The Journal of Immunology

RESEARCH ARTICLE | SEPTEMBER 01 2000

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J Immunol (2000) 165 (5): 2596–2602.
https://doi.org/10.4049/jimmunol.165.5.2596

Mycobacterium tuberculosis Infection in Complement Receptor 3-Deficient Mice[1]

Chenggang Hu,* Tanya Mayadas-Norton,† Katie Tanaka,‡ John Chan,§ and Padmini Salgame[2]*

Complement receptor type 3 (CR3) present on macrophages is used by *Mycobacterium tuberculosis* as one of its major phagocytic receptors. In this study, we examined the in vivo significance of CR3-mediated phagocytosis on the pathogenesis of disease caused by *M. tuberculosis*. The outcome of tuberculous infection in mice deficient in the CD11b subunit of CR3 ($CR3^{-/-}$) on a mixed 129SV and C57BL background and control wild-type counterparts was comparable with respect to survival, bacterial burden, granulomatous lesion development, and cytokine expression in the spleen and lungs. *M. tuberculosis* infection was also examined in $CR3^{-/-}$ mice on C57BL/6 and BALB/c backgrounds and was found to be similar. In conclusion, our results suggest that in the absence of CR3, *M. tuberculosis* is able to gain entry into host cells via alternative phagocytic receptors and establish infection. The data also indicate that absence of CR3 does not alter disease course in either the relatively resistant C57BL/6 or the relatively susceptible BALB/c strains of mice. ***The Journal of Immunology*, 2000, 165: 2596–2602.**

Tuberculosis remains a leading cause of mortality worldwide, accounting for three million annual deaths (1, 2). *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a facultative intracellular pathogen, which uses macrophages as its primary host cell. Therefore, invasion of macrophages by the tubercle bacillus is critical in the establishment of tuberculous infection; consequently, *M. tuberculosis* has evolved several strategies to parasitize its host cell. Multiple distinct macrophage receptors have the potential to recognize and bind *M. tuberculosis* in vitro (reviewed in Ref. 3); these include complement receptors (CRs)[3] 1, 3, and 4; mannose receptor (4–6) CD14 (7), surfactant protein A receptors (8), and scavenger receptors.

Two structurally different forms of CRs are present on macrophages. CR1 is a monovalent transmembrane protein (9), and CR3 and CR4 are heterodimeric proteins that belong to the integrin superfamily. Both CR3 and CR4 share a common β-chain (CD18) and contain distinct α-chains (CD11b and CD11c) (10). *M. tuberculosis* can activate the alternative pathway of the complement and become opsonized with C3b and iC3b, the degradation products of complement component C3 (11). The ligands C3b and iC3b allow the bacteria to be recognized by CR1 and CR3/CR4, respectively. *M. tuberculosis* and other pathogenic mycobacteria use yet another mechanism, a property that is not shared by other intramacrophage pathogens, for obtaining opsonic C3 peptides. A cell wall component of mycobacteria can associate with complement fragment C2a to form a C3 convertase and produce opsonic C3b (12). Thus *M. tuberculosis* has contrived several mechanisms for macrophage invasion that exploit the host CRs. In addition to the two opsonic modes of interactions of *M. tuberculosis* with the CR3 receptor, the tubercle bacillus also binds nonopsonically to CR3. The capsular polysaccharides of *M. tuberculosis* can interact with a β-glucan binding site on CR3 that is distinct from the C3bi binding site (13, 14).

In vitro studies suggest that CR3 is a predominant receptor for phagocytosis of *M. tuberculosis* by macrophages. A combination of two mAbs against CR3 significantly inhibited adherence of *M. tuberculosis* to monocytes (up to 82%) (11), suggesting that CR3 is the preferential phagocytic receptor for the tubercle bacillus. With the availability of mutant mice lacking a functional CR3, one can now examine the in vivo significance of CR3-mediated phagocytosis on the pathogenesis of tuberculosis. In this study, we assessed tuberculous infection in $CR3^{-/-}$ mice and compared it to their wild-type littermates. The results show that absence of CR3 did not alter disease pattern in either the relatively resistant C57BL/6 or the relatively susceptible BALB/c strain of mice.

Materials and Methods

Mice

CR3 null mice ($CR3^{-/-}$) generated by disrupting the gene that encodes the CD11b subunit of CR3, and their littermate wild-type controls ($CR3^{+/+}$) (15) were bred and maintained at the Longwood Medical Research Center of the Harvard Medical School (Boston, MA). For this study, mice were transferred to the Rodent Barrier Facility at Temple University School of Medicine (Philadelphia, PA). Throughout the study period, the mice were maintained under pathogen-free conditions, and the state of health was assessed by routine screening of sentinels for mouse pathogens. Sex-matched mice between 8 and 12 wk of age were used for all experiments described. IFN-$\gamma^{-/-}$ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The course of *M. tuberculosis* infection in the absence of CR3 was studied in three strains of mice. The first set of experiments was performed with $CR3^{-/-}$ mice and their wild-type littermates that were a mixed strain of 129SV and C57BL/6 ($CR3^{-/-}$.129SV/C57BL/6); the second set of experiments was performed in $CR3^{-/-}$ mice that had been backcrossed onto the C57BL/6 background for eight generations ($CR3^{-/-}$.C57BL/6); and the third set of experiments was performed in congenic BALB/c $CR3^{-/-}$ (eight backcrosses; $CR3^{-/-}$.BALB/c) and wild-type strains. All mice included in the study were CR3-genotyped.

*Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140; †Department of Pathology, Harvard Medical School, Boston, MA 02115; and Departments of ‡Pathology and §Medicine, Montefiore Medical Center, Bronx, NY 10461

Received for publication March 16, 2000. Accepted for publication June 7, 2000.

