IL12RB1 is essential for human resistance to *Mycobacterium tuberculosis* infection. In the absence of a functional IL12RB1 allele, individuals exhibit susceptibility to disseminated, recurrent mycobacterial infections that are associated with defects in both RAG1-dependent and RAG1-independent hematopoietic lineages. Despite this well-established association, a causal relationship between *M. tuberculosis* susceptibility and IL12RB1 deficiency in either RAG1-dependent or RAG1-independent lineages has never been formally tested. Here, we use the low-dose aerosol model of experimental tuberculosis (TB) to both establish that infected *il12rb1−/−* mice recapitulate important aspects of TB in IL12RB1 null individuals and, more importantly, use radiation bone marrow chimeras to demonstrate that restriction of *il12rb1* deficiency solely to *rag1*-dependent lineages (i.e., T and B cells) allows for the full transfer of the *il12rb1−/−* phenotype. We further demonstrate that the protection afforded by adaptive lymphocyte *il12rb1* expression is mediated partially through *ifng* and that, within the same infection, *il12rb1*-sufficient T cells exhibit dominance over *il12rb1*-deficient T cells by enhancing *ifng* expression in the latter population. Collectively, our data establish a basic framework in which to understand how IL12RB1 promotes control of this significant human disease.

Despite tremendous efforts in the past and present to control tuberculosis (TB), infection with *Mycobacterium tuberculosis* remains a significant source of global morbidity and mortality. It is widely recognized that controlling the spread of this organism will require a combination of approaches, including the education of affected populations, provision of adequate public health infrastructure, decreasing the incidence of the AIDS comorbidity, and developing novel therapies for tuberculous individuals (57). The last approach will likely depend on our understanding the basic mechanisms that have evolved to protect humans against mycobacterial infection, the genes that underlie these mechanisms, and the cell lineages in which these genes must be expressed.

Among the genes that are well established as being essential for controlling *M. tuberculosis* infection is *IL12RB1* (2–4, 15, 16). That *IL12RB1* is required for controlling mycobacterial infection was first demonstrated by Altare et al. and de Jong et al. (4, 16), who reported case studies of *IL12RB1* null individuals from Mediterranean regions who had suffered from disseminated *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) infections. Since that time, the same association has been observed across multiple other ethnicities (15), demonstrating that *IL12RB1*’s influence in the dissemination of mycobacterial infections is nearly ubiquitous. *IL12RB1* encodes a minimum of two protein isoforms (56), one of which is IL-12RB1 (isoform 1), the type 1 integral membrane protein that serves as a low-affinity receptor for the p40 subunit of the cytokines interleukin-12 (IL-12), IL-23, and IL-12(p40), (10, 47, 51, 72). To confer high affinity and biological responsiveness to the cytokine IL-12 or IL-23, IL-12 receptor β1 (IL-12Rβ1) must both physically associate with each cytokine and signal in complex with either IL-12Rβ2 or IL-23R, respectively (73). The second isoform expressed from *IL12RB1* (IL-12Rβ1ΔTM, or isoform 2) is a product of alternative splicing, lacks the transmembrane (TM) domain of IL-12Rβ1, and contains a C-terminal sequence that is unique from that of IL-12Rβ1 (11, 56, 63). Unlike isoform 1, isoform 2’s contribution to IL-12 and IL-23 signaling is not yet known.

Despite *IL12RB1*’s long recognition as being important for *M. tuberculosis* control, the identification of which cell types must express IL12RB1 for pathogen control to occur has never been formally tested. Deducing this based on mRNA expression alone is not possible since during active TB the lungs are infiltrated by multiple hematopoietic cells expressing *IL12RB1* (69), including both RAG1-independent cell types (e.g., NK cells and dendritic cells) and RAG1-dependent cell types (e.g., αβ-T cells and B cells). Here, we use an established model of experimental TB to specifically test whether *rag1*-dependent lineages must express *il12rb1* (the mouse homolog of human IL12RB1) for tubercular control to occur, as well as the mechanisms by which *il12rb1* promotes the same. We further determine the specific contribution T cell gamma interferon (IFN-γ) expression plays in controlling *M. tuberculosis* infection, as well as the relative dominance of *il12rb1*+/+ versus *il12rb1*−/− T cells, during the same *M. tuberculosis* infection experiment as it pertains to production of IFN-γ. Our results suggest that positive regulators of T cell *IL12RB1* expression might serve as potential adjunctive therapies for tuberculous individuals.

**MATERIALS AND METHODS**

Mice. Mice were bred at the Medical College of Wisconsin (MCW) in the MCW Biomedical Resource Center and were treated according to National Institutes of Health and MCW Institute Animal Care and Use Committee (IACUC) guidelines. C57BL/6, B6.129S7-*Rag1tm1Mom/J* (i.e., *rag1*−/− mice), B6.129S7-*Ifngtm1Z/J* (i.e., *ifng*−/− mice), and B6.SJL-Plt-Preα*Pepz/J* (Boy) mice were originally purchased...
from the Jackson Laboratory (Bar Harbor, ME). B6.129S1-Il2rb<sup>tm1jm</sup>/Il2rb<sup>−/−</sup> mice (74) were kindly provided by Andrea M. Cooper (Trudeau Institute, Saranac Lake, NY). IFN-γ–enhanced yellow fluorescent protein (eYFP) reporter mice (i.e., Yeti mice) (64) were kindly provided by Markus Mohra (Trudeau Institute, Saranac Lake, NY) and Richard Locksley (Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA).

**Genotyping and background analysis.** Il2rb<sup>−/−</sup> mice were genotyped using primers we designed specifically for this study due to difficulties we encountered amplifying wild-type (WT) Il2rb<sup>−/−</sup> with the published primer sets (data not shown). Our protocol is detailed here. To screen for the presence of a wild-type il2rb<sup>−/−</sup> allele, genomic DNA (gDNA) from tail snips was isolated using the Promega Wizard SV Genomic DNA method (Promega, Madison, WI). Amplification was performed with primer NF1 (sequence above) and NF3 (5′-TATGGTTCCGGAGGGACAAAA-3′) in a 50-μl reaction mixture comprising 2 μl of gDNA, 1.5× NEB Taq buffer (from a 10× solution), 2 mM MgCl<sub>2</sub>, 0.2 μM primer NF1, 0.2 μM primer NF2, 0.2 μM each deoxynucleoside triphosphate (dNTP), 5% dimethyl sulfoxide (DMSO), and 1 U of Taq polymerase (New England BioLabs, Ipswich, MA). For the knockout (KO) il2rb<sup>−/−</sup> allele, gDNA was amplified with primers NF1 (sequence above) and NF3 (5′-GGATGTGGAGAATGTTGCGGAGG-3′) in a 50-μl reaction mixture comprising 2 μl of gDNA, 1.0× NEB Taq buffer (from a 10× solution), 2 mM MgCl<sub>2</sub>, 0.2 μM primer NF1, 0.2 μM primer NF2, 0.2 μM each dNTP, 5% DMSO, and 1 U of Taq polymerase. The parameters for amplification of both wild-type and knockout il2rb<sup>−/−</sup> alleles were the following: one cycle of 95° for 5 min; 35 cycles of 95° for 30 s, 60° for 1 min, and 72° for 1 min; one cycle of 72° for 10 min. Amplicons were resolved on a 1% agarose gel using traditional electrophoresis techniques.

