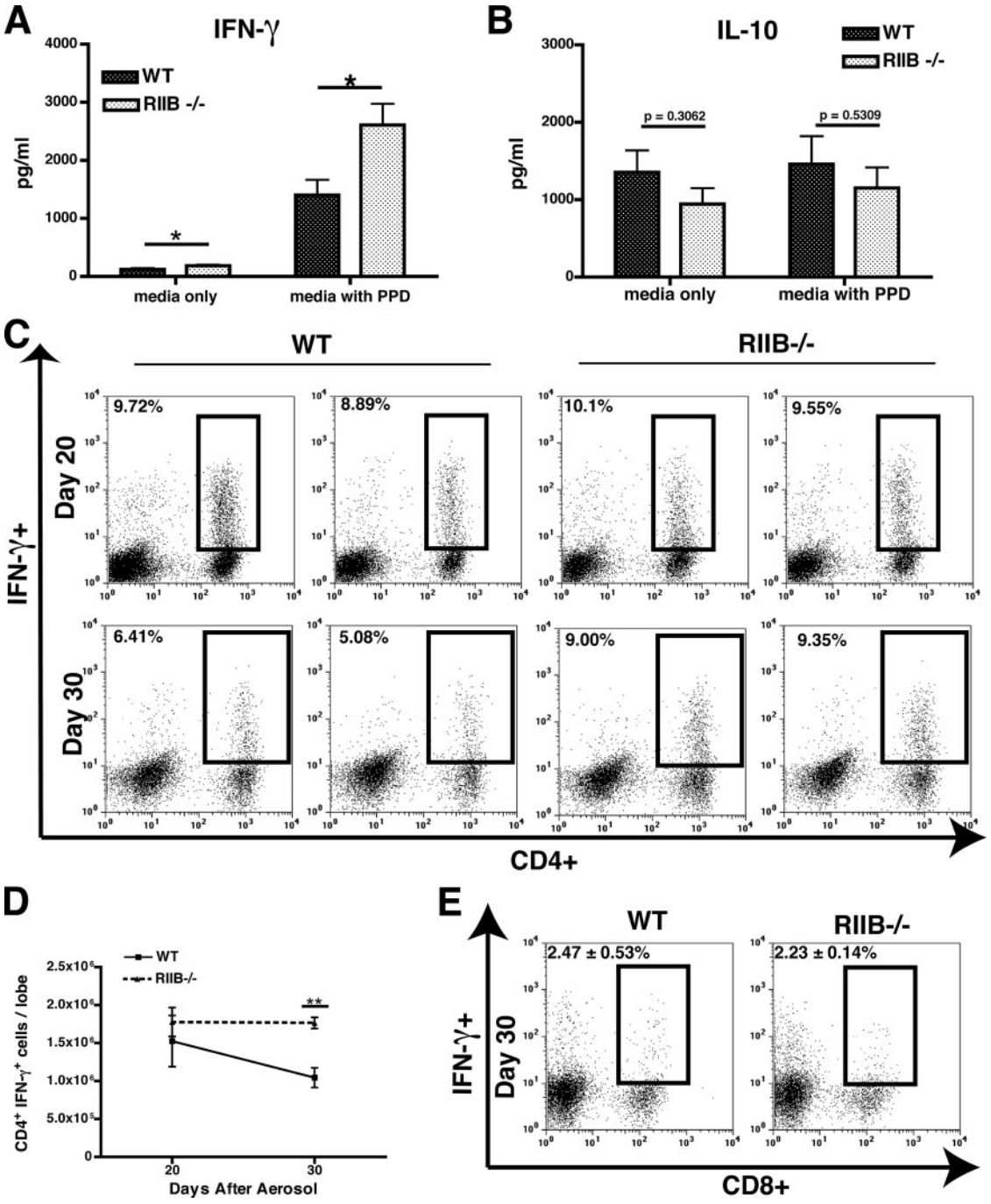

FIGURE 2. RIIB$^{-/-}$ mice have elevated IFN-γ responses in the lungs during TB. *A*, Ex vivo IFN-γ production by lung cells isolated 30 days after *M. tuberculosis* Erdman infection, cultured both in the presence and absence of PPD. *B*, Ex vivo IL-10 production by lung cells isolated 30 days after infection, cultured both in the presence and absence of PPD. *C*, Representative frequency of CD4$^+$ IFN-γ$^+$ T cells in lungs 20 and 30 days after infection. Data shown are from two representative mice per group. *D*, Calculated frequency of CD4$^+$ IFN-γ$^+$ T cells in lungs 20 and 30 days after infection. *E*, Representative dot plots of CD8$^+$ IFN-γ$^+$ T cells in lungs 30 days after infection, with mean and SE from $n = 3$ mice per group. Data are representative of three experiments ($n = 3$–5 mice per group). ∗, $p < 0.05$.

mice had significantly fewer pulmonary neutrophils compared with wild-type mice (Fig. 4*C*).

Progression of pulmonary TB in mice coincides with an increasing number of pulmonary B cells (41, 42). Consequently, we examined levels of B cells in the lungs of RIIB$^{-/-}$ mice during acute TB. CD19$^+$ B cells were significantly reduced in the lungs of RIIB$^{-/-}$ mice 30 days after infection (Fig. 4*D*). Interestingly, numbers of splenic B cells and levels of serum IgG in RIIB$^{-/-}$ mice were comparable to that in wild-type mice (data not shown), suggesting that FcγRIIB deficiency reduced pulmonary influx of B cells while maintaining similar global B cell activation as wild-type. Immunohistochemistry of lung sections cut 30 days after infection revealed that RIIB$^{-/-}$ mice had reductions in total abundance of B220$^+$ B cells and incidence of B cell aggregates characteristic of TB granulomatous pathology (Fig. 4*E*). Thus, despite the fact that RIIB$^{-/-}$ C57BL/6 mice had a heightened capacity of Th1 immunity in the lungs, deficiency of FcγRIIB did not exacerbate inflammation during TB,