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[1] This work was supported by National Institutes of Health Grant HL55972 from the National Heart, Lung, and Blood Institute.

[2] Address correspondence and reprint requests to Dr. Padmini Salgame, Temple University School of Medicine, Department of Microbiology and Immunology, 3400 North Broad Street, Kresge Room 501, Philadelphia, PA 19140. E-mail address: salgame@astro.ocis.temple.edu

[3] Abbreviations used in this paper: CR, complement receptor; H&E, hematoxylin and eosin; RPA, RNase protection assay; MOI, multiplicity of infection; m.s.t., mean survival time; E-C3bi, C3bi-coated SRBC.

0022-1767/00/$02.00

Association of complement opsonized erythrocytes with CR3$^{+/+}$ and CR3$^{-/-}$ macrophages

Complement-opsonized erythrocytes were prepared as previously described (16). Briefly, SRBC were incubated with anti-SRBC IgM/k Abs (supernatant of hybridoma S-S.3 obtained from American Type Culture Collection, Manassas, VA) at nonagglutinating titers for 40 min at room temperature. The IgM-opsonized erythrocytes were washed and resuspended in HBSS containing 10% murine serum that was deficient in C5. Following a 15-min incubation at 37°C, the C3bi-coated SRBC (E-C3bi) were washed twice and resuspended at 4×10^8 cells/ml. CR3$^{-/-}$ or wild-type bone marrow-derived macrophages (1×10^5) prepared as previously described (16) were cultured for 4 h in chamber slides (Becton Dickinson, Franklin Lakes, NJ). E-C3bi (2×10^6) were added to the macrophage monolayers. After 15 or 30 min of incubation at 37°C, the monolayers were washed three times with PBS, fixed with 4% paraformaldehyde (in PBS), and stained. Two hundred macrophages were counted in each monolayer, and the percentage of E-C3bi-associated macrophages was calculated.

Phagocytosis of M. tuberculosis *by CR3$^{+/+}$ and CR3$^{-/-}$ macrophages*

Bone marrow-derived macrophages (1×10^5) from CR3$^{-/-}$ or wild-type mice were cultured for 4 h in chamber slides. The macrophage monolayers were infected with 1×10^6 *M. tuberculosis* and incubated for an additional 2 h, or in some cases for 4 h. All infections were performed in the presence of 2% mouse serum. At the end of the infection period, the monolayers were washed three times with PBS, fixed with 4% paraformaldehyde, and Ziehl-Neelsen stained for acid-fast bacilli. To assess the efficiency of phagocytosis by CR3$^{+/+}$ and CR3$^{-/-}$ macrophages, 200 macrophages were examined in each monolayer for the presence of acid-fast bacilli. Using a similar infection protocol, we have previously demonstrated by electron microscopic examination that the macrophage-associated tubercle bacilli after washing were virtually all internalized (17). The data are presented as a percentage of *M. tuberculosis*-infected macrophages.

Bacteria and mouse infection

The virulent *M. tuberculosis* Erdman strain (Trudeau Institute, Saranac Lake, NY) obtained after mouse passage was grown in culture, titered, and stored in aliquots at −70°C as previously described (18). Before infection, aliquots were thawed, resuspended in PBS with 0.05% Tween 80 to the appropriate concentrations, and briefly sonicated. Viable CFUs of *M. tuberculosis* (2×10^5) were then delivered i.v. in a volume of 100 μl via the lateral tail vein. Animal protocols used in the studies described have been approved by the Institutional Animal Care and Use Committee.

Quantitation of viable mycobacteria in tissues

At different time points after infection, lungs, spleens, and livers were harvested. For CFU quantitation, tissues were homogenized aseptically in PBS with 0.05% Tween 80, and the number of viable mycobacteria per organ was determined by plating onto 7H10 agar plates in serial dilutions (18). Because each organ was partitioned for various studies, tissue portions were harvested for particular study based on their anatomical location. The left lobe of the lungs and livers and the middle quarter of the spleens were used for determining CFUs.

Histology

At appropriate times after infection, mice were sacrificed and the livers, spleen, and lungs were removed aseptically. Parts of the organs were procured for histopathologic studies as previously described (18). Tissues were fixed in 10% buffered formalin for 24 h before paraffin embedment. Hematoxylin and eosin (H&E) and Ziehl-Neelsen acid-fast staining of 5-μm sections from paraffin blocks were used in the histological examination to access granuloma and mycobacterial load.

Cytokine assays

A portion of the lungs and spleen was homogenized in RNAzol B (Biotex Laboratories, Houston, TX), frozen quickly, and stored at −70°C. Total RNA was extracted according to the manufacturer's protocol. The RNase protection assay (RPA) was performed using RPA kit and mouse cytokine multiprobe set mCK2b (RiboQuant; PharMingen, San Diego, CA). Protected [^{32}P]UTP-labeled probes were resolved on a 6% polyacrylamide gel and analyzed by autoradiography. The expression of specific genes was quantitated by phosphorimaging relative to the abundance of housekeeping genes GAPDH or L32. For IFN-γ ELISAs, 2×10^6 spleen cells were washed and stimulated in 24-well tissue culture plates in the presence of an equal number of *M. tuberculosis*. Supernatants from the stimulated cells were harvested 72 h later, and IFN-γ levels were measured by sandwich enzyme immunoassay using an appropriate pair of capture Abs and biotinylated detecting Abs (PharMingen).

Statistical analysis

Statistical significance was assessed by Student's *t* test.