Prior to their being used to generate radiation bone marrow chimeras, the extent to which il2rb<sup>−/−</sup> mice had been backcrossed to the C57BL/6 background was tested by the DartMouse Speed Congenic Core Facility at Dartmouth Medical School (Hanover, NH). DartMouse uses an Illumina, Inc. (San Diego, CA) GoldenGate Genotyping Assay to interrogate 1,449 single nucleotide polymorphisms (SNPs) spread throughout the genome. The raw SNP data were analyzed using DartMouse’s SNP-Map and MapSynth software, allowing the determination for each mouse of the genetic background at each SNP location. This analysis demonstrated that individual il2rb<sup>−/−</sup> mice had between 95%–98% C57BL/6 background and, consequently, were used for the generation of radiation bone marrow chimeras.

**Radiation bone marrow chimeras.** Bone marrow cells from either C57BL/6, il2rb<sup>−/−</sup>, rag1<sup>−/−</sup>, ifng<sup>−/−</sup>, or CD45.1 congenic donors were harvested via perfusion of the femur and tibia medullary cavities with complete Dulbecco’s modified Eagle’s medium (DMEM). Marrow suspensions were pelleted and subsequently resuspended in red blood cell (RBC) lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃) to remove red blood cells; following RBC lysis, the marrow was washed and resuspended at 5 × 10<sup>7</sup> cells/ml in sterile phosphate-buffered saline (PBS). As indicated in Results and figure legends, for some experiments, admixtures of select marrow preparations were made after normalizing all preparations to the same cell concentration. Recipient mice received 2.5 × 5 Gy (500 rads) of whole-body irradiation 3 h apart in a Gammachell Irradiator (1,000 rads total). Immediately following the second dose, mice were injected intravenously (i.v.) with 200 μl of marrow preparation (i.e., 1 × 10<sup>7</sup> total bone marrow cells). Mice were allowed 6 weeks to reconstitute prior to their use in experiments. Two weeks prior to experimental infection, bone marrow recipients were taken off antibiotic-containing food.

**Experimental infection.** The H37Rv strain of M. tuberculosis (kindly sent to us by Andrea Cooper) was grown in Proskauer Beck medium containing 0.05% Tween 80 to mid-log phase and frozen in 1-ml aliquots at −70°C. For aerosol delivery of ~80 bacteria, animals were placed in a Glas-Col Inhalation Exposure System (Glas-Col, Terre Haute, IN) at a maximum of 20 mice per sector. After the nebulizer (Glas-Col) was loaded with 10 ml of diluted H37Rv (5 × 10<sup>7</sup> CFU/ml in deionized water), mice were infected using the following exposure settings: 900 s for warm-up, 3,600 s to nebulize, 1,800 s for cloud decay, 900 s for UV exposure (vacuum pressure, 50; compressed-air pressure, 15). Immediately after infection, mice were placed in biocartridge unit (BCU) cages and subsequently monitored during the period of infection for outward signs of distress, per IACUC oversight. Lungs from a group of control mice were plated at day 1 postinfection to confirm the delivery ~80 CFU.

**Bacterial load determination.** Infected mice were euthanized by CO₂ asphyxiation; lungs, spleen, and liver were aseptically removed and individually homogenized in sterile normal saline using the Gentle Macs system, program RNA1.1 (Miltenyi, Bergisch Gladbach, Germany). The Gentle Macs system was used to increase containment of infectious aerosols generated during the homogenization process; efficient homogenization using RNA1.1 was ensured by comparing our M. tuberculosis CFU counts to those of other investigators using a traditional polytetrafluoroethylene (PTFE) pestle/borosilicate glass tube system (personal communication, Robert North, Trudeau Institute, Saranac Lake, NY). Serial dilutions of the organ homogeneous were plated on nutrient 7H11 agar. The number of mycobacterial CFU was determined after plates were incubated for 2 weeks at 37°C in 7% CO₂.

**Quantitative PCR analysis.** Lung RNA was extracted from infected, snap-frozen tissue by homogenizing the tissue in RT lysis buffer (Qiagen, Germantown, MD) using the Gentle Macs system, program RNA2.1 (Miltenyi). Total RNA was extracted from the lysis solution according to the manufacturer’s protocol. RNA samples from each group/time point were reverse transcribed using Fermentas reagents (Thermo Scientific, Glen Burnie, MD). cDNA was then amplified using SYBR green reagents (Fermentas) on a Bio-Rad iQ5 detection system; threshold cycle (C<sub>T</sub>) values were determined using the Bio-Rad iQ5 bundled software (Bio-Rad, Hercules, CA). The expression levels of select mRNAs (e.g., ifng) relative to expression of the housekeeping gene gapdh were determined using the ΔC<sub>T</sub> calculation recommended by the manufacturer. The forward (F) and reverse (R) primers used for real-time amplification of cDNA samples were as follows (5′→3′): gapdh, (F) CATTGGCCCTCTCGGT TCCCCTA and (R) GGCAGAOGTCAGATCCA; ifng, (F) CATTGGTCAGCTGGTTGACCAAGA and (R) TGGCTCTCGAGATTTCTTCTAG; mifn, (F) CTTGTACCGCACGGTCTTAG and (R) GGGTAGTACAGAAGTTTAC AACC; nos2, (F) GGGAGCGCTTCAGAAGCTTTG and (R) GACATT GGAAAGAAGGCTTTC; rmg1, (F) AGACAGCTATTGCTGCCTCGTA and (R) CCGTGTGTTGATGCAGTGCACAA.