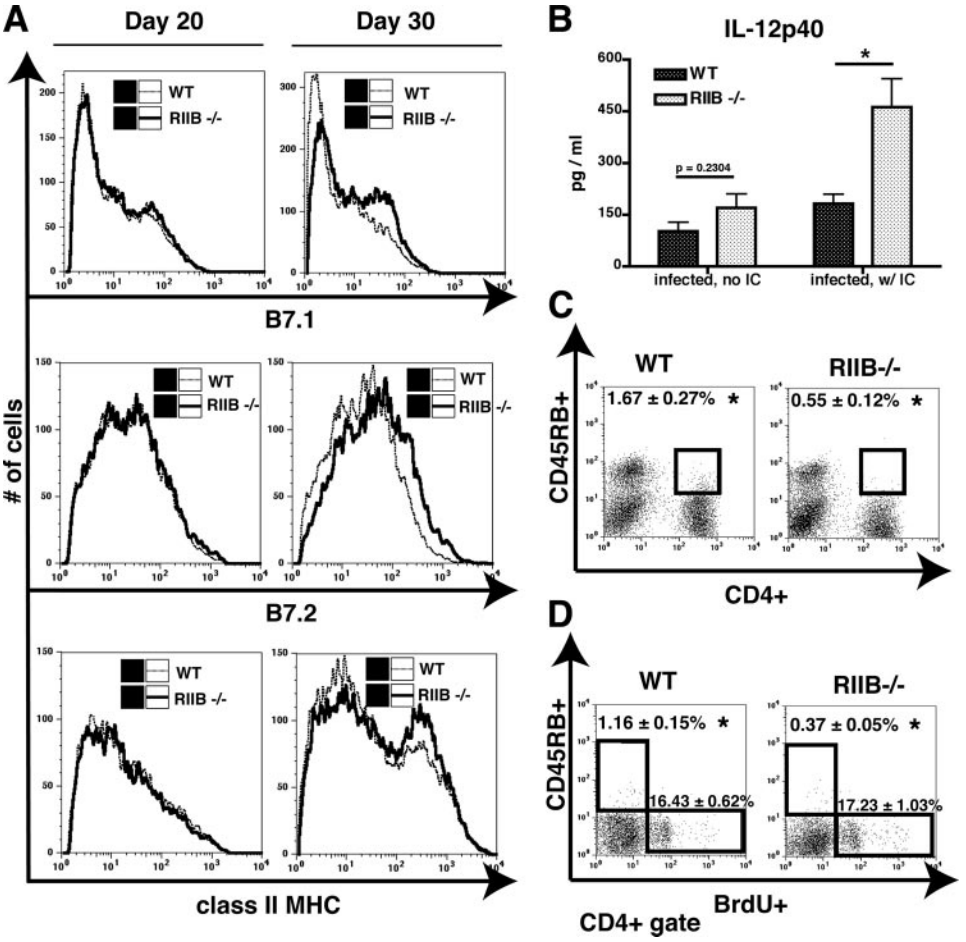

FIGURE 3. FcγRIIB regulates expression of costimulatory molecules and production of IL-12p40 during *M. tuberculosis* infection. *A*, Representative expression of B7-1, B7-2, and class II MHC 20 and 30 days after infection with *M. tuberculosis* Erdman. *B*, In vitro IL-12p40 production by bone marrow-derived macrophages infected with *M. tuberculosis*, in the presence or absence of IgG-OVA immune complex. *C*, Representative dot plots of $CD4^+CD45RB^+$ T cells in lungs after infection, with mean and SE ($n = 3$ mice per group). *D*, In vivo incorporation of BrdU 30 days after aerosol challenge, with mean and SE ($n = 3$ mice per group). Data are representative of two or three experiments ($n = 3$ or 4 mice per group). $*$, $p < 0.05$.

but conversely resulted in diminished immunopathology 30 days after infection, as evidenced by reduced cellular influx noted by immunohistochemistry and flow cytometry studies. To summarize, in the absence of the inhibitory FcγRIIB, *M. tuberculosis*-infected C57BL/6 mice exhibit reductions in organ mycobacterial burden (Fig. 1) that corresponded with the heightened Th1 responsiveness (Fig. 2), increased immune activation (Fig. 3), and reduced immunopathology (Fig. 4) noted 30 days after infection.

Mice deficient in the γ-chain shared by activation FcγR have increased susceptibility to M. tuberculosis *infection*

Although FcγRIIB is the only inhibitory FcγR, there are three known activation FcγR in mice: FcγRI, FcγRIII, and FcγRIV (16). The activation FcγR share a common γ-chain and, consequently, Fcγ-chain knockout (Fcγ-chain$^{-/-}$) mice have deficient function of the activation FcγR (43). We used Fcγ-chain$^{-/-}$ C57BL/6 mice to examine whether deficiency of activation FcγR function affected TB progression in mice.

Fcγ-chain$^{-/-}$ mice had diminished survival relative to controls upon aerosol challenge with *M. tuberculosis* Erdman (log-rank test $p < 0.05$) (Fig. 5*A*). The enhanced susceptibility of Fcγ-chain-deficient mice coincided with significantly elevated mycobacterial burden in the lungs and spleens 15, but not 3, weeks after challenge (Fig. 5*B*). Thus, the activation FcγR are required for optimal survival and bacterial containment during murine infection with *M. tuberculosis* Erdman.

C57BL/6 mice lacking FcγRIIB had heightened Th1 responses upon *M. tuberculosis* challenge (Fig. 2). Accordingly, we were curious as to whether deficiency of the activation FcγR would have a reciprocal effect and diminish Th1 responsiveness during TB. Surprisingly, no significant difference between Fcγ-chain$^{-/-}$ and wild-type C57BL/6 mice was noted in frequency of $CD4^+$, IFN-γ^+ T cells 30 days after infection (data not shown), coinciding with the finding that there was no significant increase in mycobacterial burden at approximately this same time (Fig. 5*B*). Examination of the frequency of IFN-γ-producing $CD4^+$ T cells by intracellular cytokine staining 5 mo after infection revealed that moribund Fcγ-chain$^{-/-}$ mice had significantly diminished frequency of Th1 T cells (Fig. 5*C*). In contrast, Fcγ-chain$^{-/-}$ mice not appearing moribund had comparable frequency of Th1 T cells with wild-type mice. Thus, mice deficient in activating FcγR can mount a comparable Th1 response upon *M. tuberculosis* challenge as wild type. However, Fcγ-chain$^{-/-}$ mice do not control chronic mycobacterial burden as well as wild-type mice, and manifest diminished Th1 frequency in the lungs corresponding with a moribund state. These results, together with those obtained by analysis of the response of FcγRIIB$^{-/-}$ mice to *M. tuberculosis* challenge, suggest that the inhibitory and the activating FcγR regulate distinct components of the host immune response to the tubercle bacillus and impact disease outcome.

