Airway delivery of interferon-\(\gamma\) overexpressing macrophages confers resistance to *Mycobacterium avium* infection in SCID mice

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**Abstract**  
*Mycobacterium avium* (*M. avium*) causes significant pulmonary infection, especially in immunocompromised hosts. Alveolar macrophages (AMs) represent the first line of host defense against infection in the lung. Interferon gamma (IFN-\(\gamma\)) activation of AMs enhances in vitro killing of pathogens such as *M. avium*. We hypothesized that airway delivery of AMs into the lungs of immunodeficient mice infected with *M. avium* will inhibit *M. avium* growth in the lung and that this macrophage function is in part IFN-\(\gamma\) dependent. In this study, normal BALB/c and BALB/c SCID mice received *M. avium* intratracheally while on mechanical ventilation. After 30 days, *M. avium* numbers increased in a concentration-dependent manner in SCID mice compared with normal BALB/c mice. Airway delivery of IFN-\(\gamma\)-activated BALB/c AMs or J774A.1 macrophages overexpressing IFN-\(\gamma\) into the lungs of SCID mice resulted in a significant decrease in *M. avium* growth (\(P < 0.01\), both comparisons) and limited dissemination to other organs. In addition, airway delivery of IFN-\(\gamma\) activated AMs and macrophages overexpressing IFN-\(\gamma\) increased the levels of IFN-\(\gamma\) and TNF-\(\alpha\) in SCID mice. A similar protective effect against *M. avium* infection using J774A.1 macrophages overexpressing IFN-\(\gamma\) was observed in IFN-\(\gamma\)-knockout mice. These data suggest that administration of IFN-\(\gamma\) activated AMs or macrophages overexpressing IFN-\(\gamma\) may partially restore local alveolar host defense against infections like *M. avium*, even in the presence of ongoing systemic immunosuppression.

**Introduction**  
*M. avium* causes pulmonary infections in patients with underlying lung disease and in a group of older women without apparent lung disease (Field and Cowie 2006; Glassroth 2008; Kim et al. 2008; Kikuchi et al. 2009; van Duin et al. 2010). It can also cause disseminated disease in immunocompromised hosts, such as those with AIDS.
(Horsburgh et al. 2001; Griffith et al. 2007; Johnson and Odell 2014; Orme and Ordway 2014). The portal of entry of M. avium in humans is usually the lung or gastrointestinal tract (Damsker and Bottone 1985; Bartralot et al. 2000; Field and Cowie 2003). M. avium infections are often difficult to manage due to its capability of surviving within host macrophages.

Alveolar macrophages (AMs) play a key role in pulmonary host defense against mycobacteria (Leemans et al. 2001). AMs phagocytize and kill mycobacteria by several mechanisms, including generation of reactive oxygen intermediates (ROIs) and reactive nitrogen (RNIs) intermediates (Sato et al. 1998; Murray and Nathan 1999; Denis et al. 2005; Woo et al. 2009). However, bacterial killing of M. avium is variably successful (Schlesinger et al. 1990; Fenton and Vermeulen 1996; Labro 2000; Chan et al. 2001; Pieters 2001; Flynn and Chan 2003; Karakousis et al. 2004) due to the ability of M. avium to limit macrophage production of these chemical species and/or the organism’s resistance to their cytotoxic effects (Appelberg and Orme 1993; Li et al. 2010; Miwa et al. 2014; Motamedi et al. 2014). Consequently, some organisms survive within the AM (Sangari et al. 1999).

Cell-mediated immunity, characterized by activation of Th1 subsets of CD4+ T-cells with an increase in TNF-α and IFN-γ is also important in host defense against M. avium (Jouanguy et al. 1999; Ehr et al. 2001; Manca et al. 2001; Nau et al. 2002). IFN-γ-activated macrophages have enhanced ability to kill mycobacteria (Bonay et al. 1999; Jouanguy et al. 1999; Beltan et al. 2000; Ehr et al. 2001; Giacomini et al. 2001; Hoal-van Helden et al. 2001; Manca et al. 2001; Qiao et al. 2002; Karcher et al. 2008; Herbst et al. 2011). Introducing an IFN-γ transgene into the lungs of SCID mice reduced their susceptibility to BCG infection (Xing et al. 2001). IFN-γ-deficient mice show increased susceptibility to mycobacterial infection (Flynn et al. 1993; Kamijo et al. 1993; Newport et al. 1996; Sugawara et al. 1998). Humans gene mutations that disrupt IFN-γ signaling enhances susceptibility to M. avium (Haverkamp et al. 2006). TNF-α is another key factor in controlling mycobacterial infection (Chen et al. 1992; Kasuga-aokii et al. 1999). Humans receiving anti-TNF therapies for rheumatoid arthritis or other diseases are at increased risk for infection with mycobacteria and other intracellular pathogens (Keane 2001). Thus, TNF-α and IFN-γ are important in resistance to mycobacterial infection.