**Cell preparations.** Lung cell suspensions were prepared by perfusing heparin-containing PBS through the mouse heart until the lungs appeared white, whereupon they were removed and sectioned in incomplete DMEM. Dissected lung tissue was then incubated in DMEM containing collagenase IX (0.7 mg/ml) and DNase (30 μg/ml) at 37°C for 30 min. Digested lung tissue was gently homogenized and passed through a 70-μm-pore-size nylon tissue strainer; the resultant single-cell suspension was treated with RBC lysis solution, washed, and counted. Cells prepared in this way were subsequently used for flow cytometric analyses or further magnetic purification. For collection of bronchoalveolar lavage (BAL) cells, serial inflation and flushing of the lungs (via the trachea) with 400 μl of Dulbecco’s PBS (DPBS) were performed; collected cells were directly used for staining and flow cytometric analysis.

**Flow cytometry.** All antibodies used for flow cytometric analysis were purchased from BD Pharmingen (San Diego, CA). Lung cell preparations were washed with fluorescence-activated cell sorting (FACS) buffer (2% fetal calf serum [FCS] in PBS) and stained with antibodies recognizing either Thy1 (clone 30-H12), NK1.1 (clone PK136), CD11c (clone HL3), CD45.1 (clone A20), CD45.2 (clone HIS41), αβ-T-cell receptor (TCR) (clone GL3), CD4 (clone L3T4), CD8 (clone 53-67-2), CD19 (clone MB19-1) and Gr1 (clone RB6-8C5). Acquired data were analyzed with FlowJo software (Tree Star Inc., Ashland, OR).

For intracellular cytokine analyses, cells were collected, washed, and placed in a V-bottom, 96-well plate in complete medium with 50 ng/ml
phorbol myristate acetate (PMA; Sigma-Aldrich) and 1 μg/ml ionomycin (Sigma-Aldrich). For stimulation of NK cells, both recombinant IL-12 and IL-18 were added to select wells in addition to PMA-ionomycin. Cells were placed in a 37°C incubator for 4 h; brefeldin A (Sigma-Aldrich) was added at 5 μg/ml for the final 2 h. After cells were washed, they were fixed in 4% formaldehyde in PBS. Cells were made permeable in 0.1% saponin (Sigma-Aldrich) in PBS with 2% FCS and stained with phycoerythrin (PE)-conjugated anti-IFN-γ (clone XMG1.2). Bromodeoxyuridine (BrdU) staining was performed using a fluorescein isothiocyanate (FITC) BrdU flow kit system 2 days after intraperitoneal (i.p.) administration of BrdU (BD Pharmingen); intracellular Tbet and Foxp3 staining was similarly assessed using commercially available antibodies and reagents (BD Pharmingen) according to the manufacturer’s instructions. After all staining, cells were washed twice and acquired on a biosafety cabinet (BSC)-containing Guava 8HT flow cytometer (Millipore).

**Western analysis of purified lung lineages.** For lineage purification and protein expression analysis of IL-12Rβ1 protein, cell preparations from *M. tuberculosis*-infected lungs (day 30 postinfection) were magnetically sorted using the Miltenyi system into either CD45-negative (CD45<sup>−</sup>), CD45<sup>+</sup>, I-A<sup>−</sup>+, CD11b<sup>−</sup>, Ly6G<sup>−</sup>, CD11c<sup>+</sup>, NK1.1<sup>+</sup>, or Thy1<sup>+</sup> fractions according to the manufacturer’s protocols. Afterwards, cells were lysed in β-mercaptoethanol containing lithium dodecyl sulfate (LDS) sample buffer (Invitrogen) by boiling for 1 h. Denatured lysates were then run on a 4 to 12% bis-Tris gel (Invitrogen), transferred to polyvinylidene difluoride (PVDF) membrane, and blotted with polyclonal anti-mouse IL-12Rβ1 protein, cell preparations

**Histological analysis.** Lungs were inflated through the trachea with 10% neutral buffered formalin, resected, and paraffin embedded; subsequently generated sections were stained with either Masson’s trichrome or with carbol fuchsin (i.e., Ziehl-Neelsen stain). Once slides were generated, subsequently generated sections were stained with either Masson’s trichrome or with carbol fuchsin (i.e., Ziehl-Neelsen stain). Once slides were generated, primary antibodies were probed with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody.

**Statistical analysis.** Figures were prepared using GraphPad Prism, version 5.0a. Statistical analyses used the bundled software. Bars in the figures show means plus standard deviations (SD). Numbers shown between data points represent *P* values for the comparisons indicated on the figures. Statistical comparisons involving more than two experimental groups used analysis of variance (ANOVA). All other statistical comparisons used Student’s *t* test.

**RESULTS**

**il12rb1 is required to control pulmonary *M. tuberculosis* infection.** IL12RB1 null individuals display an enhanced susceptibility to mycobacterial infections that is associated with impaired lymphocyte expression of IFNγ (16). Prior to using the low-dose aerosol mouse model of TB (48) to determine which lineages must express IL12RB1 (the mouse homolog of IL12RB1) for mycobacterial control to occur, it was necessary to first determine the extent to which *il12rb1<sup>−/−</sup>* mice recapitulated the phenotype of IL12RB1 null individuals. For this, *il12rb1<sup>−/−</sup>* mice (74) were aerogenetically infected with a low dose (~80 CFU) of the virulent *M. tuberculosis* strain H37Rv; at select times postinfection, lungs were assessed for bacterial burden, the presence of pulmonary pathology, and the expression of select genes known to promote tubercular control.

Consistent with historical observations using this model (46), *M. tuberculosis* burden progressively grew in wild-type mice for ~20 days, after which time growth was inhibited, and infection was held at a mostly stationary level (Fig. 1A). In contrast, *M. tuberculosis* growth in *il12rb1<sup>−/−</sup>* lungs continued to increase past 20 days to levels greater than those in wild-type controls (Fig. 1A). Regarding the localization of *M. tuberculosis* in the infected lungs, consistent with the extensive survey of Rhoades et al. (55), at day 20 postinfection acid-fast bacilli (AFB) in wild-type lungs were difficult to locate but, when found, were restricted to macrophages within inflamed alveoli (Fig. 2A). In *il12rb1<sup>−/−</sup>* mice, however, AFB could be found not only near alveoli (Fig. 2B and C) but also near sites of potential dissemination, including the lung vasculature (Fig. 2Bii) and bronchioles (Fig. 2Biii). While the majority of AFB in *il12rb1<sup>−/−</sup>* lungs were macrophage associated, instances of AFB associating with both bronchiolar (Fig. 2Bia and b) and alveolar epithelial cells (Fig. 2Biii) were observed. By 50 days postinfection, bacterial burdens were nearly 100-fold
higher in the lungs of il12rb1−/− mice than in wild-type controls (Fig. 1A). Following the dissemination of M. tuberculosis from the lungs (an event that occurs approximately 10 days postinfection in wild-type animals [52]), bacterial growth continued to remain unhindered in both the spleen and liver of il12rb1−/− mice (Fig. 1B and C). Collectively, these results demonstrate that il12rb1−/− mice are similar to IL12RB1 null individuals in their susceptibility to mycobacterial infection as measured by M. tuberculosis burden.