Increased immunopathology and IL-10 production in Fcγ-chain$^{-/-}$ mice during M. tuberculosis *infection*

To address whether the heightened susceptibility of Fcγ-chain$^{-/-}$ mice was associated with changes in immunopathologic progression, we analyzed lung histopathology of infected mice. Whereas only moribund Fcγ-chain$^{-/-}$ mice had greatly worsened histopathology relative to wild-type 15 wk after infection, by 30 wk the increased level of inflammation was apparent in all Fcγ-chain$^{-/-}$

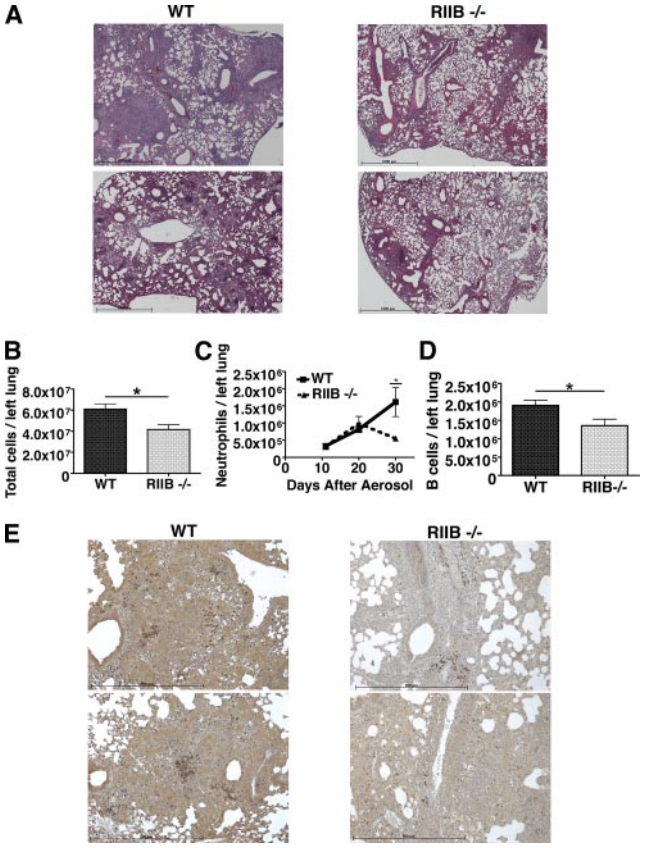

FIGURE 4. RIIB$^{-/-}$ mice have reduced immunopathology 30 days after *M. tuberculosis* infection. *A*, H&E stained lung sections cut 30 days after infection. *B*, Quantification of total cells in left lung 30 days after infection with *M. tuberculosis* Erdman. *C*, Quantification of Ly6G^{+} F4/80^{-} neutrophils in left lung at 11, 20, and 30 days after infection. *D*, Quantification by flow cytometry of CD19^{+} B cells in left lung 30 days after infection. *E*, B220 stained lung sections cut 30 days after infection. Sections and data displayed are representative of three similar experiments (n = 3–5 mice per group). ∗, $p < 0.05$.

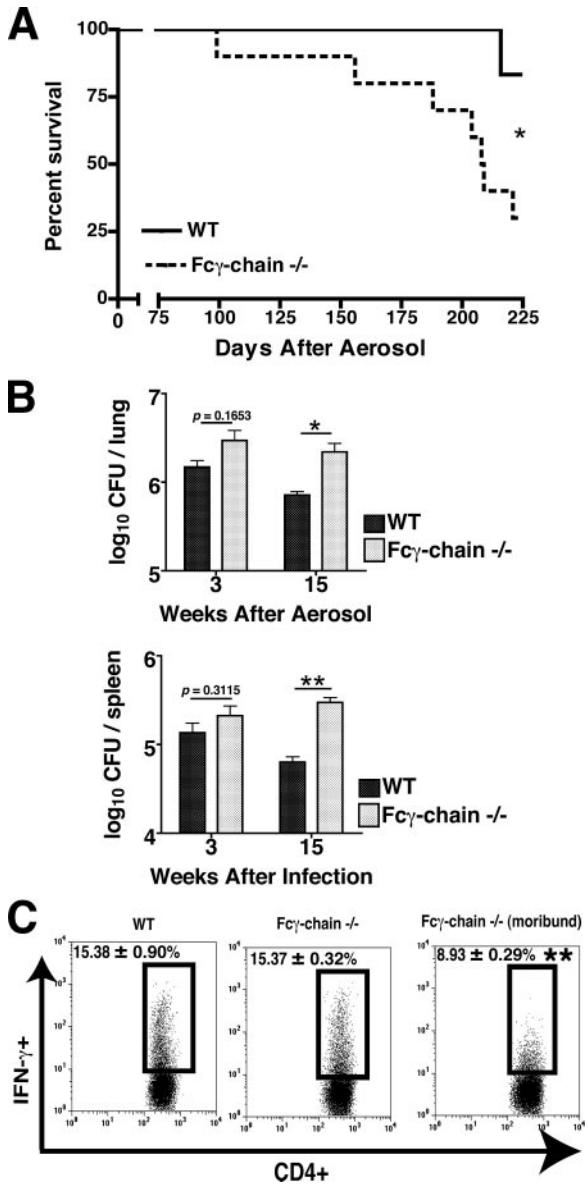

FIGURE 5. Activating FcγR are required for optimal survival and control of *M. tuberculosis*. *A*, Survival curve upon aerosol challenge with *M. tuberculosis* Erdman. Statistical significance established by log-rank test. *B*, Pulmonary and splenic bacterial burdens 3 and 15 wk after infection. *C*, Frequency of CD4^{+} IFN-γ^{+} T cells in lungs 150 days after infection (gated on CD4^{+} T cells), with mean and SE (n = 3–5 mice per group). Data are representative of three similar experiments. ∗, $p < 0.05$ ∗∗, $p < 0.01$.