Results

Phagocytosis

Monolayer cultures of CR3$^{-/-}$ and CR3$^{+/+}$ macrophages were infected with *M. tuberculosis* for 2 h at multiplicities of infection (MOIs) of either 1:2, 1:10, or 1:30 to determine whether the lack of CR3 impairs phagocytosis of the tubercle bacillus. There was a dose-dependent increase in the phagocytosis of *M. tuberculosis* by macrophages from both wild-type and CR3$^{-/-}$ macrophages (Fig. 1*A*). At all MOIs examined, a significantly lower number of CR3$^{-/-}$ macrophages phagocytized *M. tuberculosis* compared with wild type ($p < 0.05$). However, the difference in the percentage of infected macrophages between the two groups decreased

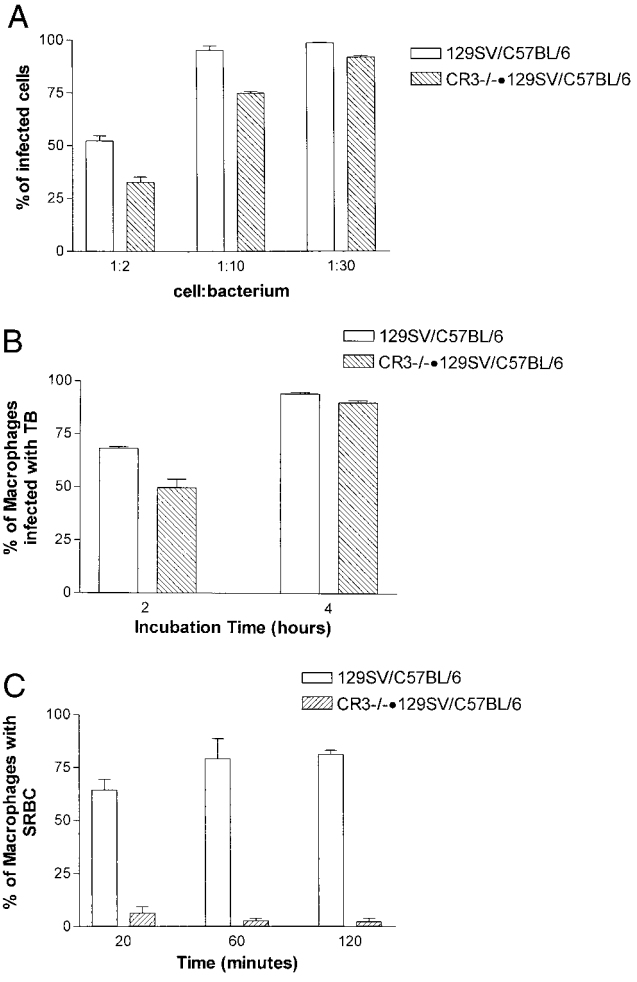

FIGURE 1. Phagocytosis of *M. tuberculosis* by CR3$^{-/-}$ macrophages and association of E-C3bi by CR3$^{-/-}$ macrophages. Bone marrow-derived macrophages from CR3$^{+/+}$ and CR3$^{-/-}$ mice were compared for their ability to associate with *M. tuberculosis*. The data are presented as percent phagocytosis. *A*, Percent phagocytosis of *M. tuberculosis* was measured in wild-type and CR3$^{-/-}$ macrophages at MOIs of 1:2, 1:10, and 1:30 in 2% mouse serum. *B*, Percent phagocytosis of *M. tuberculosis* was measured at 2 and 4 h after infection in wild-type and CR3$^{-/-}$ macrophages in 2% mouse serum. *C*, Association of E-C3bi particles in media containing 2% mouse serum. Data are the average of three experiments.

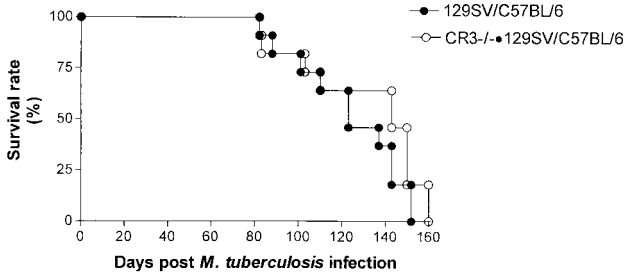

FIGURE 2. Similar course of *M. tuberculosis* infection in CR3-sufficient and -deficient mice of mixed background. $CR3^{-/-}$.129SV × C57BL/6 mice and wild-type controls were infected i.v. with 2×10^5 virulent Erdman strain of *M. tuberculosis*, and disease progression was monitored. Fig. 2 is representative of one of two experiments with similar results. Each experiment contained six mice per group.

markedly, albeit still statistically significantly, as the MOI increased: at an MOI of 30, 96 and 90% of $CR3^{-/-}$ and wild-type macrophages, respectively, were infected. Similarly, lengthening the time of infection also diminished the difference in the percentage of infected macrophages obtained from CR3-deficient and

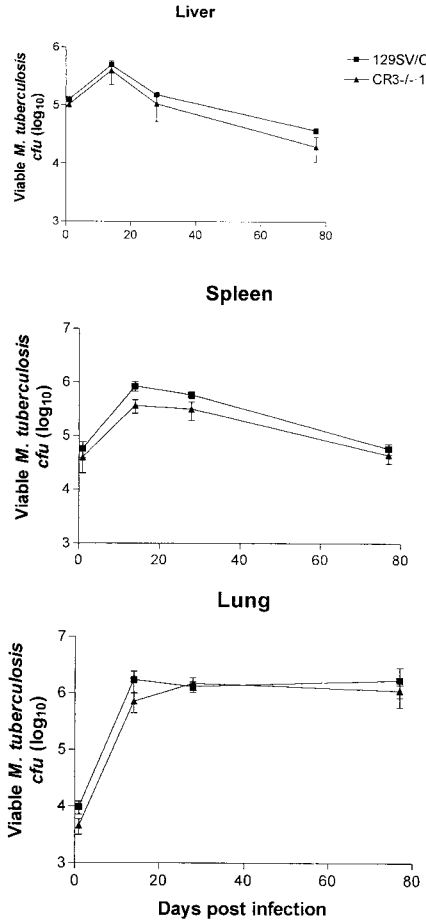

FIGURE 3. Bacterial burden in $CR3^{-/-}$.129SV × C57BL/6 and control wild-type mice infected with *M. tuberculosis*. $CR3^{-/-}$.129SV × C57BL/6 mice and littermate wild-type control mice were infected with *M. tuberculosis*, and bacterial load in lung, spleen, and liver was determined 14, 28, and 70 days postinfection. Three to four mice were included per time point for each group, and data are presented from one of two individual experiments. Between 12 and 16 h postinfection, two mice from each group were harvested for determining baseline tissue bacillary burden.