Granulomatous accumulations in il12rb1−/− mice are abnormal and associate with decreased transcription of ifng and nos2. Like individuals with functional IL12RB1 alleles, IL12RB1 null individuals develop granulomas in response to mycobacterial infection (4, 16). The composition of and number of AFB in these granulomas, however, are different than those of granulomas in individuals with intact IL12RB1 alleles (4, 16). As an additional measure of TB susceptibility in il12rb1−/− mice, therefore, we assessed the extent of pulmonary pathology in il12rb1−/− mice.
and compared it to that observed in wild-type controls (19, 37). While it is widely recognized that M. tuberculosis-infected mice do not develop classical granulomas (6), pulmonary leukocyte accumulations do, nevertheless, develop in mice that comprise many of the same lineages and proinflammatory cytokines found in human granulomas (55).

In wild-type mice, granulomatous accumulations developed over the first 50 days of infection in a manner identical to that previously described (55) (data not shown). In il12rb1−/− mice, however, these accumulations were characterized by several features not observed in wild-type controls (Fig. 3). Specifically, accumulations in il12rb1−/− mice contained necrotic centers (Fig. 3Ai and C) and abundant numbers of AFB (Fig. 3B, primarily found near a collagenous perimeter of the necrotic foci) as well as occasional pulmonary ossifications (Fig. 3Aii). As was also observed at day 20 postinfection (Fig. 2Bii to iii), AFB in il12rb1−/− mice could be seen directly associating with or near bronchial epithelia (Fig. 3D) and vascular endothelia (Fig. 3E). Regarding the expression of host-protective genes in these same lungs, beginning at day 30 postinfection, fold increase in the mRNA levels of host-protective ifng and nos2 were all lower in il12rb1−/− lungs than in those of wild-type controls (Fig. 4A and B). Notably, mRNA levels of irgm1 were unaffected by il12rb1 deficiency (Fig. 4C), and levels of tnfa mRNA were increased compared to those expressed in wild-type animals at days 40 and 50 postinfection (Fig. 4D). These data, when combined with that of bacterial burden (Fig. 1), collectively demonstrate that il12rb1 is required to both control pulmonary M. tuberculosis infection and regulate the development of pulmonary pathology. Furthermore, given the similar disease characteristics in il12rb1−/− mice and IL12RB1 null individuals, we also conclude that the mouse model is suitable for testing which lineage(s) must express IL12RB1 for tubercular control to occur.

**il12rb1 expression by rag1-dependent lineages is required to control M. tuberculosis infection.** The functions of rag1-dependent lineages are compromised in the absence of IL12RB1 (14, 36). Whether il12rb1 expression by rag1-dependent lineages is required to control M. tuberculosis infection, however, has never been formally tested. To do this, we generated radiation bone marrow chimeras in which il12rb1 deficiency was restricted to

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**FIG 3** Granulomatous accumulations in il12rb1−/− mice are distinct from those found in wild-type mice. Fifty days following aerogenic infection with M. tuberculosis, lung sections from C57BL/6 and il12rb1−/− mice were stained with Masson’s trichrome in order to visually assess the degree of pulmonary pathology. Shown are representative micrographs of features observed in il12rb1−/− mice that were not present in C57BL/6 controls. (A) Micrograph at a ×4 magnification of an il12rb1−/− lung at day 50 postinfection showing the presence of two densely red staining regions, one of which is shown at ×10 magnification in the inset below (i). Shown in inset ii is a ×40 magnification of a pulmonary ossification found in the same il12rb1−/− lung. (B) An acid-fast stain of a serial section located adjacent to the trichrome-stained region shown in panel Ai was used to visualize the localization of AFB relative to the collagenous (i.e., blue) perimeter seen in panel Ai. (C to E) Micrographs at ×100 magnification of select features observed in panel B, namely, the presence of AFB in the necrotic center (C), AFB on or near bronchial epithelium (D), and AFB near the vascular epithelium (E).

**FIG 4** M. tuberculosis-induced expression of host-protective ifng and nos2 is lower in il12rb1−/− mice. mRNA from the lungs of M. tuberculosis-infected C57BL/6 and il12rb1−/− mice was collected at select times postinfection; subsequently generated cDNA was used for real-time quantitation of ifng (A), nos2 (B), irgm1 (C), and tnfa (D) expression levels. Expression of each gene was normalized to that of gapdh in the same sample; data were analyzed via ΔΔCt analysis (7) and is expressed as fold expression over the levels found in genotype-matched uninfected controls. Each data point represents the mean ± SD of the values at each time point and show one experiment representative of a total of three. For the difference between values of il12rb1−/− relative to C57BL/6 mice, *P* = 0.05 (*) by Student’s t test.
**FIG 5** il12rb1 expression by rag1-dependent lineages is required to control *M. tuberculosis* infection. Lethally irradiated rag1−/− mice were reconstituted with one of either of the following bone marrow preparations: 100% C57BL/6, 80% rag1−/− and 20% il12rb1−/− cells (T/Bil22rb1WT mice), 100% il12rb1−/−, or 80% rag1−/− and 20% il12rb1−/− (T/Bil22rb1KO mice) cells. All chimeras were simultaneously infected via aerosol with *M. tuberculosis* H37Rv. Fifty days postinfection with *M. tuberculosis*, bacterial burdens in the lungs (A), spleen (B), and liver (C) were determined. Each bar represents the mean ± SD of the values observed and show one experiment representative of a total of three. ∗, *P* ≤ 0.05, between the values of two groups as determined by ANOVA; ns, not significant. (D and E) To confirm that innate lineages retained il12rb1 expression in T/Bil22rb1KO mice, CD45.1 congenic mice were used as il12rb1−/− donors so as to determine, via flow cytometry of both lung and BAL cell preparations, the percentage of NK1.1+ (D, top row) and CD11c+ (D, bottom row) lineages that were both significantly higher than those observed in T/Bil22rb1WT mice (Fig. 5Band C). In the spleen and liver, bacterial burdens in T/Bil22rb1KO mice were also above those of T/Bil22rb1WT mice and equal to those levels found in Rag1−/− mice. For controls, we used lethally irradiated Rag1−/− mice reconstituted with either 100% C57BL/6 bone marrow, 100% il12rb1−/− bone marrow, or a bone marrow admixture comprising 80% rag1−/− and 20% il12rb1−/− cells (T/Bil22rb1WT mice). For generating all chimeras, we confirmed that the majority of both NK1.1+ and CD11c+ lineages in T/Bil22rb1KO mice were il12rb1−/− sufficient (Fig. 5D); Western analysis of magnetically sorted lineages also confirmed that innate I-Aα+, CD11b+, Ly6G+, CD11c+, and NK1.1+ lineages of infected T/Bil22rb1KO mice expressed IL-12Rβ1 protein (Fig. 5E). As anticipated, Thy1+ cells in the same T/Bil22rb1KO mice did not express IL-12Rβ1; supporting a role for hematopoietic il12rb1 expression, the CD45+ fraction of *M. tuberculosis*-infected lungs did not express IL-12Rβ1 protein (Fig. 5E). From these data, we conclude that il12rb1 expression by rag1-dependent lineages is required to control *M. tuberculosis* infection and that restriction of il12rb1 deficiency solely to rag1-dependent lineages allows for the full transfer of the il12rb1−/− phenotype.