mice (Fig. 6*A*). The enhanced level of inflammation observed in the Fcγ-chain$^{-/-}$ strain is not surprising because the lung bacterial burden in these mice was significantly elevated compared with that detected in wild-type mice. Because the number of neutrophils present in situ correlates well with the severity of tissue pathology in *M. tuberculosis* infection (38, 39), studies were undertaken to enumerate this immune cell in the lungs of infected Fcγ-chain$^{-/-}$ mice. Not unexpectedly, quantification of neutrophil influx into the lungs revealed a significant increase in Fcγ-chain$^{-/-}$ mice relative to wild-type mice up to at least 15 wk after infection (Fig. 6*B*). Surprisingly, this increase is apparent as early as 3 wk after inoculation, when the lung bacterial burden as well as immunopathology are comparable among the Fcγ-chain$^{-/-}$ and wild-type mice (Fig. 5*B* and data not shown). Thus, the absence of the Fcγ-chain predisposes C57BL/6 mice to have increased level of pulmonic neutrophils, immune cells known to exacerbate inflammation, which may therefore contribute to accelerated inflammatory progression during chronic TB relative to wild-type mice.

Previously, we have reported elevated IL-10 production associated with heightened immunopathology in B cell$^{-/-}$ mice upon 300 CFU aerosol challenge with *M. tuberculosis* Erdman (3). Moreover, it is known that FcγR engagement can influence IL-10 production in bone marrow macrophages (44). Thus, we examined whether activation FcγR deficiency would affect IL-10 levels in the lungs that have been shown to display increased levels of inflammation. IL-10 production was elevated in PPD stimulated ex vivo cultures of Fcγ-chain$^{-/-}$ lung cells up to 15 wk after infection (Fig. 6*C*). Coinciding with an elevated production of IL-10, cell surface expression of the IL-10R was up-regulated in Fcγ-chain$^{-/-}$ lungs relative to wild-type mice (Fig. 6*D*). In contrast, no difference in IL-10R expression was noted in the lungs of RIIB$^{-/-}$ mice relative to wild-type mice (data not shown), demonstrating that levels of IL-10 and its receptor were affected by deficiency in the Fcγ-chain but not FcγRIIB.

In addition to diminishing survival and elevating mycobacterial burden, the absence of activation FcγR resulted in accelerated inflammatory progression and a propensity toward increased production of IL-10 and its receptor in TB lungs. These data strongly suggest that IL-10 signaling is remarkably enhanced in the lungs of

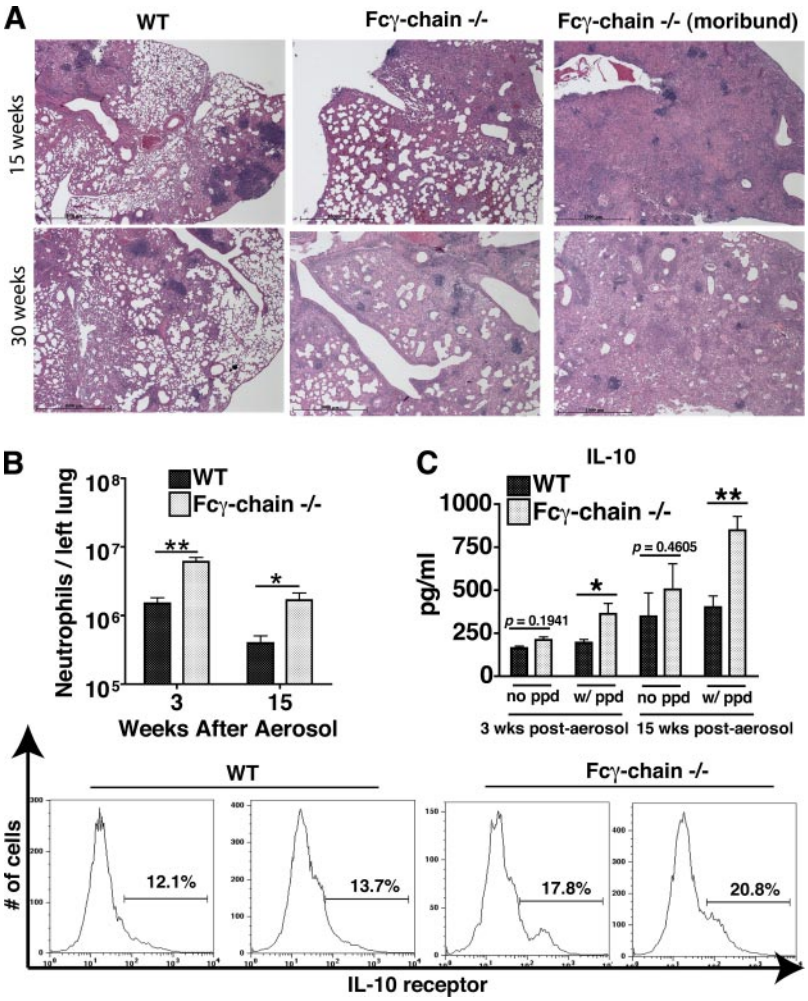

FIGURE 6. Increased immunopathology and IL-10 production in lungs of Fcγ-chain$^{-/-}$ mice during *M. tuberculosis* infection. *A*, H&E stained lung sections cut 15 or 30 wk after aerosol infection with *M. tuberculosis*. *B*, Quantification of Ly6G^{+} F4/80^{-} neutrophils in left lung 3 and 15 wk after infection. *C*, Ex vivo IL-10 production by lung cells isolated 3 and 15 wk after infection, in the presence of absence of PPD. *D*, Cell surface expression of the IL-10R on lung cells 5 mo after infection. Data are representative of two similar experiments. *, $p < 0.05$; **, $p < 0.01$.

M. tuberculosis-infected Fcγ-chain$^{-/-}$ mice. Surprisingly, the increased pulmonic expression of IL-10 in Fcγ-chain$^{-/-}$ mice was already apparent by 3 wk after infection, at a time when levels of lung immunopathology and bacterial burden are similar to that observed in infected wild-type strains. These data suggest that the activation FcγR regulate IL-10 production in the lung of tuberculous C57BL/6 mice independent of the level of inflammation. Based on the immunosuppressive effect of IL-10, and its ability to down-regulate antimycobacterial activity in TB mice, it is possible that the enhanced production of this cytokine in the lungs of Fcγ-chain$^{-/-}$ mice contribute to their enhanced susceptibility to *M. tuberculosis* (Fig. 5, *A* and *B*).