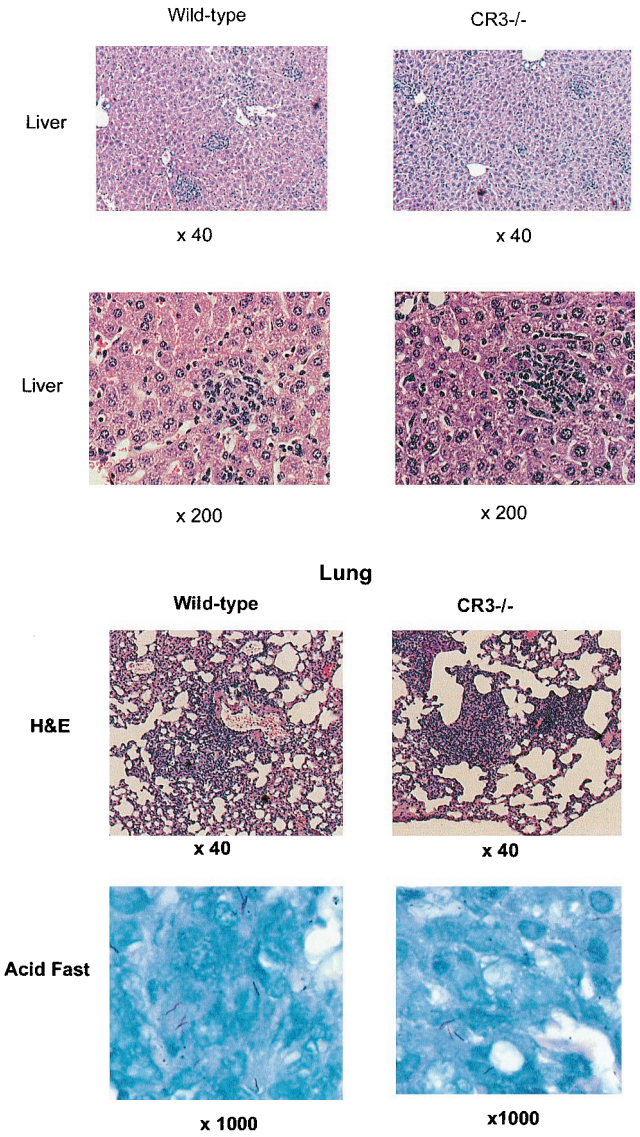

FIGURE 4. Granuloma formation in $CR3^{-/-}$.129SV × C57BL/6 and wild-type control mice. Staining of liver and lung revealed no differences between $CR3^{-/-}$ and wild-type mice infected with *M. tuberculosis*. Sections represent tissues obtained from mice 2 wk postinfection. The first three rows of panels are H&E staining; the fourth row of panels is Zhiel-Neelson staining for acid-fast bacilli.

wild-type mice. At an MOI of 10, increasing the infection period from 2 to 4 h resulted in a decrease in the difference of phagocytosis from 27 to 5% (Fig. 1*B*). By contrast, in studies using E-C3bi particles, the time of incubation did not alter the marked deficiency of the $CR3^{-/-}$ macrophages in associating with the coated red cells ($p < 0.001$, Fig. 1*C*). Together, these results indicate that in this in vitro model, 1) CR3 contributes to the uptake of *M. tuberculosis* by macrophages; and 2) the defect in the phagocytosis of tubercle bacilli by $CR3^{-/-}$ macrophages can be overcome by increasing the MOI or the time of contact between the phagocyte and the bacteria.

Course of M. tuberculosis *infection in 129SV/C57BL/6.$CR3^{-/-}$ mice and control littermates*

To rigorously test the significance of the defect in phagocytosis of tubercle bacilli by $CR3^{-/-}$ macrophages, disease outcome in *M. tuberculosis*-infected $CR3^{-/-}$ mice was examined. Results in Fig.