Both AMs and T-cell-derived cytokines are necessary for optimal host defense against mycobacteria. However, how these two host defense systems interface remains unclear. Cell-mediated immunity can be restored by reconstituting normal cells into immunodeficient mice. CD4+ cells reconstituted into SCID mice resulted in resolution of P. carinii infection (Theus et al. 1995; Keane 2001). Activated macrophages have been reconstituted into humans for cancer therapy (Eyward et al. 1996; Monnet et al. 2002; Suzuki et al. 2014). However, this approach has not been evaluated for treatment or prevention of pulmonary M. avium infection.

Although present, AMs are not fully functional in SCID mice and are felt to contribute to the susceptibility of these mice to interfere with M. avium (Armstrong and Cushion 1994). The aim of this study was to evaluate whether airway delivery of normal AMs or macrophages overexpressing IFN-γ decreases susceptibility to M. avium lung infection in SCID mice and IFN-γ knockout mice (IFN-γ KO). We found that transfer of IFN-γ ex vivo activated AMs or macrophages overexpressing IFN-γ, but not unstimulated macrophages, into lungs of SCID or IFN-γ KO mice markedly enhances the ability of these animals to control local pulmonary M. avium infection, as well as limit disseminated disease. This protective effect is associated with a significant increase in lung levels of IFN-γ and TNF-α.

**Materials and Methods**

**M. avium cultivation and isolation**

*M. avium* ATCC strain 25291 (Rockville, MD) was grown in Middlebrook 7H9 broth containing albumin (50 g/L), dextrose (20 g/L), and catalase (0.03 g/L) as enrichments (BD Diagnostics, Franklin Lakes, NJ). *M. avium* was cultured at 37°C in dispersed form and harvested in mid-log phase. The mixture was centrifuged (3500 x g for 30 min) at 4°C, washed with saline, and sonicated (15 sec at 20 W, GenProbe, San Diego, CA) to disrupt bacterial clumps, as described in our previous studies (Pasula et al. 2002b). The purity of the *M. avium* cultures was verified by Kinyoun staining (Midlantic Biomedical Inc., Paulsboro, NJ). The final bacterial suspension was adjusted to contain 1 x 10⁷ organisms/mL using a Spectronic 20 as previously described (Dhople and Ibanez 1995). The accuracy of these measurements was confirmed with a serial dilution and determination of colony-forming units (CFUs).

**Labeling of M. avium with FITC**

*M. avium* were labeled with fluorescein isothiocyanate (FITC) (Sigma Chemical Co. St. Louis, MO) as described (Ezekowitz et al. 1991; Weaver et al. 1996). Briefly, *M. avium* was isolated as described in the preceding section. A final concentration of 1 x 10⁶/mL *M. avium* was suspended in PBS (pH 7.2) containing FITC (0.1 mg/mL) and incubated for 4 h in the dark at 37°C with occasional
agitation/shaking. After the incubation, the labeled M. avium was centrifuged at 3000 × g for 20 min. The supernatant was discarded and the pellet was washed three times with PBS to remove unincorporated FITC. The labeling was verified by the direct examination under the fluorescent microscope. The final pellet was resuspended in PBS.

**Mice**

Pathogen-free 6–8-week-old BALB/c, BALB/c SCID (Harlan Sprague-Dawley, Indianapolis, IN) and IFN-γ KO mice with the respective BALB/c background mice were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed and maintained under pathogen-free conditions. All animal procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee. The mice were housed in a barrier facility, maintained in sterile conditions in microisolator cages, and supplied with sterilized food and water. All experimental animals were maintained in microisolator cages until use.

