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## Interleukin-10 Promotes *Mycobacterium tuberculosis* Disease Progression in CBA/J Mice<sup>1</sup>

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### Abstract

IL-10 is a potent immunomodulatory cytokine that affects innate and acquired immune responses. The immunological consequences of IL-10 production during pulmonary tuberculosis (TB) are currently unknown, although IL-10 has been implicated in reactivation TB in humans and with TB disease in mice. Using *Mycobacterium tuberculosis*-susceptible CBA/J mice, we show that blocking the action of IL-10 in vivo during chronic infection stabilized the pulmonary bacterial load and improved survival. Furthermore, this beneficial outcome was highly associated with the recruitment of T cells to the lungs and enhanced T cell IFN- $\gamma$  production. Our results indicate that IL-10 promotes TB disease progression. These findings have important diagnostic and/or therapeutic implications for the prevention of reactivation TB in humans.

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Interleukin-10 is a potent immunomodulatory cytokine that has been shown in vitro to directly or indirectly affect multiple cell types, including macrophages, monocytes, dendritic cells, CD4 T cells, and CD8 T cells (1). The dominant function of IL-10 is to deactivate macrophages, resulting in diminished Th1 cytokine production (2,3), decreased production of reactive nitrogen or oxygen species (4), and limited Ag presentation (5), which may have far-reaching consequences on both innate and acquired immunity in vivo.

IL-10 has been identified as a correlate of susceptibility for tuberculosis (TB)<sup>3</sup> in both mice and humans (6–11), as well as susceptibility to *Mycobacterium avium* infection in murine models (12). In humans, IL-10 can be found in the serum (6,7) and bronchoalveolar lavage

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#### Disclosures

Rene de Waal Malefyt is an employee of Schering-Plough Biopharma.

<sup>3</sup>Abbreviations used in this paper: TB, tuberculosis; *M.tb.*, *Mycobacterium tuberculosis*.

fluid (8) of active TB patients and may be an important clinical biomarker of disease progression (9). In the murine model, IL-10-deficient C57BL/6 mice have a subtle yet increased early resistance to infection with *Mycobacterium tuberculosis* (*M.tb*) (10,11). C57BL/6 mice are relatively resistant to infection with *M.tb* (13,14) and a lack of IL-10 does not alter chronic infection in this strain (10,11). Genetically manipulated C57BL/6 mice may not, therefore, accurately reflect a role for IL-10 in driving *M.tb* infection. We, and others, have previously defined the CBA/J mouse strain as relatively susceptible to *M.tb* infection, as it has increased bacterial load within the lung as infection progresses (13) and earlier mortality than does the C57BL/6 strain (14). Furthermore, disease progression in CBA/J mice has been associated with abundant IL-10 within the lung tissue (15). We have hypothesized that the increased production of IL-10 during late stages of a chronic *M.tb* infection in CBA/J mice exacerbates disease progression. This is supported by our previous findings that C57BL/6 mice engineered to overexpress IL-10 had accelerated disease progression following *M.tb* infection (15), confirming a clear relationship between IL-10 and poor disease outcome.

The increased amount of IL-10 produced by CBA/J mice during *M.tb* infection provides us with a natural model in which the role of IL-10 can be more accurately defined. In this study we used CBA/J mice to determine whether IL-10 can accelerate disease progression during an established chronic *M.tb* infection. Using anti-IL-10R1 Ab delivery starting at day 90 postinfection, we show that blockade of IL-10 activity led to improved control of *M.tb* bacterial load within the lung and enhanced Th1 immunity. These data demonstrate that increased IL-10 production exacerbates TB disease and provides supportive evidence that IL-10 can be a biomarker of TB disease progression in humans.

## Materials and Methods

### Mice

Specific pathogen-free 8-wk-old female CBA/J and C57BL/6 mice (National Cancer Institute, Frederick, MD) were maintained in biosafety level 3 facilities and provided with sterile food and water ad libitum. All protocols were approved by The Ohio State University's Institutional Laboratory Animal Care and Use Committee.

### *M.tb* infection and quantification of bacterial load

*M.tb* Erdman (ATCC no. 35801) was obtained from American Type Culture Collection. Stocks were grown and mice infected with *M.tb* Erdman using an inhalation exposure system (Glas-Col) calibrated to deliver 50–100 CFU to the lungs of each mouse, as previously described (16). At specific time points postinfection, the *M.tb* pulmonary load was determined as previously described (18).

### Quantification of immune mediators

Lung homogenates were clarified for cytokine ELISAs using Ab pairs (BD Biosciences) as previously described (16). Nitrites/nitrates in lung tissue homogenates were quantified using the Greiss reaction. Briefly, Greiss reagent was added to equal volumes of clarified lung homogenate, absorbance was read at 550 nm, and values were compared with a sodium nitrite standard curve.