*ifng* expression by rag1-dependent lineages contributes to control of *M. tuberculosis* infection. A well-known consequence of IL-12 signaling in hematopoietic lineages is production of IFN-γ. IFN-γ is essential for control of experimental TB (12, 22) and, as demonstrated by flow cytometric analysis of Yeti mouse lungs (64), is solely produced by hematopoietic cells following *M. tuberculosis* infection (Fig. 6A and B), including CD4+ T cells, CD8+ T cells, NK1.1+ cells, and GR1+ granulocytes (Fig. 6C and D). The contribution of each lineage to the total IFN-γ response changes over the course of infection (Fig. 6D). Of particular note is the decline in NK cell representation following *M. tuberculosis* infection and the concomitant increase in representation of CD4+, CD8+, and GR1+ cells (Fig. 6D). That most NK cells con-
sentative histograms for each subset. Shown are data from one experiment that
above) radiation bone marrow chimeras comprising D20, day 20 postinfection; D50, day 50 postinfection; UI, un-
and 20% significantly higher than those of T/B
controls beginning 30
these data
demonstrate that
is representative of a total of two (four mice per experiment). (D) Cumulative
data demonstrating the percentage of CD45 YFP + cells expressing either of
the following lineage markers at indicated times following infection: CD4,
CD8, NK1.1, GR1, CD19, or γδ TCR. Data are combined from two separate
experiments, with four mice per time point per experiment; bars represent
the mean percentage of YFP + events expressing each marker ± SD. A significant
difference (P ≤ 0.05) between two time points (for a given lineage) is indicated
by an asterisk. D20, day 20 postinfection; D50, day 50 postinfection; UI, un-
ected during the first 21 days of infection. In the low-dose aerogenic model of
TB, however, ~20 days postinfection is the very earliest point at
which all IFN-γ-producing T cells have arrived into the lungs of
M. tuberculosis-infected animals (31, 53). More importantly, sig-
ificant differences between wild-type and T-cell-deficient (or even
ifngr-deficient) mice have historically not been until
after the 20-day time point (Fig. 8A) (17, 41). To resolve this
issue and directly test whether IFN-γ expression by rag1-dependent
lineages was required to control primary M. tuberculosis infection, we generated (in a manner analogous to that described
above) radiation bone marrow chimeras comprising rag1-deficient
hosts reconstituted with a bone marrow admixture of either 80%
rag1-deficient and 20% ifng KO cells (T/B ifng KO mice) or 80% rag1-deficient and 20%
ifng KO cells (T/B ifng WT mice). As is shown in Fig. 8A,
at 20 days postinfection, lung bacterial burdens in T/B ifng KO and
T/B ifng WT mice were equal. By 40 days postinfection, however,
T/B ifng KO mice exhibited burdens in the lung that were signifi-
cantly higher than those of T/B ifng WT controls (Fig. 8A). A similar pattern was observed in the spleen and liver following M. tubercu-
losis dissemination to these organs, with T/B ifng KO mice exhibiting
bureaus in the spleen (Fig. 8B) and liver (Fig. 8C) that were signi-
icantly higher than those of T/B ifng WT controls beginning 30
days postinfection. Despite being higher than T/B ifng WT controls,
however, bacterial burdens in T/B ifng KO mice were significantly
less than those in complete (100%) ifng KO mice in all organs
tested (Fig. 8A to C). The gross visible abnormalities associated
with M. tuberculosis-infected ifng KO lungs (22) were also not
observed in T/B ifng KO mice (data not shown). Collectively, these data
demonstrate that ifng expression by rag1-dependent lineages con-
tributes to tubercular control; however, the absence of ifng
in rag1-defendent lineages does not completely account for the
phenotype of ifng KO mice.

FIG 6 ifng is expressed by multiple hematopoietic subsets during experimen-
tal TB. C57BL/6 and IFN-γ–eYFP reporter mice (i.e., Yeti mice) (54) were
infected via aerosol with M. tuberculosis. At 50 days postinfection, lung cell
preparations were stained with a panel of antibodies for FACS determination
of which cells were expressing ifng at that point in time. Using a gating strategy
based on their forward scatter and side scatter characteristics, CD45-stained
C57BL/6 cells served as a negative control for YFP fluorescence (A) while Yeti
cells demonstrated all YFP signal to be found among CD45 YFP + cells (B). (C)
Among CD45 YFP + cells, we surveyed the extent to which CD4 +, CD8 +,
NK1.1 +, GR1 +, CD19 +, or γδ TCR + events were present. Shown are repre-
sentative histograms for each subset. Shown are data from one experiment that
is representative of a total of four (two mice per experiment). (D) Cumulative
T cells are dominant over \textit{il12rb1}\textsuperscript{−/−} T cells during \textit{M. tuberculosis} infection. Given the importance of T-cell \textit{ifng} expression to \textit{M. tuberculosis} control (Fig. 8), we wished to determine the relative dominance of \textit{il12rb1}\textsuperscript{−/−} T cells versus \textit{il12rb1}\textsuperscript{+/+} T cells as it pertains to production of IFN-\(\gamma\). In other words, when \textit{il12rb1}\textsuperscript{−/−} and \textit{il12rb1}\textsuperscript{+/+} T cells coexist within the same infection, does the presence of \textit{il12rb1}\textsuperscript{−/−} T cells influence the expression of IFN-\(\gamma\) by \textit{il12rb1}\textsuperscript{+/+} T cells, and vice versa?