Discussion

Ab-mediated protection is a hallmark of vaccination and long-term immunity against infectious agents (45), yet very little is understood regarding the humoral arm of the immune response during TB. Knowing that a substantial Ab response is mounted during *M. tuberculosis* infection (23, 24) and that adoptively transferred B cells confer protection to B cell$^{-/-}$ mice (3), we were interested in understanding whether humoral immunity regulated the well-studied protective cellular immune response during TB. Engagement of Ab complexes regulates activity and maturation of leukocytes via FcγR, with broad effects upon the host response including activation of T lymphocytes by APCs (14–16). Accordingly, targeting FcγR is an intriguing therapeutic and vaccination strategy against intracellular pathogens, like mycobacteria, that may be contained by effective cellular immunity (46). Therefore, we used gene-deleted mice to study the roles of activation and inhibition FcγR, individually, upon TB progression in mice.

Coinciding with heightened IFN-γ responses, increased immune activation, and diminished immunopathology, RIIB$^{-/-}$ mice had significant reductions in mycobacterial burden in the lungs and spleens 30 days after *M. tuberculosis* Erdman infection. Other reports have previously implicated FcγR as affecting host defense in infectious disease models (17–22, 47). Interestingly, lupus-associated polymorphisms in FcγRIIB have been correlated with reduced susceptibility to malaria in humans (21), suggesting that infectious diseases may provide selective pressure for autoimmunity.

It has previously been demonstrated with in vivo murine models and in vitro human studies that the absence of FcγRIIB leads to greater activation of APCs and heightened T cell responses (26, 27, 34). Thus, our observations of enhanced IFN-γ production and increased frequency of Th1 T cells in TB lungs of RIIB$^{-/-}$ mice corroborate these findings. The correlation of reduced mycobacterial burden with heightened Th1 responses in RIIB$^{-/-}$ mice is not surprising given the well-established role of IFN-γ in immunity against *M. tuberculosis* (30–33). B7 and class II MHC levels were elevated relative to wild-type levels in RIIB$^{-/-}$ lungs 30 days after infection, demonstrating that increased Th1 responses correlated with elevated activation of APCs. Of interest, reductions in mycobacterial burden, increases in Th1 responses, and up-regulation of activation markers of APCs in RIIB$^{-/-}$ mice are all apparent 30, but not 20 days, after aerosol challenge. We hypothesize that this delay follows a time course of humoral feedback regulation

upon T cell immunity, and it may take several weeks for such effects upon adaptive immunity to manifest.

Increased production of IL-12 in the absence of FcγRIIB has also been reported previously (26, 27). IL-12 and IL-23 share the common subunit IL-12p40 and both promote the polarization of naive $CD4^+$ T cells into Th1 effectors (48). Thus, enhanced IL-12p40 production in the absence of FcγRIIB supports the observation of increased Th1 responses noted in $RIIB^{-/-}$ mice. Lower expression of CD45RB on $CD4^+$ T cells in TB lungs of the receptor-deficient C57BL/6 mice relative to controls may indicate a greater extent of T cell activation (31). The increase in Th1 cells noted in TB lungs of $RIIB^{-/-}$ mice was not associated with a similar increase in BrdU incorporation. This observation could be due to more effective recruitment of IFN-γ-producing $CD4^+$ T cells from lymph nodes to the lungs of infected $RIIB^{-/-}$ mice. Alternatively, the result may be indicative of selective Th1 effector polarization or perpetuation locally in the lungs of $RIIB^{-/-}$ mice, rather than a general increase in proliferation of $CD4^+$ T cells. Indeed, the results of enhanced pulmonic expression of IFN-γ as well as costimulatory and MHC class II molecules by *M. tuberculosis*-infected $RIIB^{-/-}$ mice suggests that the local environment in the lungs of the FcγRIIB-deficient strain is conducive to fostering Th1 immunity. A similar phenomenon has been demonstrated for the pulmonary pathogen *Aspergillus fumigatus*, where incremental $CD4^+$ effector T cell commitment involves Th1 differentiation locally within the lungs (49). By limiting levels of B7-1, B7-2, and MHC class II as well as IL-12p40, FcγRIIB may curb Th1 immunity within the lungs of infected C57BL/6 mice, consequently allowing more mycobacteria to persist.

TB immunopathology results from cumulative inflammatory damage by the host response against a persistent pathogen (38, 50). Thus, we were curious to know whether absence of inhibitory FcγRIIB exacerbates immunopathology upon *M. tuberculosis* challenge, especially considering our findings of increased Th1 responsiveness in lungs of these mice, which can enhance tissue inflammation. Surprisingly, we noted that pathology appeared to be improved in $RIIB^{-/-}$ mice 30 days after infection, as evidenced by histopathologic examination, and by immunohistochemistry and flow cytometric studies that revealed diminished pulmonary infiltrate as well as fewer neutrophils and B cells in the lungs, two leukocyte subsets associated with worsened TB pulmonary immunopathology (39–42, 51). It is conceivable that diminished inflammation and leukocyte influx in the lungs of $RIIB^{-/-}$ mice is a result of their ability to mount a more efficient Th1 response than wild-type C57BL/6 mice. This enhanced pulmonary response against *M. tuberculosis* can be predicted to require fewer cells to achieve mycobacterial containment, resulting in the decreased cellularity and inflammation of $RIIB^{-/-}$ lungs. It is important to note that TB immunopathology in $RIIB^{-/-}$ mice was not assessed beyond the chronic phase of infection, because these mice spontaneously develop autoimmunity as they age (29), thus introducing an additional confounding variable that would make data interpretation difficult, if not impossible. Although it remains to be confirmed and further analyzed, the result of a preliminary study has revealed that in the chronic phase of infection, *M. tuberculosis*-infected $RIIB^{-/-}$ C57BL/6 mice exhibited increased mortality compared with wild-type controls (P.J. Maglione and J. Chan, unpublished results). Detailed analysis of the role of FcγRIIB in regulating the host immune response to the tubercle bacillus in the chronic phase of tuberculosis will require studying mice that do not develop aging-related autoimmunity in the absence of this receptor, such as the BALB/c strain (29).