### Ab administration

Anti-IL-10R1 mAb (1B1.3A) (Schering-Plough Biopharma) and whole rat IgG (Jackson ImmunoResearch Laboratories) or rat IgG1 isotype control mAb (GL113) (Schering-Plough Biopharma) was diluted to 2 mg/ml in PBS and stored at  $-80^{\circ}\text{C}$ . Thawed stocks were maintained at  $4^{\circ}\text{C}$  for up to 1 mo. Ab administration was modified based on Silva et al. (17)

as follows: at day 90 postinfection, 1 mg of anti-IL-10R1 (1B1.3A) or control Ab was injected into the peritoneal cavity of each mouse, followed by 0.2 mg at weekly intervals thereafter until the designated experimental time points. At the initiation of Ab treatment CBA/J mice consistently harbor  $6.59 \pm \text{SE } 0.12 \log_{10} M.tb$  CFU (18). For animals in survival studies, Ab injection was continued weekly until signs of morbidity developed and mice were then euthanized.

### Lung cell isolation and cell culture

Mice were euthanized by CO<sub>2</sub> asphyxiation and single-cell suspensions obtained from the lung using collagenase/DNase as previously described (16). Viable cells were determined by trypan blue exclusion, counted, and resuspended at  $2 \times 10^6/\text{ml}$ . Lung cells ( $2.5 \times 10^5$ ) were cultured in duplicate with 10  $\mu\text{g}/\text{ml}$  of OVA (Sigma-Aldrich), *M.tb* culture filtrate protein (National Institutes of Health, National Institute of Allergy and Infectious Diseases), or Con A (Sigma-Aldrich) for 72 h at 37°C. Supernatants were stored at -80°C until analysis. Cytokine production from cell culture supernatants was quantified by ELISA using Ab pairs and standards from BD Biosciences as described previously (16).

### Flow cytometry

Isolated lung cells were suspended in deficient RPMI (Irvine Scientific) supplemented with 0.1% sodium azide (Sigma-Aldrich). Following 5–10 min of incubation at room temperature with Fc Block (clone 2.462; BD Biosciences), surface and intracellular targets were detected as previously described (16). Specific Abs and isotype controls were purchased from BD Biosciences: PerCP-Cy5.5 anti-CD3 $\epsilon$  (145-2C11), allophycocyanin-Cy7 anti-CD4 (GK1.5), PeCy7 anti-CD8 (53-6.7), FITC anti-CD11a (2D7), allophycocyanin anti-CD11c (HL3), allophycocyanin anti-TCR- $\beta$ -chain (H57597), PerCP anti-CD8 (53-6.7), anti-I-A/I-E (2G9), and PeCy7 anti-IFN- $\gamma$  (XMG1.2). IFN- $\gamma$  was determined according to the manufacturers instructions for intracellular cytokine staining (Cytotfix/Cytoperm fixation/permeabilization solution kit with BD GolgiStop, BD Biosciences), following a 4-h incubation with anti-CD3 (145-2C11) and anti-CD28 (37.51). Samples were read using an LSRII flow cytometer and analyzed with FACSDiva software (BD Biosciences). For some experiments 100  $\mu\text{l}$  of BrdU (FITC BrdU flow kit, BD Biosciences) was injected into the peritoneal cavity of each mouse 24 h before euthanasia. BrdU incorporation was detected by flow cytometry as per the manufacturer's instructions (FITC BrdU flow kit).

### Histology

The right cranial lung lobes from individual mice were inflated with 10% neutral buffered formalin before paraffin embedding, sectioning at 5  $\mu\text{m}$ , and staining with H&E. Samples were evaluated in a double-blind fashion by a board-certified veterinary pathologist and graded as previously described (19).

### Real-time PCR

Right medial lung lobes were homogenized in 1 ml of UltraSpec (Biotecx Laboratories) and frozen rapidly at -80°C. Total RNA was isolated by following the manufacturer's instructions, and 1  $\mu\text{g}$  was reverse transcribed using an Omniscript RT kit (Qiagen). Real-time PCR was performed with an iQ5 real-time PCR detection system (Bio-Rad) using Taq-Man gene expression assays for CHIITA, IFN regulatory factor-1, or the 47-kDa GTPase LRG-47 (Applied Biosystems). The  $\Delta\Delta\text{Ct}$  method was used for relative quantification of mRNA expression; in this analysis 18S was used as an endogenous normalizer.

## Statistics

Pairwise comparisons used the Student's *t*-test; significance was defined as \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , or \*\*\*,  $p < 0.001$ . Multigroup comparisons used one-way ANOVA with Tukey's posttest; significance was defined as +,  $p < 0.05$ , ++,  $p < 0.01$ , or +++,  $p < 0.001$ . Log-rank analysis was used for survival studies.