\textit{il12rb1}\textsuperscript{+/+} T cells are dominant over \textit{il12rb1}\textsuperscript{−/−} T cells during \textit{M. tuberculosis} infection. Given the importance of T-cell \textit{ifng} expression to \textit{M. tuberculosis} control (Fig. 8), we wished to determine the relative dominance of \textit{il12rb1}\textsuperscript{+/+} T cells versus \textit{il12rb1}\textsuperscript{−/−} T cells as it pertains to production of IFN-\(\gamma\). In other words, when \textit{il12rb1}\textsuperscript{+/+} and \textit{il12rb1}\textsuperscript{−/−} T cells coexist within the same infection, does the presence of \textit{il12rb1}\textsuperscript{+/+} T cells influence the expression of IFN-\(\gamma\) by \textit{il12rb1}\textsuperscript{−/−} T cells, and vice versa?

**FIG 7** \textit{il12rb1} positively regulates T cell \textit{ifng} expression following \textit{M. tuberculosis} infection. Lung cell preparations from C57BL/6 and \textit{il12rb1}\textsuperscript{−/−} mice that were either uninfected (A) or \textit{M. tuberculosis} infected (50 days postinfection) (B) were stained for T-cell marker Thy1 and intracellular IFN-\(\gamma\). Isotype control staining (i.e., the top row) was used to discriminate positive staining for IFN-\(\gamma\). Shown are representative FACS dot plots from each condition; inside the plots of \textit{il12rb1}\textsuperscript{−/−} cells (i.e., the bottom row) are box gates indicating the percentage of Thy1\textsuperscript{+} cells positive for IFN-\(\gamma\). (C) Cumulative data demonstrating the percentage of Thy1\textsuperscript{+} cells expressing IFN-\(\gamma\), as determined by intracellular cytokine staining, in both C57BL/6 and \textit{il12rb1}\textsuperscript{−/−} lungs after 50 days of \textit{M. tuberculosis} infection (Mtb; solid bars). Data from uninfected (UI) lungs (open bars) are also shown. Data are combined from three separate experiments, with four mice per time point per experiment; bars represent the mean percentage ± SD. A significant difference (\(P \leq 0.05\)) between uninfected and infected animals is indicated by an asterisk.

**FIG 8** \textit{ifng} expression by \textit{rag1}-dependent lineages contributes to \textit{M. tuberculosis} control. (A) Lethally irradiated \textit{rag1}\textsuperscript{−/−} mice were reconstituted with either 100% C57BL/6 or 100% \textit{ifng}\textsuperscript{−/−} bone marrow; 4 weeks later, these chimeras were aerosol infected with \textit{M. tuberculosis} H37Rv alongside \textit{ifng}\textsuperscript{−/−} controls. At select times postinfection, lung \textit{M. tuberculosis} burdens were determined. *, statistically significant difference from C57BL/6 or \textit{rag1}\textsuperscript{−/−} controls. (B to D) Lethally irradiated \textit{rag1}\textsuperscript{−/−} mice were reconstituted with one of either of the following bone marrow preparations: 100% C57BL/6, 80% \textit{rag1}\textsuperscript{−/−} and 20% \textit{ifng}\textsuperscript{−/−} cells (T/B\textit{ifngWT} mice), or 80% \textit{rag1}\textsuperscript{−/−} and 20% \textit{ifng}\textsuperscript{−/−} (T/B\textit{ifngKO} mice). All chimeras were simultaneously infected via aerosol with \textit{M. tuberculosis} H37Rv. At indicated times following infection, \textit{M. tuberculosis} burdens in the lungs (B), spleen (C), and liver (D) were determined. Each data point in panels A to D represents the mean ± SD of the values observed, and panels show one experiment representative of a total of two. *, \(P \leq 0.05\), between indicated groups as determined by ANOVA of all data points collected at that particular time.
**FIG 9** *il12rb1*+/* T cells exhibit dominance over *il12rb1*−/* T cells during *M. tuberculosis* infection. Lethally irradiated *rag1*−/− mice were reconstituted with one of either of the following bone marrow preparations: 80% *rag1*−/− and 20% *CD45.1*::*il12rb1*+/* (T/B*il12rb1WT*), 80% *rag1*−/− and 20% *il12rb1*−/* (T/B*il12rb1KO*), or 80% *rag1*−/−, 10% *CD45.1*::*il12rb1*+/*, and 10% *il12rb1*−/* (T/B*il12rb150/50*) cells. Following *M. tuberculosis* infection, we determined the relative percentage of IFN-γ cells among cells of either the *il12rb1*+/* (i.e., CD45.1*np*so*) or *il12rb1*−/* (i.e., CD45.1*n*eg*) genotype at day 50 postinfection. Shown in panel A is our gating strategy for identification of T lymphocytes. Examination of lung T lymphocytes in *M. tuberculosis*-infected T/B*il12rb1WT* (B) and T/B*il12rb1KO* (C) mice established the percentages of T cells expressing IFN-γ in the absence of T cells of a different genotype, whether they be CD45.2*neg* CD45.1*np* *il12rb1*+/* cells (F) or CD45.2*np* CD45.1*neg* *il12rb1*−/* cells (G). (D and E) The same analysis was extended to T/B*il12rb150/50* mice that had been either left uninfected (D) or *M. tuberculosis* infected (E) at the same time as the controls shown in panels B and C. Box gates in panels B to E indicate the percentages of IFN-γ events among T cells of each genotype. (H) IFN-γ expression by lung NK cells 30 days after *M. tuberculosis* infection in both T/B*il12rb1WT* (left) and T/B*il12rb1KO* (right) chimeric
test this, we generated and infected bone marrow chimeras in which half of all T/B-cell lineages were il12rb1+/+ and half were il12rb1−/− (hereinafter referred to as T/B il12rb1+/+10G to L) T cells were examined in this manner. Regarding CD4 (Fig. 9E and I). Unexpectedly, we also observed that expression of the master transcription factors Tbet and Foxp3, il12rb1 WT mice were simultaneously generated alongside control chimeras comprising il12rb1−/− mice reconstituted with either 80% rag1−/− (Fig. 9F) or il12rb1−/− (Fig. 9G) genotype, respectively. IFN-γ expression levels by il12rb1+/+ NK cells in both T/B il12rb1+/+10G to L and T/B il12rb1−/− (T/B il12rb1−/−KO) bone marrow. Consistent with the phenotype of T cells of nonchimeric il12rb1+/+ and il12rb1−/− mice (Fig. 7), at 50 days postinfection T/B il12rb1−/−KO mice produced fewer IFN-γ+ T cells than T/B il12rb1+/+10G controls (Fig. 9A to C). Staining for CD45.1 allowed for FACS discrimination of T cells of either the il12rb1+/+ (Fig. 9F) or il12rb1−/− (Fig. 9G) genotype, respectively. IFN-γ expression levels by il12rb1+/+ NK cells in both T/B il12rb1+/+10G to L and T/B il12rb1−/− (T/B il12rb1−/−KO) mice were comparable (Fig. 9H); NK cells derived from il12rb1−/− bone marrow comprised approximately 10% of total lung NK cells (Fig. 9H, right). When this analysis was extended to T/B il12rb1−/−KO mice, we observed that, compared to their counterparts in T/B il12rb1−/−KO mice, the percentage of IFN-γ+ il12rb1−/− T cells increased in the presence of il12rb1+/+ T cells (Fig. 9E and I). Unexpectedly, we also observed that il12rb1+/+ T cells modestly increased their own production of IFN-γ when il12rb1−/− T cells were present (Fig. 9E and I). Both il12rb1+/+ and il12rb1−/− T cells increased their expression levels of IFN-γ over their counterparts in uninfected T/B il12rb1−/−KO mice (controls (Fig. 9D and I). We conclude from this analysis that il12rb1−/− T cells exhibit dominance over il12rb1+/− T cells during M. tuberculosis infection by both promoting IFN-γ production by il12rb1−/− T cells and enhancing their own production of this significant cytokine.