**Isolation and labeling of AMs**

Alveolar macrophages were isolated from normal BALB/c or BALB/c SCID mice by bronchoalveolar lavage (BAL), as described previously (Pasula et al. 1997, 2009). Briefly, mice were killed and a 20-gauge angiocath (Becton Dickinson, Sandy, UT) was inserted into the trachea. Following BAL with ten 1 mL aliquots, BAL cells were harvested by centrifugation, washed, and then enumerated by hemocytometer (Reichert, Buffalo, NY). AM viability was determined to be >98% using trypan blue exclusion. Cytopreparation smears were made and stained with Hema III stain (Biochemical Sciences Inc., Swedesboro, NJ) to determine cellular differentials. Only the cellular suspensions that contained >98% pure AM population were used.

In some experiments, donor AMs were labeled with DiI as previously described (Pasula et al. 2002a). In selected studies, donor AMs from BALB/c mice, were incubated in the presence or absence of IFN-γ (100 U/mL) for 18 h at 37°C. The production of genetically engineered J774A.1 macrophages overexpressing IFN-γ from our laboratory was described previously (Wu et al. 2001). Donor AMs or AMs labeled with DiI and or J774A.1 macrophages overexpressing IFN-γ were intratracheally (I.T.) administered at a concentration of 5 × 10⁶/50 μL into the mechanically ventilated SCID mice as described below. Our previous studies demonstrated that AMs labeled with DiI retain the label fastidiously in the lung (Pasula et al. 2002a), similar to reports of others using DiI as cell marker in vivo (Honig and Hume 1986, 1989; Heredia et al. 1991; Ragnarson et al. 1992) and that DiI labeling of AMs and airway delivery of AMs does not alter the function of these AMs in vivo (Pasula et al. 2002a).

**Administration of AMs, IFN-γ-treated AMs, or J774A.1 macrophages overexpressing IFN-γ into recipient mice**

Alveolar macrophages were administered into the lungs of mice as described in our earlier studies (Pasula et al. 2002a). Briefly, mice were anesthetized with isoflurane provided by the Laboratory Animal Medical Services at the University of Cincinnati, College of Medicine, following which the trachea was exposed using blunt dissection. When the mice had reached a sufficient level of anesthesia, they were surgically prepared, and given preemptive analgesics subcutaneously (Buprenorphine). A midline neck incision was made, the trachea was exposed using blunt dissection, and a 20-gauge angiocath was inserted into the trachea. The mice experienced no discernible pain. The mice were connected to the ventilator (Analytic Specialties, St. Louis, MO) that was directly connected to the airway catheter. The ventilator is time-cycled with pressure limits set at a maximum peak inspiratory pressure of 4 cmH₂O, a respiratory rate of 150 breaths per minute, and an inspiratory to expiratory ratio of 1:2. This results in a tidal volume of 150–250 μL per breath. Mice were ventilated for 10 min and then allowed to recover. Mechanical ventilation insures adequate delivery and distribution of mycobacteria or macrophages to the lower respiratory tract and avoids problems associated with uneven recovery from anesthesia among the animals. The ventilator has proved very safe for the mice from lung-related injury from manipulation and the recovery is typically very fast (Martin et al. 2005). The AMs (5 × 10⁶) with or without DiI labeling in 50 μL HBSS were administered into the lungs via the tracheal catheter. The cell suspension was followed by a 200 μL of air to clear the angiocath and upper respiratory tract of the mice and the mice were ventilated for 10 min during the administration. Selected groups of mice were administered with IFN-γ AMs or J774A.1 macrophages overexpressing IFN-γ or control BALB/c AMs or J774A.1 macrophages.

**Infection of mice with M. avium**

Specific concentrations of M. avium (5 × 10⁵ to 1 × 10⁶) suspended in 50 μL HBSS were I.T. administered into the lungs of the mice via a tracheal catheter (Pasula et al. 2009). The mice were ventilated for another 10 min and then allowed to recover. In experiments to determine the ability of AMs or ex vivo IFN-γ-activated AMs
et al. 1999, 2009). Sparks, MD), as described in our earlier studies (Pasula et al. 2001). Freshly isolated AMs from BALB/c and BALB/c SCID mice were incubated with M. avium at 1:10 ratio (AM:M. avium) for 2 h at 37°C in tissue culture glass slides (BD Falcon, Becton and Dickinson). The slides were washed to remove any unbound M. avium. The adherent AMs on the glass slides containing bound M. avium were identified by Auramine O stain (Scientific Device Laboratory, Inc. Des Plaines, IL). The dye binds to the mycolic acid in the mycobacteria cell wall, and the organisms appear as bright yellow, luminous rods against a dark background. The AMs were visualized under fluorescent microscope (Orca ER Zeiss, Thornwood, NJ) to determine the attached/phagocytosed M. avium by the AMs and calculated as number of bacilli per AM. Five fields of 100 AMs were counted and the mean percentage of M. avium attached/phagocytosed by AMs was determined.