## Results

### Anti-IL-10R1 treatment stabilized *M.tb* growth and improved survival in CBA/J mice

IL-10 production in the lungs of *M.tb*-infected CBA/J and C57BL/6 mice was determined. In comparison to the relatively resistant C57BL/6 mouse strain, IL-10 protein in CBA/J lungs was significantly higher at days 60, 120, and 150 days after *M.tb* infection (Fig. 1A). Furthermore, IL-10 continued to increase substantially over time during late infection (120 and 150 days) in CBA/J mice, while the levels stabilized in C57BL/6 mice (Fig. 1A). Similar increases were also detected in lung IL-10 mRNA (data not shown). Therefore, the immunocompetent inbred CBA/J mouse strain produces IL-10 during *M.tb* infection (15), allowing for the in vivo assessment of IL-10 function in a natural model of heightened *M.tb* susceptibility (14). To determine whether IL-10 directly influenced TB disease progression, we blocked the signaling chain of the IL-10 receptor by administering anti-IL-10R1 (1,17) during chronic *M.tb* infection in CBA/J mice. *M.tb*-infected CBA/J mice were administered anti-IL-10R starting at day 90 postinfection. Day 90 was specifically chosen as the start point because this occurs before the substantial increase in lung IL-10 (Fig. 1A) and before loss of controlled *M.tb* growth (18) associated with increased morbidity in CBA/J mice (data not shown). Anti-IL-10R1 treatment was continued weekly throughout the experimental infection. The results showed a significant and predictable increase in bacterial burden in the lungs (15) of CBA/J mice that received control Ab (Fig. 1B), which approached  $10^8$  *M.tb* CFU, a level at which CBA/J mice rarely survive (data not shown). In contrast, treatment with anti-IL-10R1 stabilized the pulmonary bacterial load for up to 150 days (Fig. 1B). A separate group of mice was assigned to survival studies (Fig. 1C). Weekly Ab treatment was continued throughout survival experiments and mice were euthanized according to established morbidity criteria (13). Mice treated with anti-IL-10R1 survived ~70 days longer than did mice receiving control Ab (median survival of 196 days for anti-IL-10R1 treated vs 118 days for control antibody). Statistical significance was not achieved in the survival studies ( $p = 0.11$ ); however, the small group size may have precluded this finding. Overall, these data indicate that blocking the activity of IL-10 in vivo in the CBA/J mouse strain during chronic infection with *M.tb* led to control of bacterial growth within the lung and extended survival. As expected, IL-10 blockade in the relatively resistant C57BL/6 mouse strain did not alter the *M.tb* lung load during the time frame studied (data not shown). This finding is consistent with results suggesting that IL-10 does not contribute to disease progression in C57BL/6 mice (10,11).

### Anti-IL-10R1-treated CBA/J mice have increased cell density in the lung

We next determined whether improved control of infection was associated with increased T cell numbers within the lung. After 115 days no significant differences in total numbers of viable cells, CD4 T cells, or CD8 T cells were observed (Fig. 2). In contrast, at 150 days postinfection the total numbers of lung cells, CD4 T cells, and CD8 T cells were increased in mice that received anti-IL-10R1, although statistical significance was not reached. To determine whether increased cell numbers in anti-IL-10R1-treated CBA/J mice resulted from T cell proliferation, mice were injected with BrdU 24 h before euthanasia, and BrdU incorporation was determined by intracellular flow cytometry. Data from three independent experiments failed to identify a consistent increase in the number of proliferating CD4 or CD8 T cells in the lung (data not shown). To determine whether cell recruitment contributed to the increased number of T cells in anti-IL-10R1-treated mice, T cells from the lung were analyzed

for CD11a<sup>bright</sup> expression. At day 150 postinfection, the numbers of CD4 and CD8 T cells expressing CD11a<sup>bright</sup> (Fig. 3) were significantly increased in mice treated with anti-IL-10R1. In contrast, mice treated with control Ab showed moderate increases in CD11a<sup>bright</sup> T cells. Importantly, within both CD4 and CD8 T cell populations, multivariate analysis showed a significant increase in CD11a<sup>bright</sup> expression across all groups and time points as a result of anti-IL-10R1 treatment. These data strongly suggest that IL-10 blockade promotes T cell recruitment into the lungs.

### IFN- $\gamma$ production from lung cells of anti-IL-10R1-treated CBA/J mice

Two methods were used to determine the functional effects of IL-10 blockade on lung cells during chronic *M.tb* infection. First, lung cell suspensions were cultured for 4 h with anti-CD3 and anti-CD28 to stimulate TCR-mediated crosslinking and signaling in the presence of GolgiStop to allow for intracellular accumulation of IFN- $\gamma$ . The proportion (%) of IFN- $\gamma$ <sup>+</sup> producing CD4 and CD8 T cells within the lungs of mice treated with anti-IL-10R1 was not consistently statistically increased (data not shown). Additionally, when the absolute numbers of IFN- $\gamma$ <sup>+</sup> T cells were calculated at day 115, there were no significant differences between control and anti-IL-10R1-treated mice (Fig. 4, A and B). In contrast, after 150 days of infection there was a substantial increase in the number of IFN- $\gamma$ <sup>+</sup> T cells in the lungs of anti-IL-10R1-treated CBA/J mice. Statistical significance between anti-IL-10R1-treated and control Ab treated mice was observed in the CD8 T cell population (Fig. 4B), and a trend for more IFN- $\gamma$ <sup>+</sup> cells was observed within the CD4 T cells population (Fig. 4A,  $p = 0.08$ ). These data indicate that anti-IL-10R1 treatment during chronic *M.tb* lung infection increases the number of CD4 and CD8 T cells within the lung that have the potential to produce IFN- $\gamma$ .