il12rb1−/− T cells exhibit decreased proliferative capacity and altered polarization following M. tuberculosis infection. Since impaired T-cell expression of IFN-γ did not fully account for the phenotypic differences between T/B and T/B il12rb1−/−KO mice, we determined, via flow cytometry, whether other phenotypic differences (beyond IFN-γ) existed between il12rb1+/+ and il12rb1−/− T cells. Specifically, we infected T/B il12rb1−/−KO mice and, at 50 days postinfection, assessed proliferative capacity (via BrdU incorporation), activation status (CD44), expression of the master transcription factors Tbet and Foxp3, and intracellular levels of effector cytokines TNF-α and IL-17. As before (Fig. 9), using CD45 congenic mice as il12rb1+/+ donors afforded the ability to directly compare il12rb1−/− (CD45.2-positive [CD45.2pos]) and il12rb1+/+ (CD45.2neg) T cells within the same infected mouse. Both CD4 (Fig. 10A to F) and CD8 (Fig. 10G to L) T cells were examined in this manner. Regarding CD8+ T cells, despite being activated to a similar degree (Fig. 10A), CD45.2neg cells displayed a greater proliferative capacity than CD45.2pos cells (Fig. 10B). The polarization of CD45.2pos cells was also altered relative to CD45.2neg cells, with CD45.2pos cells expressing lower levels of T11-associated proteins Tbet (Fig. 10C) and tumor necrosis factor alpha (TNF-α) (Fig. 10E), and increased levels of regulatory T cell (Treg)-associated transcription factor Foxp3 (Fig. 10D). Levels of proinflammatory IL-17 were similarly low in CD4+ T cells of either genotype (Fig. 10F). Regarding CD8+ T cells, CD45.2neg and CD45.2pos cells expressed similar levels of CD44 (Fig. 10G). However, despite the appearance of a more discrete CD45.2+ BrdU+ subset (Fig. 10H), il12rb1−/− CD8+ cells displayed a lower proliferative potential relative to their il12rb1+/− counterparts when the data are expressed as the percentage of CD8+ cells taking up BrdU (Fig. 10H). CD8 expression of Tbet (67) and TNF-α was also lower among CD45.2pos cells (Fig. 10I and K); Foxp3 and IL-17 expression levels were equally low in CD45.2neg and CD45.2pos cells (Fig. 10J and L). The cumulative data from both CD4+ and CD8+ T cells are shown in Fig. 10M to N. Collectively, these data demonstrate that both CD4+ and CD8+ il12rb1−/− T cells exhibit decreased proliferative capacities and altered polarization following M. tuberculosis infection.

DISCUSSION
IL-12 family members [IL-12, IL-23, and IL-12(p40)] directly and indirectly influence the function of nearly all hematopoietic cell types, including those derived from myeloid progenitors (erythrocytes [42, 43], thrombocytes [9], mast cells [75], basophils [60], neutrophils [44], eosinophils [40], macrophages [58], and dendritic cells [33]) as well as lymphoid progenitors (NK-cells [125], NK T cells [73], B cells [39], γδ T cells [71], CD4+ αβ T cells [68], and CD8+ αβ T cells [20, 49]). The effects of IL-12 family members on nonhematopoietic lineages are less known although bronchial epithelial cells have been demonstrated to express the receptor for IL-12 (1). The activity of each IL-12 family member is dependent on the products of IL2RB1, a gene that in both humans and mice (il12rb1) expresses multiple mRNA isoforms, including the type I integral membrane protein IL-12RB1. IL-12RB1 serves as a low-affinity receptor for the p40 subunit of each IL-12 family member (11, 51). The extracellular portion of IL-12RB1 contains the cytokine-binding region essential for physical association with IL-12 or IL-23, while the cytoplasmic portion acts in concert with IL-12RB2/IL-23R to transmit intracellular signals via preassociated Janus kinases TYK2 and JAK2. Transcription of IL12RB1 is increased during active tuberculosis in humans (69) and is critical for the control of both tuberculous and nontuberculous forms of mycobacterial disease (3, 16).

Here, we have demonstrated that in the early stages of experimental tuberculosis, il12rb1 functions primarily through rag1-dependent lineages to control mycobacterial growth and limit pul-
monary pathology. This *rag1*-dependent lineage likely represents αβ-T cells, given the extensive analysis of North et al., who demonstrated the essential role of αβ-T cells have in containing bacteria during this phase of experimental TB (41, 45, 46) and the similarly nonessential role of γδ-T cells (41), NK T cells (5, 18, 66), and B cells (30) in the same mouse model.