Fcγ-chain-deficient C57BL/6 mice succumb to infection with *M. tuberculosis* quicker than wild-type controls. Deficiency of the Fcγ-chain was previously reported to affect the progression of infection with other intracellular pathogens, including influenza and *Leishmania* species (17–22). Diminished survival was associated with statistically significantly elevated bacterial burden in the lungs and spleens 15 wk, but not 3 wk, after infection. The enhanced susceptibility of Fcγ-$chain^{-/-}$ mice may not be apparent 3 wk after infection because this time point may be too early to detect the inhibitory effects of humoral immunity via selective FcγRIIB-mediated feedback. Moreover, changes in Ab isotype predominance, which can influence affinity for activating or inhibiting FcγR (16), and FcγR expression kinetics may influence the impact of activation FcγR deficiency. The important information that is pertinent to the regulation of the host response by the interaction of Ab complex and FcγR during *M. tuberculosis* remains to be determined.

It is difficult to draw definitive conclusions from our finding that the frequency of $CD4^+$ T cells in lungs producing IFN-γ is diminished in moribund, but remains similar to wild-type in healthier Fcγ-$chain^{-/-}$ mice. Diminished Th1 responsiveness in the lungs of moribund Fcγ-$chain^{-/-}$ mice may be the cause of the heightened susceptibility and increased mycobacterial burden of these mice, especially given the finding that Th1 immunity is elevated in conjunction with improved mycobacterial control in $RIIB^{-/-}$ mice. However, it is also possible that waning Th1 immunity in Fcγ-$chain^{-/-}$ mice may simply be a characteristic of multiple organ failure in moribund mice.

Our data demonstrated that Fcγ-$chain^{-/-}$ lung cells, when stimulated with PPD ex vivo 3 or 15 wk after infection, produced elevated levels of IL-10 relative to wild-type levels. The inability to contain infection in Fcγ-$chain^{-/-}$ mice may be related to the increased IL-10 response in these mice, as IL-10 is an immunosuppressive cytokine that can subvert optimal *M. tuberculosis* containment (52, 53). Although the impact of IL-10 elevations in Fcγ-chain-deficient mice awaits further investigation, it is possible that the absence of the Fcγ-chain predisposes FcγR-bearing leukocytes, such as dendritic cells and macrophages, to produce higher levels of this cytokine as well as promote the polarization of a greater number of IL-10-producing lymphocytes compared with wild-type mice. Up-regulation of the IL-10R in the lungs of Fcγ-$chain^{-/-}$ mice relative to wild-type C57BL/6 mice further indicates the more extensively IL-10-dominated microenvironment of these mice during TB. Thus, the absence of inhibitory FcγRIIB enhances IFN-γ production, whereas deficiency in the activation FcγR conversely affects immune activation such that IL-10 is increased. Our efforts to identify the specific immune cells producing IL-10 (such as regulatory T cells) by intracellular cytokine staining have failed, this is likely indicative of the notorious difficulty in measuring this cytokine in situ. In fact, the difficulty in detecting IL-10 production in vivo has motivated the development of mouse models allowing more sensitive tracking of IL-10-producing cells (54, 55).

Worsened lung pathology and increased pulmonary influx of neutrophils indicate that Fcγ-$chain^{-/-}$ mice have inflammatory exacerbation coinciding with enhanced susceptibility. As discussed concerning the $RIIB^{-/-}$ mice, less efficient immunity results in greater immunopathology, as more leukocytes are required in the lungs to contain infection. By this logic, recruitment of leukocytes to the lungs would be intensified in Fcγ-$chain^{-/-}$ mice to control *M. tuberculosis* because Fcγ-chain deficiency compromises containment, as evidenced by increased mycobacterical burdens 15 wk after infection. The increased bacterial burden may itself contribute to the enhanced immunopathology observed in Fcγ-$chain^{-/-}$ mice by providing greater antigenic stimulus of inflammation. Future experiments are required to determine which

factors, in the absence of the Fcγ-chain, determine the stimulus for inflammatory exacerbation.

Building upon our previous work (3), this study demonstrates specific mechanisms by which humoral immunity influences TB cellular immunity. An immune-inhibitory receptor that limits the development of autoimmune disease, FcγRIIB also subverts optimal immunity against *M. tuberculosis* by limiting Th1 activation and consequential mycobacterial containment by regulating the capacity of APCs to polarize T cells. Activating FcγR conversely promote optimal immune activation, as impaired mycobacterial containment and heightened disease susceptibility occurred in their absence, corresponding with polarization toward a less protective IL-10 response. Hence, activating and inhibiting FcγR have reciprocal functions during *M. tuberculosis* Erdman infection of C57BL/6 mice, with opposing effects upon immune activation and TB vulnerability. Activation of distinct cytokine responses are dependent upon signals mediated by Abs via FcγR, with profound effects upon tissue mycobacterial burden, inflammatory progression, and disease outcome during *M. tuberculosis* infection of C57BL/6 mice.

Acknowledgments

We thank Matthew Scharff for critically reading portions of this manuscript and establishing mouse colonies used in these studies, Al Watford for assistance with mouse breeding, and Laureen Ojalvo for valuable discussion.

Disclosures

The authors have no financial conflict of interest.