To evaluate Ag-specific responses ex vivo, lung cells were cultured with *M.tb* culture filtrate protein and IFN- $\gamma$  production was determined by ELISA. As anticipated (15), Ag-specific IFN- $\gamma$  production was low in lung cultures from CBA/J mice that had received control Ab at each time point tested (Fig. 4C). At day 115 postinfection, lung cell cultures from anti-IL-10R1-treated mice had significantly increased IFN- $\gamma$  production relative to the control group (Fig. 4C), suggesting that IL-10R1 treatment enhanced numbers/function of lung Ag-specific cells during the first 25 days of anti-IL-10R1 therapy. Interestingly, IFN- $\gamma$  production in anti-IL-10R1-treated mice declined to levels similar to the controls by day 150.

### Altered cytokine production in the lung following anti-IL-10R1 treatment

To quantify cytokine production within the lung during infection, cytokine levels were directly measured in lung homogenates. Mice receiving control Ab showed stable but elevated levels of IFN- $\gamma$  (Fig. 5A) within the lung at both time points analyzed. Mice receiving anti-IL-10R1 treatment had significantly less IFN- $\gamma$  protein within the lung after 115 days infection relative to isotype-treated mice; however, these levels increased significantly by 150 days (Fig. 5A). Therefore, anti-IL-10R1 treatment led to more T cells capable of Ag-specific IFN- $\gamma$  production (Fig. 4), but these cells were not continually producing IFN- $\gamma$  in vivo (Fig. 5A).

No consistent differences were found for IL-12p40 protein (Fig. 5B), TNF (data not shown), or reactive nitrogen species (data not shown) attributable to anti-IL-10R1 treatment. Additionally, the numbers of lung CD11c<sup>+</sup> cells and their activation status were assessed. There were no consistent statistically significant differences in proportions or absolute numbers of lung CD11c<sup>+</sup> (data not shown). To assess whether in vivo IL-10 blockade increased MHCII expression on CD11c<sup>+</sup> cells, the mean fluorescent intensity of surface MHCII was determined; however, IL-10 blockade did not affect MHCII expression at the level of global CD11c<sup>+</sup> lung cells. Overall, these results suggest that macrophage function remains intact during IL-10 blockade in *M.tb*-infected CBA/J mice or alternatively reflects the limitations of detection of our assays. As anticipated (15), IL-10 protein levels within the lungs of mice receiving control

Ab significantly increased between 115 and 150 days (Fig. 5C). In contrast, anti-IL-10R1-treated mice did not exhibit an increase in IL-10 protein in the lung, perhaps reflecting a feedback loop in IL-10 regulation (Fig. 5C).

To determine potential mechanisms by which increased T cell-derived IFN- $\gamma$  could affect *M.tb* burden during IL-10 blockade, downstream effectors of IFN- $\gamma$  signaling were quantified by RT-PCR. No significant differences in mRNA levels for CIITA, IFN regulatory factor 1, or the 47-kDa GTPase LRG-47 were detected (data not shown). These results suggest that, at least at the tissue level, IL-10 blockade does not affect transcription of these molecules.

## Discussion

Our data provide evidence that IL-10 production during chronic *M.tb* infection promotes bacterial growth and exacerbation of disease in a susceptible CBA/J mouse model of infection. This was shown by blocking the biological action of IL-10 in vivo using anti-IL-10R1 Abs that were delivered during chronic *M.tb* infection. Anti-IL-10R1 treatment led to a stabilization of the lung bacillary load, increased median survival time, and an increased number of cells within the lung by day 150 of infection. Enhanced cellularity within the lung was associated with augmentation of CD8 and CD4 T cells that expressed CD11a<sup>bright</sup> and/or were capable of secreting IFN- $\gamma$ . Overall, these data indicate that IL-10 production during infection with *M.tb* leads to the poor T cell responses and impaired control of infection in CBA/J mice. Furthermore, these deficiencies can be overcome by blocking the action of IL-10 in vivo.

Treatment with anti-IL-10R1 led to an increased number of CD4 and CD8 T cells within the lung that were capable of producing IFN- $\gamma$ , which for CD8 T cells was significantly higher than for cells from control-treated mice. Analysis of IFN- $\gamma$  within the whole-lung homogenate supported our findings, with a significant increase in IFN- $\gamma$  between days 115 and 150 of infection in anti-IL-10R1-treated mice. Why control treated mice had increased IFN- $\gamma$  in whole-lung homogenates at day 115 is uncertain. It is possible that IFN- $\gamma$  was increased as a result of subtle differences in lung bacterial growth or turnover that were not detected by the CFU assay. Importantly, Ag-specific IFN- $\gamma$  secretion ex vivo showed that lung cells from anti-IL-10R1 mice secreted significantly more IFN- $\gamma$  after 115 days of infection than did lung cells from control mice. We can hypothesize that this early availability (day 115) of Ag-specific T cells contributed to the stable bacillary load within the lungs of CBA/J mice treated with anti-IL-10R1 Ab. Why we saw a significant decrease in Ag-specific IFN- $\gamma$  secretion during the later (day 150) time point is unclear. This finding, however, was seen in multiple experiments and could reflect a restoration of phenotype to the control Ab-treated group. Alternatively, our data suggest that a reduced bacterial load in anti-IL-10R1-treated mice led to fewer actively secreting Ag-specific cells in the lung or that CD8 T cells were the dominant source of IFN- $\gamma$  within the lung as infection progressed.