*il12rb1* KO T cells have a decreased capacity to express protective *ifng*, which partially accounts for the elevated bacterial burdens in T/B.*il12rb1KO* mice. *il12rb1* KO T cells increase their own expression of IFN-γ. The mechanism behind this and its significance to TB control will be a focus of future investigations.

Given the propensity of *IL12RB1* null individuals to develop disseminated forms of mycobacterial disease, we found it very...
interesting that *M. tuberculosis* is more readily found near sites of potential dissemination in *il12rb1*−/− mice than in *il12rb1*+/+ controls. This was independent of bacterial burden, as localization in/near the vascular and airway epithelia was observed as early as day 20 postinfection in *il12rb1*−/− lungs (at this time, bacterial burdens were equivalent between *il12rb1*+/+ and *il12rb1*−/− mice, independent of the homogenization method utilized) (Fig. 6A and data not shown). Regarding the identity of the IL-12 family member promoting this containment, it is likely that IL-12 itself, rather than IL-23 or IL-12(p40)2, is the primary cytokine promoting *M. tuberculosis* containment, given the well-known role IL-12 has in promoting T-cell expression of IFN-γ, which itself can directly activate nonhematopoietic lineages to restrict pathogen growth (21, 50). However, as is clear from the different phenotypes of *M. tuberculosis*-infected *il12b*−/− mice [which lack the IL-12p40 subunit required for IL-12, IL-23, and IL-12(p40)2 activity] and *il12a*−/− mice [which lack the IL-12p35 subunit required for IL-12 and IL-35 activity] (13), the loss of IL-12-signaling alone does not fully explain the phenotype of *il12rb1*−/− mice as reported here. Thus, while IL-12 may be the primary cytokine promoting *M. tuberculosis* containment in the C57Bl/6 model (34), what we observe in *il12rb1*−/− mice reflects the concomitant loss of IL-12, IL-23, and IL-12(p40)2 bioactivities. Each of these cytokines is host protective in the context of experimental tuberculosis (23, 27, 32, 34).

IFN-γ is well established as being essential for controlling *M. tuberculosis* infection (12, 22) and is expressed by multiple leukocyte subsets during experimental TB (Fig. 6C). In contrast to the recent suggestion of Sharma et al. (61), flow cytometric analysis of Yeti lungs did not demonstrate CD45− cells to be a source of IFN-γ during experimental TB (Fig. 6B). Regarding the specific role of T-cell IFN-γ, radiation bone marrow chimeras demonstrate that IFN-γ produced by T cells does significantly contribute to the control of primary *M. tuberculosis* infection. These results complement those of Gallegos et al. (24), who demonstrated that adoptive transfer of *ex vivo* differentiated T cells prior to infection provides protection that is independent of IFN-γ up to 20 days following infection. That experimental approach, which is shared by other laboratories investigating which cytokines T cells must express for pathogen control (65), is fundamentally a vaccination protocol; the animals used in our study are not exposed to *M. tuberculosis* antigens prior to their infection. Consequently, the study by Gallegos et al. (24) is important for future investigations of *M. tuberculosis* vaccination (70). It has been demonstrated that the relative contribution CD4+ and CD8+ T cells make to the total level of IFN-γ expressed during experimental TB fluctuates depending on the stage of infection examined (35). The mechanisms driving this flux have yet to be completely defined, but they are possibly related to variation in the level of *M. tuberculosis* antigens present during different stages of infection (35). Nevertheless, chimeras in which *ifng* deficiency was restricted to *rag1*-dependent cells (i.e., T/B/RgKO mice) did not fully recapitulate the phenotype of either *ifng*−/− mice or *rag1*−/− chimeras reconstituted with *ifng*−/− bone marrow. The simplest explanation for these data is that *rag1*-independent lineages (e.g., NK cells and granulocytes) are an additional source of protective *ifng* during experimental TB. This conclusion is supported by previous comparisons of *M. tuberculosis*-infected *rag1*−/− and *ifng*−/− mice (46) and suggests that the phenotype of T/B/RgKO mice is not solely due to impaired *ifng* expression but, rather, reflects a lack of multiple effector mechanisms (Fig. 10).

Less certain still is the role that each individual IL12RB1 isoform plays in controlling *M. tuberculosis* infection in both humans and the mouse model. Human IL12RB1 and mouse *il12rb1* produce at least two unique isoforms (56), only one of which has been fully described (i.e., isoform 1, or IL-12Rβ1) (11, 51). The second isoform (i.e., isoform 2, or IL-12Rβ1ΔTM) is the result of alternative splicing (56) and contains a C-terminal sequence that is unlike that of isoform 1. To date, the function of isoform 2 has been examined only in the context of dendritic cell function (56). However, given our demonstration here that *il12rb1* must be expressed by T cells (and not dendritic cells) for TB control to occur, the impetus now should be to determine the relative functions each *il12rb1* isoform has specifically in αβ-T-cell lineages. Since the *il12rb1* knockout mouse used in this study is deficient in both of these isoforms (due to the genomic location at which neo is inserted [74]), such investigations will be greatly assisted by the generation of a mouse deficient in only isoform 2.

Finally, it is worth noting that our results do not rule out a role for *il12rb1* expression by other cell types, including nonhematopoietic lineages, during latent stages of infection. Latent TB is the form of disease that the majority of *M. tuberculosis*-infected individuals have and can be modeled in mice using either the low-dose aerogenic infection or Cornell model (38, 59, 62). Using radiation bone marrow chimeras of *ifngr*−/− mice reconstituted with *ifng*−/− bone marrow, Desvignes and Ernst (17) recently used the low-dose model to demonstrate that nonhematopoietic IFN-γR expression regulates the development of pulmonary immunopathology during latent *M. tuberculosis* infection. This important phenotype is otherwise masked, given the more dominant role hematopoietic IFN-γR expression plays in the early stages of *M. tuberculosis* infection (17). Nonhematopoietic lineages are the most abundant cell type in the lung and, at least in the absence of *il12rb1*, clearly come into contact with *M. tuberculosis* (Fig. 2B). Bronchial epithelia have been demonstrated to express IL-12R, while type II pneumocytes express markers of stress during pulmonary tuberculosis (29). Whether nonhematopoietic lineages in the lungs of humans and mice express *il12rb1* during latent infection, as well as whether the isoforms produced by this gene contribute to immunopathogenesis during latent *M. tuberculosis* infection, is under investigation.

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