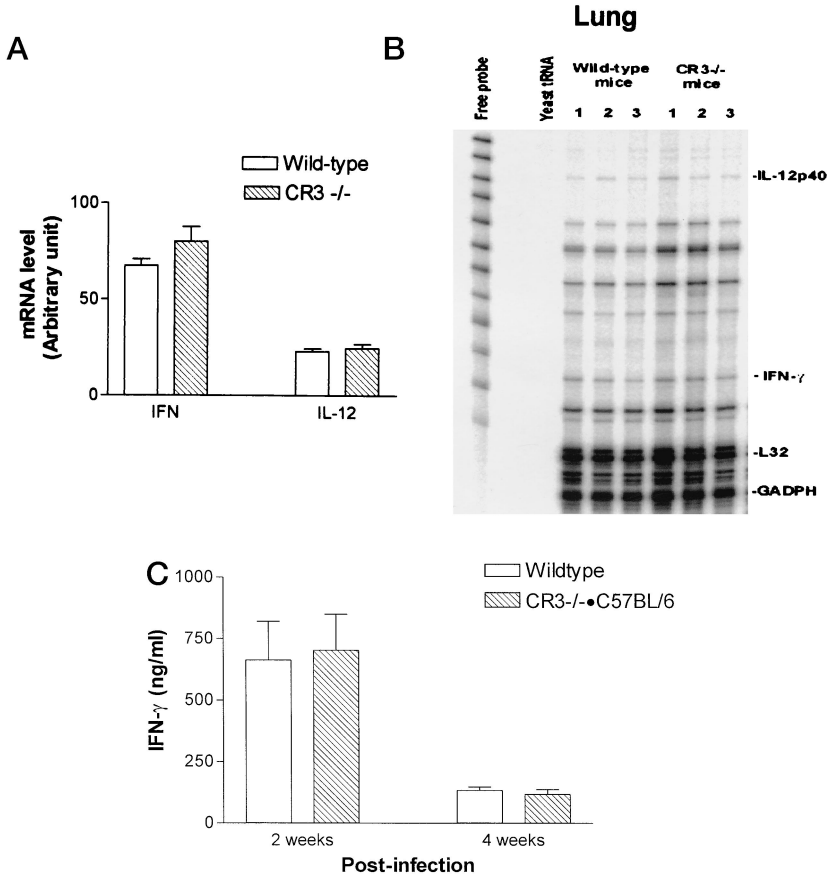

FIGURE 5. Cytokine production. *A*, In vivo expression of IL-12 and IFN-γ. Total RNA was isolated from spleen and lungs of 2-wk-infected animals, analyzed for mRNA expression levels for IL-12p40 and IFN-γ by RPA using the mck2b probe kit (PharMingen), and quantitated by phosphorimaging (*B*). *B*, Representative autoradiogram of gel; *A*, quantification of mRNA shown in *B*. *C*, Ag-specific in vitro IFN-γ production by splenocytes. Splenocytes were obtained from $CR3^{-/-}$ and wild-type mice 2 and 4 wk postinfection and stimulated in vitro with viable *M. tuberculosis*. Supernatants were harvested 72 h later, and the presence of IFN-γ protein was measured by sandwich ELISA. Data are presented as picograms per milliliter of secreted IFN-γ.

2 indicated that there was no difference in the susceptibility of $CR3^{-/-}$ and wild-type littermates to *M. tuberculosis* infection as assessed by mortality rate. The mean survival time (m.s.t.) for the $CR3^{-/-}$ mice was 123 ± 7 days, and that for the wild types was 130 ± 9 days ($p > 0.05$). Mice in both groups begin to succumb to the infection at ~80 days postinfection. By 160 days after inoculation of *M. tuberculosis*, there was 100% mortality in both groups. In agreement with these results, assessment of bacterial burdens in the lungs, liver, and spleen revealed no significant difference between the two groups (Fig. 3; $p > 0.05$). Similarly, examination of H&E-stained tissue sections by light microscopy revealed no difference in histopathology between the wild-type and $CR3^{-/-}$ mice examined at 2, 4, and 10 wk after infection with *M. tuberculosis*. The granulomatous response, characterized by well-demarcated conspicuous lymphoid aggregates (which have a minor monocytic component) among areas of consolidation resulting from a diffuse interstitial infiltration of lymphocytes and histocytic cells, was comparable in the lungs of the two groups of mice. There was also no apparent difference in the distribution of tissue bacilli; virtually all acid-fast organisms were intragranulomatous (Fig. 4). In sum, these results suggest that CR3 does not play a significant role in modulating the cause of tuberculous infections in mice.

Cytokine expression in M. tuberculosis*-infected $CR3^{-/-}$ mice*

Expression of mRNA for IFN-γ and IL-12 in the lungs and spleens from 2-wk-infected mice was examined by RPA. No significant differences in the expression of cytokine mRNA was observed (Fig. 5, *A* and *B*). Spleen cells were obtained at 2 and 4 wk after infection from both wild-type and $CR3^{-/-}$ mice and stimulated in vitro with live *M. tuberculosis* (5×10^6 bacteria/ml). Supernatants were tested for IFN-γ protein levels, and there was no statistical difference ($p > 0.5$) between the two groups at both time points (Fig. 5*C*).

Survival of $CR3^{-/-}$.C57BL/6 mice and control littermates following M. tuberculosis *infection*

Because the experiments described above were performed using mice with a mixed SV129 and C57BL/6 genetic background, the apparent lack of modulation of *M. tuberculosis* infection by CR3 might be the result of factors independent of the CR. Consequently, the significance of CR3 in murine tuberculosis was examined using $CR3^{-/-}$ mice that are the progeny of eight backcrosses with the C57BL/6 strain. Survival rates following *M. tuberculosis* infection in these $CR3^{-/-}$ mice and the C57BL/6 wild type were first compared. Mice from both groups were observed for 150 days, and no mortality was observed (Fig. 6*A*). The IFN-$\gamma^{-/-}$ mice on the C57BL/6 background, included as a control for the virulence of the *M. tuberculosis* Erdman stock used, all succumbed to the infection by 18 days postinfection. Bacterial burden in the spleen, lungs, and liver at 2 and 4 wk postinfection were also compared and found to be similar in the two groups (Fig. 6*B*). Histological examination of these organs from both the $CR3^{-/-}$.C57BL/6 and the wild type revealed similar granulomatous response (Fig. 6*C*). These data strongly suggest that CR3 does not play a significant role in affecting disease outcome in the relatively resistant C57BL/6 strain of mice.