IL-10 is an antiinflammatory cytokine that can dampen an overly exuberant immune response and potentially prevent tissue damage (1). Indeed, the increased cellularity within the lungs of anti-IL-10R1-treated CBA/J mice alludes to a role for IL-10 in preventing detrimental pulmonary inflammation. Anti-IL-10R1 treatment did not, however, lead to any overt histopathological changes in the lung (data not shown), and therefore we consider these increases to be reflective of enhanced protective immunity rather than a failure to dampen inflammatory responses. Furthermore, the lung cell numbers recovered from anti-IL-10R1-treated CBA/J mice were comparable to C57BL/6 mice (data not shown), an inbred mouse strain with relative resistance to *M.tb*. In fact, we have previously shown that C57BL/6 mice have more CD11a<sup>bright</sup> CD4 T cells in the lungs during *M.tb* infection than do CBA/J mice (13) and, again, the increased number of CD11a<sup>bright</sup> T cells in anti-IL-10R1-treated CBA/J mice suggests acquisition of a protective immune response. Furthermore, Ely et al. have

implied that CD11a<sup>bright</sup> T cells localize to lung tissue during an Ag-specific immune response (20). In conjunction with our findings that IL-10 blockade did not consistently alter lung T cell proliferation, these results suggest that IL-10 may be a significant factor in the inhibition of T cell recruitment to the lung in CBA/J mice.

The failure to reach statistical significance in some of our data may be a consequence of the variable time frame that CBA/J mice progress to active disease (14), and the choice of only two selected time points for analysis, which cannot account for the wax and wane of immunity that has been described by others (21). Additionally, the extensive Ab treatments may have stimulated anti-rat Ab production and a loss of functional activity of the anti-IL-10R Ab, reducing the long-term biological impact of the Ab treatment (preliminary studies showed some mice generated anti-rat Abs after multiple injections (data not shown)). We can only speculate as to the outcome of infection/survival if anti-IL-10R1 treatment remained effective for the long term. Alternative strategies to study the role of IL-10 during chronic *M.tb* infection, such as IL-10-deficient CBA/J mice, could provide valuable tools to assess the relative role of IL-10 in disease progression.

It is important to acknowledge that we are manipulating a complex in vivo system, and our readout of a protective response is based on what is currently known about immunity during *M.tb* infection. Molecules such as IL-12, TNF, and NO are essential for control of *M.tb* infection (22–25), and consequently these were the parameters that we measured. We found little difference in the expression of these known protective molecules when we manipulated the action of IL-10. What is significant in this study, however, is that despite failing to find significant differences in some immunological parameters, we could significantly modify the course of infection (lung bacterial load) by blocking the action of IL-10. The possibility therefore exists that enhanced protection is associated with alternate cells/molecules that have yet to be clearly defined.

In summary, we have shown that modulation of IL-10 in vivo using a mouse strain that naturally produces abundant IL-10 during infection with *M.tb* leads to stabilization of the lung bacterial load, extended survival, and enhanced T cell responses within the lung. It is important to recognize that the detection of an improved phenotype following the in vivo manipulation of one single immunological mediator is a highly significant finding. To date, in vivo removal of cytokines during *M.tb* infection have rarely shown a beneficial effect (26,27), and the depletion or absence of a single cytokine typically impairs resistance (22,23,25,28,29). Our finding that IL-10 blockade altered the CBA/J susceptibility phenotype of *M.tb* infection has particular relevance to studies in humans where a spectrum of susceptibility and immune responses can often be observed.