References

1. Glatman-Freedman, A., and A. Casadevall. 1998. Serum therapy for tuberculosis revisited: reappraisal of the role of antibody-mediated immunity against *Mycobacterium tuberculosis*. *Clin. Microbio. Rev.* 11: 514–532.
2. Johnson, C. M., A. M. Cooper, A. A. Frank, C. B. Bonorino, L. J. Wysoki, and I. M. Orme. 1997. *Mycobacterium tuberculosis* aerogenic rechallenge infections in B cell-deficient mice. *Tuber. Lung. Dis.* 78: 257–261.
3. Maglione, P. J., J. Xu, and J. Chan. 2007. B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *J. Immunol.* 178: 7222–7234.
4. Bosio, C. M., D. Gardener, and K. L. Elkins. 2000. Infection of B cell-deficient mice with CDC 1551, a clinical isolate of *Mycobacterium tuberculosis*: delay in dissemination and development of lung pathology. *J. Immunol.* 164: 6417–6425.
5. Vordermeier, H. M., N. Venkataprasad, D. P. Harris, and J. Ivanyi. 1996. Increase of tuberculosis infection in the organs of B cell-deficient mice. *Clin. Exp. Immunol.* 106: 312–316.
6. Teitelbaum, R., A. Glatman-Freedman, B. Chen, J. B. Robbins, E. Unanue, A. Casadevall, and B. R. Bloom. 1998. A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. *Proc. Natl. Acad. Sci. USA* 95: 15688–15693.
7. Reljic, R., and J. Ivanyi. 2006. A case for passive immunoprophylaxis against tuberculosis. *Lancet Infect. Dis.* 6: 813–818.
8. Hamasur, B., M. Haile, A. Pawlowksi, U. Schroder, G. Kallenius, and S. B. Svenson. 2004. A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab′) fragment prolong survival of mice infected with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* 138: 30–38.
9. Hamasur, B., M. Haile, A. Pawlowski, U. Schröder, A. Williams, G. Hatch, G. Hall, P. Marsh, G. Källenius, and S. B. Svenson. 2003. *Mycobacterium tuberculosis* arabinomannan-protein conjugates protect against tuberculosis. *Vaccine* 21: 4081–4093.
10. Bayry, J., S. Lacroix-Desmazes, M. D. Kazatchkine, O. Hermine, D. F. Tough, and S. V. Kaveri. 2005. Modulation of dendritic cell maturation and function by B lymphocytes. *J. Immunol.* 175: 15–20.
11. Amigorena, S., and C. Bonnerot. 1999. Fc receptor signaling and trafficking: a connection for antigen processing. *Immunol. Rev.* 172: 279–284.
12. Casadevall, A., and L. A. Pirofski. 2006. A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. *Adv. Immunol.* 91: 1–44.
13. Radaev, S., and P. Sun. 2002. Recognition of immunoglobulins by Fcγ receptors. *Mol. Immunol.* 38: 1073–1083.
14. Bajtay, Z., E. Csomor, N. Sandor, and A. Erdei. 2006. Expression and role of Fc- and complement-receptors on human dendritic cells. *Immunol. Lett.* 104: 46–52.
15. Bolland, S., and J. V. Ravetch. 2001. IgG Fc receptors. *Annu. Rev. Immunol.* 19: 275–290.
16. Nimmerjahn, F., and J. V. Ravetch. 2006. Fcgamma receptors: old friends and new family members. *Immunity* 24: 19–28.
17. Moore, T., G. A. Ananaba, J. Bolier, S. Bowers, T. Belay, F. O. Eko, and J. U. Igietseme. 2002. Fc receptor regulation of protective immunity against *Chlamydia trachomatis*. *Immunology* 105: 213–221.
18. Huber, V. C., J. M. Lynch, D. J. Bucher, J. Le, and D. W. Metzger. 2001. Fc receptor-mediated phagocytosis makes a significant contribution to clearance of influenza virus infections. *J. Immunol.* 166: 7381–7388.
19. Kima, P. E., S. L. Constant, L. Hannum, M. Colmenares, K. S. Lee, A. M. Haberman, M. J. Shlomchik, and D. McMahon-Pratt. 2000. Internalization of *Leishmania mexicana* complex amastigotes via the Fc receptor is required to sustain infection in murine cutaneous leishmaniasis. *J. Exp. Med.* 191: 1063–1068.
20. Woelbing, F., S. L. Kostka, K. Moelle, Y. Belkaid, C. Sunderkoetter, S. Verbeek, A. Waisman, A. P. Nigg, J. Knop, M. C. Udey, and E. von Stebut. 2006. Uptake of *Leishmania major* by dendritic cells is mediated by Fcγ receptors and facilitates acquisition of protective immunity. *J. Exp. Med.* 203: 177–188.
21. Clatworthy, M. R., L. Willcocks, B. Urban, J. Langhorne, T. N. Williams, N. Peshu, N. A. Watkins, R. A. Floto, and K. G. Smith. 2007. Systemic lupus erthematosus-associated defects in the inhibitory receptor FcγRIIb reduce susceptibility to malaria. *Proc. Natl. Acad. Sci. USA* 104: 7169–7174.
22. Uppington, H., N. Menager, P. Boross, J. Wood, M. Sheppard, S. Verbeek, and P. Mastroeni. 2006. Effect of immune serum and role of individual Fcγ receptors on the intracellular distribution and survival of *Salmonella enterica serovar Typhimurium* in murine macrophages. *Immunology* 119: 147–158.
23. Greenaway, C., C. Lienhardt, R. Adegbola, P. Brusasca, K. McAdam, and D. Menzies. 2005. Humoral response to *Mycobacterium tuberculosis* antigens in patients with tuberculosis in the Gambia. *Int. J. Tuberc. Lung Dis.* 9: 1112–1119.
24. Davidow, A., G. V. Kanaujia, L. Shi, J. Kaviar, X. Guo, N. Sung, G. Kaplan, D. Menzies, and M. L. Gennaro. 2005. Antibody profiles characteristic of *Mycobacterium tuberculosis* infection state. *Infect. Immun.* 73: 6846–6851.
25. Song, X., S. Shapiro, D. L. Goldman, A. Casadevall, M. Scharff, and S. C. Lee. 2002. Fcγ receptor I- and III-mediated macrophage inflammatory protein 1α induction in primary human and murine microglia. *Infect. Immun.* 70: 5177–5184.
26. Kalergis, A. M., and J. V. Ravetch. 2002. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. *J. Exp. Med.* 12: 1653–1659.
27. Dhodapkar, K. M., J. L. Kaufman, M. Ehlers, D. K. Banerjee, E. Bonvini, S. Koenig, R. M. Steinman, J. V. Ravetch, and M. V. Dhodapkar. 2005. Selective blockade of inhibitory Fcγ receptor enables human dendritic cell maturation with IL-12p70 production and immunity to antibody-coated tumor cells. *Proc. Natl. Acad. Sci. USA* 102: 2910–2915.
28. Mogues, T., M. E. Goodrich, L. Ryan, R. LaCourse, and R. J. North. 2001. The relative importance of T cell subsets in immunity and immunopathology of airborne *Mycobacterium tuberculosis* infection in mice. *J. Exp. Med.* 193: 271–280.
29. Bolland, S., and J. V. Ravetch. 2000. Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity* 13: 277–285.
30. Flynn, J. L., J. Chan, K. J. Triebold, D. K. Dalton, T. A. Stewart, B. R. Bloom. 1993. An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178: 2249–2254.
31. Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme. 1993. Disseminated tuberculosis in interferon γ gene-disrupted mice. *J. Exp. Med.* 178: 2243–2244.
32. Doffinger, R., S. Y. Patel, and D. S. Kumararatne. 2006. Host genetic factors and mycobacterial infections: lessons from single gene disorders affecting innate and adaptive immunity. *Microbes Infect.* 8: 1141–1150.
33. Condos, R., W. N. Rom, and N. W. Schluger. 1997. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-γ via aerosol. *Lancet* 349: 1513–1515.
34. Desai, D. D., S. O. Harvers, M. Flores, L. Colonna, M. P. Downie, A. Bergtold, S. Jung, and R. Clynes. 2007. Fcγ receptor IIB on dendritic cells enforces peripheral tolerance by inhibiting effector T cell responses. *J. Immunol.* 178: 6217–6226.
35. Blander, J. M., D. B. Sant'Angelo, D. Metz, S. W. Kim, R. A. Flavell, K. Bottomly, and C. A. Janeway Jr. 2003. A pool of central memory-like CD4 T cells contains effector memory precursors. *J. Immunol.* 170: 2940–2948.
36. Clynes, R., J. S. Maizes, R. Guinamard, M. Ono, T. Takai, and J. V. Ravetch. 1999. Modulation of immune complex-induced inflammation in vivo by the coordinate expression of activation and inhibitory Fc receptors. *J. Exp. Med.* 189: 179–186.
37. Petkova, S. B., K. N. Konstantinov, T. J. Sproule, B. L. Lyons, M. A. Awwami, and D. C. Roopenian. 2006. Human antibodies induce arthritis in mice deficient in the low-affinity inhibitory IgG receptor Fcγ RIIB. *J. Exp. Med.* 203: 275–280.
38. Dheda, K., H. Booth, J. F. Huggett, M. A. Johnson, A. Zumla, and G. A. Rook. 2005. Lung remodeling in pulmonary tuberculosis. *J. Infect. Dis.* 192: 1201–1209.
39. Eruslanov, E. B., I. V. Lyadova, T. K. Kondratieva, K. B. Majorov, I. V. Scheglov, M. O. Orlova, and A. S. Apt. 2005. Neutrophil responses to *Mycobacterium tuberculosis* infection in genetically susceptible and resistant mice. *Infect. Immun.* 73: 1744–1753.
40. Brahmbhatt, S., G. F. Black, N. M. Carroll, N. Beyers, F. Salker, M. Kidd, P. T. Lukey, K. Duncan, P. van Helden, and G. Walzl. 2006. Immune markers measured before treatment predict outcome of intensive phase tuberculosis therapy. *Clin. Exp. Immunol.* 146: 243–252.
41. Turner, J., A. A. Frank, J. V. Brooks, M. Gonzalez-Juarrero, and I. M. Orme. 2001. The progression of chronic tuberculosis in the mouse does not require the participation of B lymphocytes or interleukin-4. *Exp. Gerontol.* 36: 537–545.