Susceptibility of $CR3^{-/-}$.BALB/c mice and control littermates to M. tuberculosis *infection*

C57BL/6 mice are relatively resistant to *M. tuberculosis*. Therefore, the possibility exists that this relative resistance could have masked the influence of CR3 on the progression of tuberculous infection. Consequently, we tested the effect of CR3 deficiency in

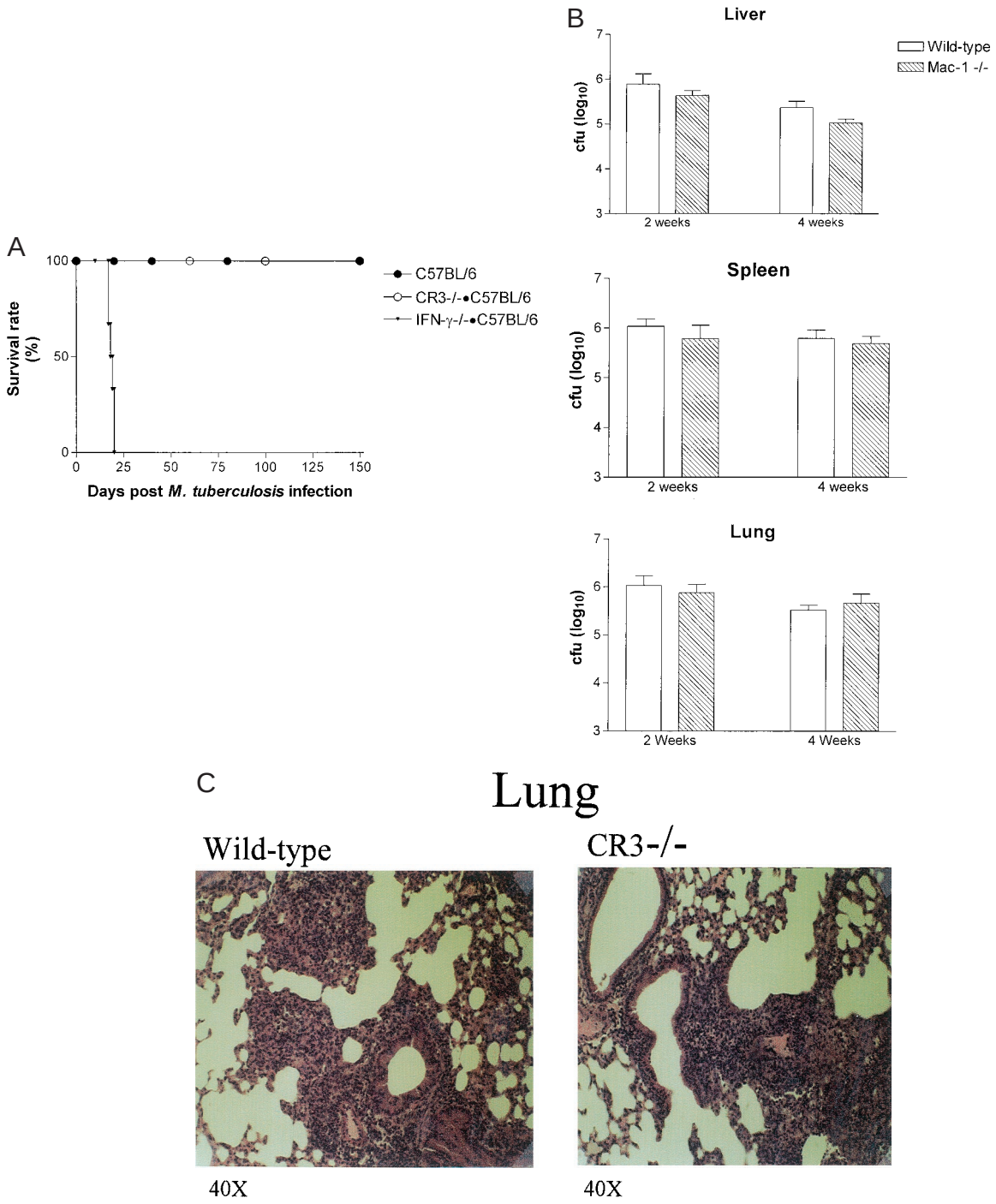

FIGURE 6. Similar course of *M. tuberculosis* infection in CR3-sufficient and -deficient mice on pure C57BL/6 background. $CR3^{-/-}$.C57BL/6 mice and their respective wild-type littermate controls and IFN-γ-deficient mice were infected i.v. with 2×10^5 virulent Erdman strain of *M. tuberculosis*, and disease progression was monitored. *A*, Mortality rate; *B*, bacterial load in liver, spleen, and lung of $CR3^{-/-}$.C57BL/6 mice at 2 wk after infection. *C*, Granuloma formation in $CR3^{-/-}$.C57BL/6 and wild-type control mice. Staining of lung revealed no differences between $CR3^{-/-}$.C57BL/6 and wild-type mice infected with *M. tuberculosis*. Sections represent tissues obtained from mice 2 wk postinfection.

BALB/c mice, a strain that is relatively susceptible to tuberculosis. Conversely, based on previous reports that ligation of CR3 can down-regulate IL-12 production from macrophages (16, 19, 20), it is possible that the absence of CR3 in the relatively susceptible BALB/c mice may render them more resistant to *M. tuberculosis* infection. Data presented in Fig. 7 demonstrate that even in the absence of CR3 the survival of BALB/c mice was not prolonged. The m.s.t. for the wild-type and the $CR3^{-/-}$.BALB/c mice were 105 ± 22 and 110 ± 12 days, respectively ($p > 0.05$), with groups reaching 100% mortality by 145 days postinfection.