For the in vivo assessment, recipient mice were killed and BAL was obtained at 1, 7, 14, or 28 days following AM administration. BAL containing both administered DiI labeled AMs and resident unlabeled AMs associated with FITC-labeled M. avium organisms was analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Suspensions of unlabeled AMs, DiI-labeled AMs, and FITC-labeled M. avium organisms were independently analyzed as standards for the flow cytometry studies. The percentage of M. avium associated with the total cells in suspension belonging to each group was determined.

Assay of IFN-γ and TNF-α

IFN-γ and TNF-α concentrations were measured in cell-free supernatants of BAL samples. The IFN-γ or TNF-α levels were determined by using commercially available

Quantitation of colony-forming units

Bacterial growth was also assessed by measuring M. avium colony-forming units (CFU) in the tissue homogenates of infected mice. Serial dilutions from the tissue homogenates were prepared and plated on 7H11 agar in triplicate. The plates were incubated at 37°C, 5% CO2, for 2–3 weeks. The colonies were counted manually.

Attachment/phagocytosis of M. avium by AMs

The attachment/phagocytosis of M. avium by AMs was evaluated both in vitro and in vivo. To determine the difference in the ability of normal AMs and SCID AMs to attach/phagocytose M. avium, an assay of attachment/phagocytosis of M. avium by AMs was performed as previously described (Pasula et al. 1997, 2002b; Hartmann et al. 2001). Freshly isolated AMs from BALB/c and BALB/c SCID mice were inoculated with M. avium as the initial inoculum that was used to infect the mice for each experiment was verified routinely by plating onto 7H11 Middlebrook plates. To assess M. avium growth, the lungs, liver, spleen, and kidneys were removed aseptically at specified time points. The mice were not sick at the time of measuring M. avium burden from the tissues as the mice were administered with the sublethal concentrations of M. avium. The organs were cut into small pieces and homogenized using a Tissue Tearor (Dremel, Racine, WI). M. avium numbers in the organ homogenates of infected mice were measured using both conventional colony-forming units (CFU) and the radiometric BACTEC methods (BACTEC 460 TB system, Becton Dickenson Diagnostic Instrument Systems, Sparks, MD), as described in our earlier studies (Pasula et al. 1999, 2009).

Radiometric BACTEC assay

The BACTEC method is based on the metabolism of the 14C substrate present in the medium by viable mycobacteria and subsequent release of 14CO2 into the atmosphere above the medium. The organ homogenates (0.5 mL) of infected mice were inoculated into BACTEC 12 Middlebrook vials (Becton Dickenson Diagnostic Instrument Systems, Sparks, MD) containing 0.1 mL PANTA, an antibiotic supplement (Becton Dickenson Diagnostic Instrument Systems) which prevents the growth of any contaminating organisms. The vials were incubated at 37°C, with growth of M. avium determined every 24 h by a 460 BACTEC TB instrument (Becton Dickenson Diagnostic Instrument Systems) and expressed as a growth index from 0 to 999, with an index >10 indicating significant growth. In selected experiments, we used this BACTEC method, which is more sensitive than CFU when the number of surviving organisms is low and provides more rapid detection. However, for most of the studies, we used the standard conventional agar and colony counting method to provide more accurate quantification.
ELISA kits (R&D System, Minneapolis, MN) according to the manufacturer’s instructions. Cytokine levels were expressed as pg/ml of the returned BAL. Specific mRNA transcripts for IFN-γ or TNF-α were also determined by PCR from the lung homogenates (IDT, Coralville, IA). Specific cytokine in RNA was also determined by PCR from the lung homogenates (IDT, Coralville, IA). The expression of IFN-γ mRNA was detected by RT-PCR (Kuroda et al. 2002). The total RNA was extracted from the lungs using RNeasy Plus Mini Kit (Qiagen, Valencia, CA). The concentrations and purities of the RNA were determined by spectrophotometer (GeneQuant II, Pharmacia Biotech, San Francisco, CA) using absorbance at 260 and 280 nm (A260/A280 ratio). cDNA was synthesized using SuperScript® III First-Strand Synthesis System (Invitrogen, CA). The resultant cDNA was subjected to PCR using the following primers. The cDNA was generated from each RNA sample (5 μg) was reverse transcribed using 2 μL of oligo(dT) 12-18 primer, 1 μL (200 U) of SuperScript II reverse transcriptase, 1 μL of 5 mmol/L (each) deoxynucleoside triphosphate, 5 μL of 0.1 mol/l dithiothreitol, 10 μL of 5× enzyme buffer (Gibco BRL, Grand Island, NY), and 22 μL of DEPC-treated water (total reaction volume, 50 μL). Negative controls with all components but without reverse transcriptase were also determined (RNA from Gibco BRL) were used for establishing reaction conditions.