42. Tsai, M. C., S. Chakravarty, G. Zhu, J. Xu, K. Tanaka, C. Koch, J. Tufariello, J. Flynn, and J. Chan. 2006. Characterization of the tuberculosis granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. *Cell Microbiol.* 8: 218–232.
43. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J. V. Ravetch. 1994. FcRγ chain deletion results in pleiotrophic effect cell defects. *Cell* 76: 519–529.
44. Gerber, J. S., and D. M. Mosser. 2001. Reversing lipopolysaccharide toxicity by ligating the macrophage Fcγ receptors. *J. Immunol.* 166: 6861–6868.
45. Gourley, T. S., E. J. Wherry, D. Masopust, and R. Ahmed. 2004. Generation and maintenance of immunological memory. *Semin. Immunol.* 16: 323–333.
46. Igietseme, J. U., F. O. Eko, Q. He, and C. M. Black. 2003. Antibody regulation of T-cell immunity: implications for vaccine strategies against intracellular pathogens. *Expert Rev. Vaccines* 3: 23–34.
47. Saeland, E., J. H. Leusen, G. Vidarsson, W. Kuis, E. A. Sanders, I. Jonsdottir, and J. G. van de Winkel. 2003. Role of leukocyte immunoglobulin G receptors in vaccine-induced immunity to *Streptococcus pneumoniae*. *J. Infect. Dis.* 187: 1686–1693.
48. Watford, W. T., M. Moriguchi, A. Morinobu, and J. J. O'Shea. 2003. The biology of IL-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev.* 14: 361–368.
49. Rivera, A., G. Ro, H. L. Van Epps, T. Simpson, I. Leiner, D. B. Sant'Angelo, and E. G. Pamer. 2006. Innate immune activation and $CD4^+$ T cell priming during respiratorhy fungal infection. *Immunity* 25: 665–675.
50. Casadevall, A., and L. A. Pirofski. 2003. The damage-response framework of microbial pathogenesis. *Nat. Rev. Microbiol.* 1: 17–24.
51. Ulrichs, T., G. A. Kosmiadi, V. Trusov, S. Jorg, L. Pradl, M. Titukhina, V. Mishenko, N. Gushina, and S. H. Kaufmann. 2004. Human tuberculosis granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defense in the lung. *J. Pathol.* 204: 217–228.
52. Turner, J., M. Gonzalez-Juarrero, D. L. Ellis, R. J. Basaraba, A. Kipnis, I. M. Orme, and A. M. Cooper. 2002. In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J. Immunol.* 169: 6343–6351.
53. Redpath, S., P. Ghazal, and N. R. Gascoigne. 2001. Hijacking and exploitation of IL-10 by intracellular pathogens. *Trends Microbiol.* 9: 86–92.
54. Kamanaka, M., S. T. Kim, Y. Y. Wan, F. S. Sutterwala, M. Lara-Tejero, J. E. Galan, E. Harhaj, and R. A. Flavell. 2006. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 25: 941–952.
55. Maynard, C. L., L. E. Harrington, K. M. Janowski, J. R. Oliver, C. L. Zindl, A. Y. Rudensky, and C. T. Weaver. 2007. Regulatory T cells expressing interleukin 10 develop from $FoxP3^+$ and $FoxP3^-$ precursor cells in the absence of interleukin 10. *Nat. Immunol.* 8: 931–941.