Discussion

In this study, we demonstrate that macrophages lacking CR3, compared with cells from wild-type mice, are deficient in phagocytizing *M. tuberculosis* in vitro. Results derived from the in vivo studies using a murine tuberculosis model that used $CR3^{-/-}$ mice

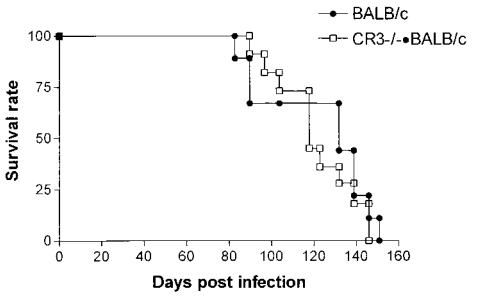

FIGURE 7. Similar course of *M. tuberculosis* infection in CR3-sufficient and -deficient mice on BALB/c background. $CR3^{-/-}$.BALB/c mice and their respective wild-type controls were infected i.v. with 2×10^5 virulent Erdman strain of *M. tuberculosis*, and m.s.t. was determined.

suggested that lack of this receptor did not alter the course of tuberculosis infection in the mouse, as assessed by tissue bacterial load, mortality, granulomatous response, and the production of IFN-γ and IL-12, the two cytokines critical in imparting protective immunity in tuberculosis.

With respect to the use of CR3 by the tubercle bacillus to enter macrophages, results obtained from the in vitro macrophage system is in agreement with that reported previously, which showed that in the presence of Abs to CR3, uptake of *M. tuberculosis* by human monocytes is substantially impaired (11). However, this latter study did not address the effects of MOI or the length of infection time on the uptake of *M. tuberculosis* by CR3-blocked macrophages. Results reported in this study demonstrate that the apparent deficiency in *M. tuberculosis* phagocytosis by $CR3^{-/-}$ macrophages appears to be largely reversible with increased MOI and the time of contact between macrophages and bacteria. This latter observation suggests that in the absence of CR3, *M. tuberculosis* can gain entry into macrophages via alternative receptors, albeit with less efficiency. Apparently, the phagocytosis via these less efficient alternative receptors can approach the efficiency of that mediated by CR3 with increased MOI or increased contact time between the phagocytes and the bacilli. In support of this notion, it has been well documented that the tubercle bacillus can be internalized by macrophage/monocytes through interactions with multiple receptors (3, 21).

Extrapolating these in vitro observations to situations in vivo, one can expect two possible scenarios regarding the initial phase of *M. tuberculosis* infection in $CR3^{-/-}$ animals: 1) due to the deficiency in internalizing *M. tuberculosis* by macrophages in the absence of CR3, a portion of the inoculum will remain extracellular; and 2) given adequate time for the bacillus to interact with the phagocytes, compensatory non-CR3 receptors will be able to internalize *M. tuberculosis* at levels comparable to that of wild-type cells. It is likely that in the first scenario, the outcome of the infection will depend on the fate of extracellular organisms, an area that has not been well defined. We favor the second scenario because the results obtained from the in vivo studies demonstrated that virtually all bacilli observed in acid-fast bacilli-stained tissue sections obtained from $CR3^{-/-}$ mice were intragranulomatous, and the bacillary load in these animals was similar to that in controls, suggesting that, in vivo, macrophages of $CR3^{-/-}$ mice could indeed phagocytize *M. tuberculosis* at a level comparable to that of wild types. Our in vivo observation that progression of tuberculous infection, as assessed by mortality, bacterial burden, and tissue pathological response, is comparable in $CR3^{-/-}$ and wild-type mice supports and extends a previous study (22), which reported that not only entry into, but also subsequent survival and replication of, the tubercle bacillus inside macrophages remained similar whether phagocytosis occurred in the presence or absence of Abs to CR3 (22). Finally, a recent report examining the role of CRs in *Mycobacterium avium* infection (23) supports our present findings. *M. avium* infection in CD18-deficient mice was comparable to the control wild types, implicating that, in vivo, this mycobacterium uses receptors other than CR3 and CR4 to gain entry into tissue macrophages (23). Indeed, intracellularly grown *M. avium* (derived from macrophages) use noncomplement-mediated mechanisms for subsequent infection into fresh macrophages (24).

Several studies have speculated that routing into macrophages via CR3 may be advantageous to the pathogen. Interaction of CR3 with its ligand does not trigger a respiratory burst (25), and thus entry via this receptor may be less toxic to the pathogen. In addition, several groups have demonstrated that signaling through CR3 suppresses IL-12 production by macrophages (16, 19, 20), a cytokine that drives naive T cells to differentiate into Th1-type cells (26), the latter required for host defense against a number of intracellular pathogens. In this study, we show that the production of IFN-γ and IL-12, the cytokines critical in engendering protective immunity in tuberculosis, are similar in the two groups of mice. These results strongly suggested that effective antimycobacterial immune responses can be elicited from macrophages and T cells independent of the CR3-mediated route of entry. This is further supported by the fact that the lack of CR3 did not influence the course of tuberculous infection.

Worthy of note is our in vivo finding that the lack of modulation of the progression of *M. tuberculosis* infection by CR3 can be observed in three different strains of mice (SV129, C57BL/6, and BALB/c) lacking CR3. Of the three studied, C57BL/6 is relatively resistant to *M. tuberculosis* infection compared with the more susceptible BALB/c, as attested by the shorter m.s.t. of the latter strain (compare Fig. 6*A* and 7). Thus, even in the genetic background of the relatively susceptible BALB/c strain, a role for CR3 in influencing disease outcome and the immune response of the host could not be appreciated. These results strongly suggest that in the murine experimental tuberculosis model used, CR3 does not play a major role in affecting the course of tuberculous infection. However, data derived from mice with gene disruption must be interpreted with caution. A major caveat is the possibility that $CR3^{-/-}$ mice might have developed compensatory *M. tuberculosis* uptake mechanisms that are qualitatively and/or quantitatively different from those used by wild-type animals. Caveat notwithstanding, the data we present and those reported by Bermudez and coworkers (23) should prompt a re-examination of the role of CRs as portals of entry for *M. tuberculosis* into tissue macrophages in vivo. Future studies using mice lacking CR4, CD18, and the third component of complement, as well as those involving in vivo blocking of CR3 in wild-type tuberculous mice, will further our understanding of the role of complement and its receptors in establishing *M. tuberculosis* infection in vivo.