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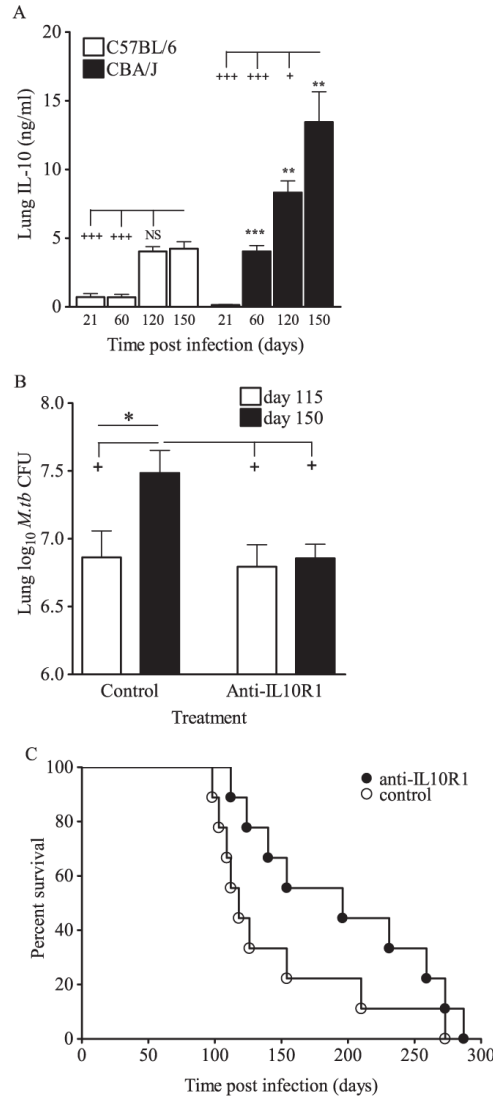
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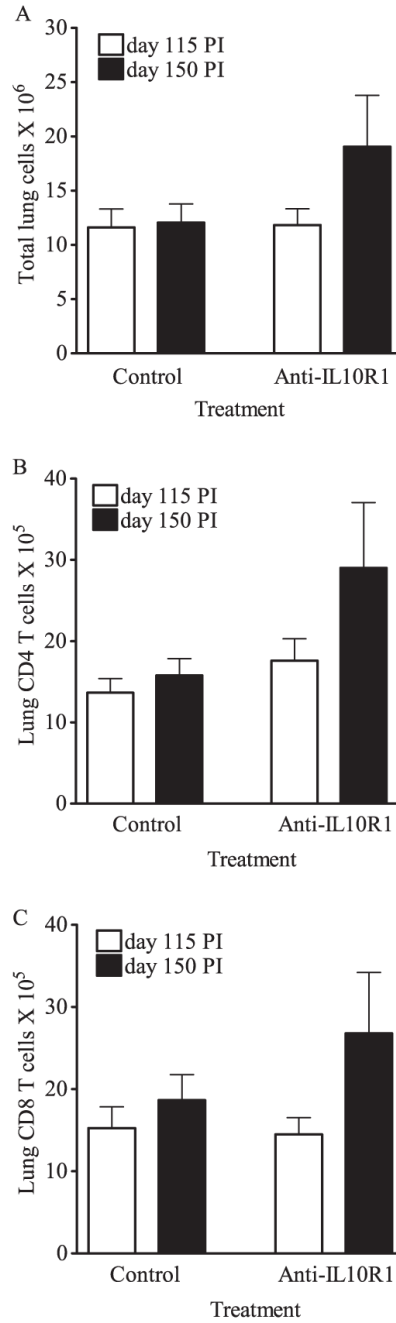
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**FIGURE 1.**

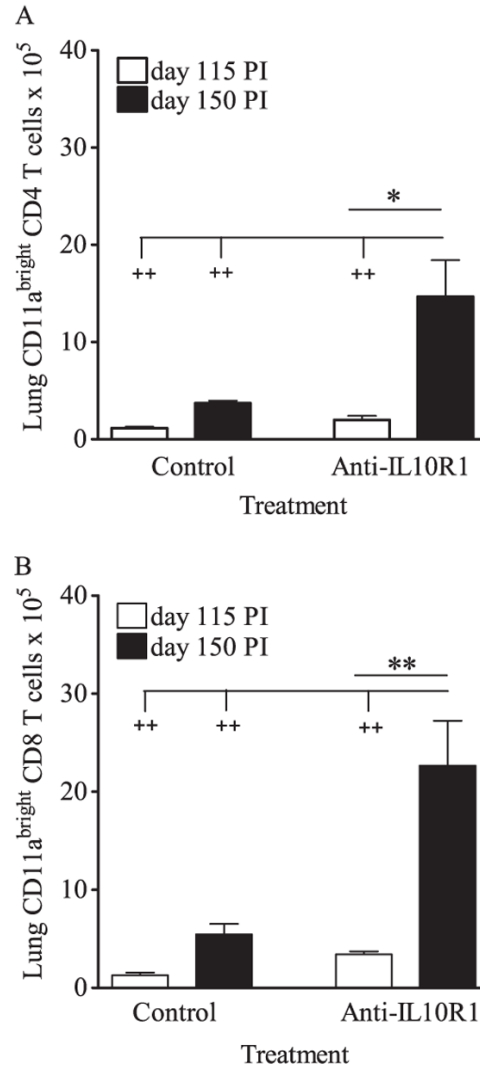
A role for IL-10 in driving disease progression in CBA/J mice. *A*, CBA/J and C57BL/6 mice were aerogenically infected with *M.tb* Erdman, euthanized at 21, 60, 120, and 150 days postinfection, lungs were homogenized, and IL-10 was detected by ELISA in clarified homogenates. Data are the means  $\pm$  SD of one experiment with four mice per strain per time point analyzed by Student's *t* test (\*\*,  $p < 0.01$  and \*\*\*,  $p < 0.001$ ) at each time point and by one-way ANOVA with Tukey's posttest for multigroup comparisons over time (+,  $p < 0.05$  and +++,  $p < 0.001$ ). *B*, CBA/J mice were aerogenically infected with *M.tb* Erdman and injected weekly with anti-IL-10R1 or control Ab starting at day 90 postinfection. Mice were euthanized at days 115 and 150, lungs homogenized and plated onto 7H11 agar, and *M.tb* CFU were determined after 21 days incubation at 37°C. Data are the means  $\pm$  SEM of five independent experiments each with three to six mice per treatment group per time point, analyzed pairwise by Student's *t* test (\*,  $p < 0.05$ ) and by one-way ANOVA with Tukey's posttest for multigroup comparisons (+,  $p < 0.05$ ). Total numbers of mice: control group 115 days,  $n = 26$ ; control group 150 days,  $n = 25$ ; anti-IL-10R1 group 115 days,  $n = 22$ ; anti-IL-10R1 group day 150,  $n = 22$ . For survival, separate groups of anti-IL-10R1-treated mice and controls were euthanized