Acknowledgments

We thank David Mosser for critical reading of the manuscript and Margaret Mentinck-Kane for help with the E-C3bi experiments.

References

1. Bloom, B. R., and C. J. L. Murray. 1992. Tuberculosis: commentary on a reemergent killer. *Science 257:1055.*
2. Raviglione, M. C., J. D. E. Snider, and A. Kochi. 1995. Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. *J. Am. Med Assoc. 273:320.*
3. Ernst, J. D. 1998. Macrophage receptors for *Mycobacterium tuberculosis. Infect. Immun. 66:1277.*
4. Schlesinger, L. S. 1993. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. *J. Immunol. 150:2920.*

5. Schlesinger, L. S., S. R. Hull, and T. M. Kaufman. 1994. Binding of the terminal mannosyl units of lipoarabinomannan from a virulent strain of *Mycobacterium tuberculosis* to human macrophages. *J. Immunol. 152:4070.*
6. Schlesinger, L. S., T. M. Kaufman, S. Iyer, S. R. Hull, and L. K. Marchiando. 1996. Differences in mannose receptor-mediated uptake of lipoarabinomannan from virulent and attenuated strains of *Mycobacterium tuberculosis* by human macrophages. *J. Immunol. 157:4568.*
7. Pugin, J., I. D. Heumann, A. Tomasz, V. V. Kravchenko, Y. Akamatsu, M. Nishijima, M. P. Gluaser, P. S. Tobias, and R. J. Ulevitch. 1994. CD14 is a pattern recognition receptor. *Immunity 1:509.*
8. Pasula, R., J. F. Downing, J. R. Wright, D. L. Kachel, J. T. E. Davis, and W. J. N. Martin. 1997. Surfactant protein A (SP-A) mediates attachment of *Mycobacterium tuberculosis* to murine alveolar macrophages. *Am. J. Respir. Cell. Mol. Biol. 17:209.*
9. Ahearn, J. M., and D. T. Fearon. 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol. 46:183.*
10. Sanchez-Madrid, F., A. Nagy, E. Robbins, P. Simon, and T. A. Springer. 1983. A human leukocyte antigen family with distinct α subunits and a common β subunit. *J. Exp. Med. 158:1785.*
11. Schlesinger, L. S., C. G. Bellinger-Kawahara, N. R. Payne, and M. A. Horwitz. 1990. Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement C3. *J. Immunol. 144:2771.*
12. Schorey, J. S., M. C. Carroll, and E. J. Brown. 1997. A macrophage invasion mechanism of pathogenic mycobacteria. *Science 277:1091.*
13. Cywes, C., N. L. Godenir, H. C. Hoppe, R. R. Scholle, L. M. Steyn, R. E. Kirsch, and M. R. Ehlers. 1996. Nonopsonic binding of mycobacterium tuberculosis to human complement receptor type 3 expressed in Chinese hamster ovary cells. *Infect. Immun. 64:5373.*
14. Cywes, C., H. C. Hoppe, M. Daffe, and M. R. Ehlers. 1997. Nonopsonic binding of *Mycobacterium tuberculosis* to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. *Infect. Immun. 65:4258.*
15. Coxon, A., P. Rieu, F. J. Barkalow, S. Askari, A. H. Sharpe, U. H. von Andrian, M. A. Arnaout, and T. N. Mayadas. 1996. A novel role for the β_2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. *Immunity 5:653.*
16. Sutterwala, F. S., G. J. Noel, R. Clynes, and D. M. Mosser. 1997. Selective suppression of interleukin-12 induction after macrophage receptor ligation. *J. Exp. Med. 185:1977.*
17. Chan, J., J. Xing, R. S. Magliozzo, and B. R. Bloom. 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med. 175:1111.*
18. Chan, J., K. Tanaka, D. Carrol, J. Flynn, and B. R. Bloom. 1995. Effects of nitric oxide synthase inhibitors on murine infection with *M. tuberculosis. Infect. Immun. 63:736.*
19. Karp, C. L., M. Wysocka, L. M. Wahl, J. M. Ahearn, P. J. Cuomo, B. Sherry, G. Trinchieri, and D. E. Griffin. 1996. Mechanism of suppression of cell-mediated immunity by measles virus. *Science 273:228.*
20. Marth, T., and B. L. Kelsall. 1987. Regulation of interleukin-12 by complement receptor 3 signaling. *J. Exp. Med. 185:1987.*
21. Hirsch, C. S., J. J. Ellner, D. G. Russell, and E. A. Rich. 1994. Complement-receptor-mediated uptake and tumor necrosis factor-α-mediated growth inhibition of *Mycobacterium tuberculosis* by human alveolar macrophages. *J. Immunol. 152:743.*
22. Zimmerli, S., S. Edwards, and J. D. Ernst. 1996. Selective receptor blockade during phagocytosis does not alter the survival and growth of *Mycobacterium tuberculosis* in human macrophages. *Am. J. Respir. Cell. Mol. Biol. 15:760.*
23. Bermudez, L. E., J. Goodman, and M. Petrofsky. 1999. Role of complement receptors in uptake of *Mycobacterium avium* by macrophages in vivo: evidence from studies using CD18-deficient mice. *Infect. Immun. 67:4912.*
24. Bermudez, L., E. A. Parker, and J. Goodman. 1997. Growth within macrophages increases the efficiency of *Mycobacterium avium* in invading other macrophages by a complement receptor-independent pathway. *Infect. Immun. 65:1916.*
25. Wright, S. D., and S. C. Silverstein. 1983. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J. Exp. Med. 158:2016.*
26. Trinchieri, G. 1993. Interleukin-12 and its role in the generation of Th1 cells. *Immunol. Today 14:335.*