when signs of morbidity developed (*C*) and statistical significance was determined by log-rank analysis. Survival data are combined from two independent experiments.

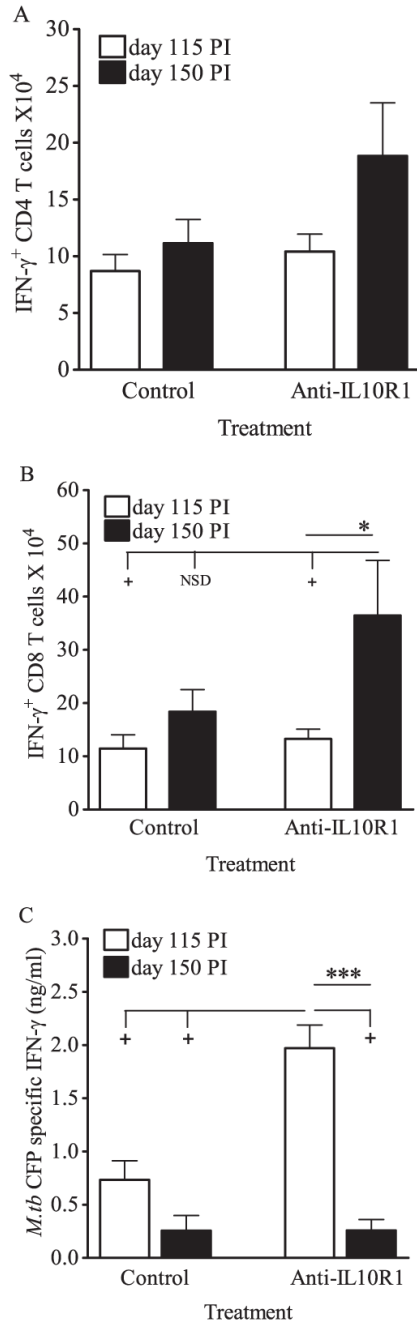
**FIGURE 2.**

Anti-IL-10R1 treatment increased lung cellularity during chronic *M.tb* infection in CBA/J mice. Mice were aerogenically infected with *M.tb* Erdman, injected weekly with anti-IL-10R1 or control Ab starting at day 90 postinfection, and euthanized at days 115 and 150. Isolated lung cells were counted, fixed, and labeled with anti-CD3, anti-CD4, and anti-CD8 for flow cytometric analysis. Total number of cells isolated (A), number of CD4 T cells (B), and number of CD8 T cells (C) are the means  $\pm$  SEM of five independent experiments, each with three to six mice per group per time point, analyzed by Student's *t* test for pairwise comparisons and one-way ANOVA with Tukey's posttest for multigroup comparisons. Total numbers of mice:

control group 115 days,  $n = 27$ ; control group 150 days,  $n = 24$ ; anti-IL-10R group 115 days,  $n = 24$ ; anti-IL-10R group day 150,  $n = 22$ .

**FIGURE 3.**

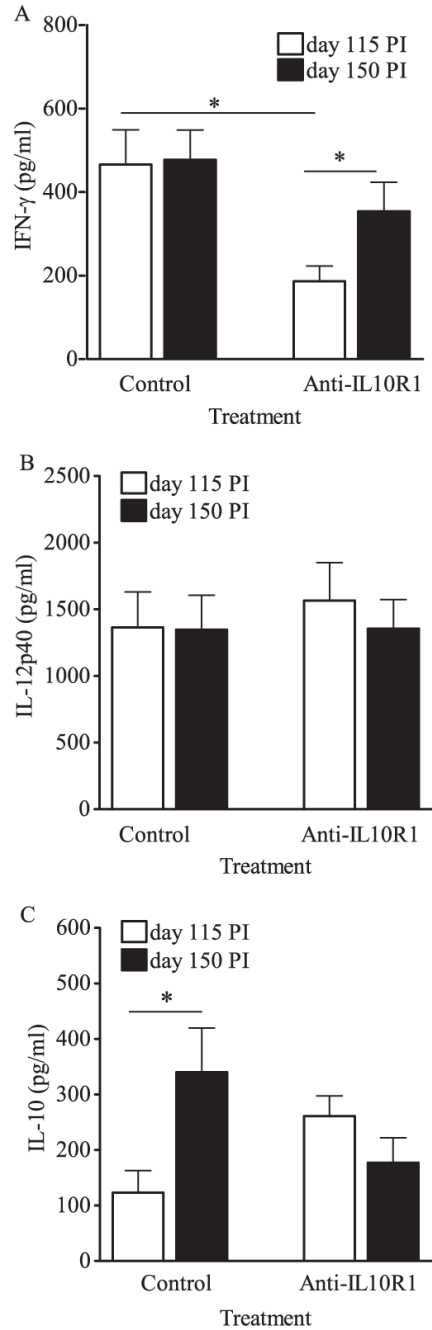
Anti-IL-10R1 treatment of CBA/J mice increased the numbers of CD11a<sup>bright</sup> T cells in the lung during chronic *M.tb* infection. Mice were aerogenically infected with *M.tb* Erdman, injected weekly with anti-IL-10R1 or control Ab starting at day 90 postinfection, and euthanized at days 115 and 150. Isolated lung cells were counted, fixed, and labeled with anti-CD3, anti-CD4, anti-CD8, and anti-CD11a for flow cytometric analysis. Numbers of CD11a<sup>bright</sup> CD4 (A) and CD8 (B) T cells are the means  $\pm$  SEM and are representative of two independent experiments, each with four to five mice per group per time point, with pairwise analyses using Student's *t* test (\*,  $p < 0.05$  and \*\*,  $p < 0.001$ ) or multigroup analysis using one-way ANOVA with Tukey's posttest (+,  $p < 0.05$ ; ++,  $p < 0.01$ ; +++,  $p < 0.001$ ).

**FIGURE 4.**

Anti-IL-10R1 treatment improved IFN- $\gamma$  production during chronic *M.tb* infection of CBA/J mice. Mice were aerogenically infected with *M.tb* Erdman, injected weekly with anti-IL-10R1 or control Ab starting at day 90 postinfection, and euthanized at days 115 and 150. Isolated lung cells were stimulated with anti-CD3 and anti-CD28 for 4 h, fixed, labeled with anti-TCR $\beta$ , anti-CD4, and anti-CD8, permeabilized, and labeled with anti-IFN- $\gamma$  for intracellular flow cytometry. Numbers of IFN- $\gamma$ <sup>+</sup> CD4 T cells (A) and IFN- $\gamma$ <sup>+</sup> CD8 T cells (B) are the means  $\pm$  SEM of four independent experiments, each with three to six mice per group per time point, with pairwise analyses using Student's *t* test (\*,  $p < 0.05$ ) and multigroup comparisons using one-way ANOVA (+,  $p < 0.05$ ; NSD, no significant difference). Total numbers of mice: control

group 115 days,  $n = 19$ ; control group 150 days,  $n = 16$ ; anti-IL-10R group 115 days,  $n = 20$ ; anti-IL-10R group day 150,  $n = 18$ . *C*, Isolated lung cells were stimulated *ex vivo* with *M.tb* culture filtrate protein, and secreted Ag-specific IFN- $\gamma$  in supernatants was quantified by ELISA. Data are the means  $\pm$  SEM of two independent experiments, each with four to five mice per treatment group per time point, with pairwise analyses by Student's *t* test (\*\*\*,  $p < 0.001$ ) and multigroup comparisons using one-way ANOVA (+,  $p < 0.05$ ). Total numbers of mice: control group 115 days,  $n = 9$ ; control group 150 days,  $n = 8$ ; anti-IL-10R group 115 days,  $n = 9$ ; anti-IL-10R group day 150,  $n = 8$ .



**FIGURE 5.**

Cytokines in lung tissue following anti-IL-10R1 treatment during chronic *M.tb* infection. CBA/J mice were aerogenically infected with *M.tb* Erdman and injected weekly with anti-IL-10R1 or control Ab starting at day 90 postinfection. Mice were euthanized at days 115 and 150, lungs homogenized, and IFN- $\gamma$  (A), IL-12p40 (B), and IL-10 (C) quantified by ELISA in clarified supernatants. Data shown are the means  $\pm$  SEM of three (A and C) or four (B) independent experiments, each with three to six mice per treatment group per time point, and analyzed in pairs by Student's *t* test (\*,  $p < 0.05$ ). Total numbers of mice (A and C): control group 115 days,  $n = 17$ ; control group 150 days,  $n = 17$ ; anti-IL-10R group 115 days,  $n = 14$ ; anti-IL-10R group

day 150,  $n = 14$ . Total numbers of mice ( $B$ ): control group 115 days,  $n = 22$ ; control group 150 days,  $n = 18$ ; anti-IL-10R group 115 days,  $n = 22$ ; anti-IL-10R group day 150,  $n = 17$ .