The amplification mixture for each sample was made to a total volume of 50 μL. The reaction mixture contained 0.5 μL (1 μg/μL) each of a 3′ and a 5′ gene-specific primer. For IFN-γ, the sequences used were 5′-TGA ACG CTA CAC ACT GCA TCT TGG (sense) and CGA CTC CTT TTC CGC TTC CTG AG-3′ (anti-sense); TNF alpha 5′-GAG TGA CAA GCC TGT AGC CCA TGT TGT AGC A-3′ (sense) and GCA ATG ATC CCA AAG TAG ACC TGG GAC GAC ATG GAG AA (sense) and GTG GTG GTG AAG CTG TAG CC -3′ (anti-sense) (Integrated DNA technologies, Inc, Coralville, IA), 0.5 μL (5 U/μL) of Taq DNA Syber green polymerase (Gibco BRL), 2.5 μL of cDNA, 21 μL of DEPC-treated water, and 25 μL of the corresponding premix tube of FailSafe PCR (Epicentre Technologies, Madison, WI). A control RNA was used to verify the reaction is working. PCR was performed using cDNA from AMs or GAPDH cDNA using an ABI 7700 real time system (Applied Biosystems, Foster city, CA) under the following conditions: 38 cycles of 30 sec denaturation at 94°C for 5 min, 94°C for 1 min, primer annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were visualized on a 1.2% agarose gel stained with ethidium bromide.

**Statistical analysis**

All experiments were performed in triplicate, with each experiment repeated three times. All animal experiments were carried out using groups of at least five mice and were repeated three times. The results were expressed as means ± SEM. The statistical difference between the control and experimental data were determined with the Student’s t test (Fig. 1) or ANOVA (Figs. 2, 3, 5, 6 and Table 1). A P value of less than 0.05 was considered to be statistically significant (Kuebler and Smith 1976).

**Results**

**Effect of AMs on M. avium infection in SCID mice**

*M. avium* infection levels in the lungs of SCID mice increased significantly (*P* < 0.05) with the concentration of the initial airway-delivered inoculum compared and at each inoculum were significantly greater (*P* < 0.05) than seen with wild-type mice (Fig. 1). Thus, this model of *M. avium*-infected SCID mice allowed us to test the hypothesis that normal immunocompetent AMs with or without activation by IFN-γ were important in the control of *M. avium* lung infection.
To determine the impact of the presence of normal AMs in the control of *M. avium* in SCID mice, AMs from BALB/c mice were instilled into the lungs of recipient SCID mice followed 10 min later by airway delivery of *M. avium*. Four weeks later, there was a slight, but not significant, decrease in *M. avium* numbers in the lungs of the AM-treated SCID mice relative to untreated control (Fig. 2A).

To test the hypothesis that IFN-γ activation of AMs was necessary for effective host defense, AMs from normal BALB/c mice with or without treatment with IFN-γ were I.T. administered into the airways of BALB/c or BALB/c SCID mice followed by airway delivery of *M. avium*. BALB/c SCID mice received I.T. AMs from normal BALB/c mice treated in vitro with IFN-γ (0–1000 U/mL) followed 10 min later by I.T. *M. avium* (1 × 10⁶). *M. avium* growth was measured by BACTEC assay and expressed as growth index. BALB/c AMs treated with 10 U/mL of IFN-γ administered to SCID mice showed a minimal (statistically not significant) decrease in *M. avium* growth. However, normal AMs activated with either 100, 500, and 1000 U/mL of IFN-γ showed a significant decrease in *M. avium* growth compared with level of infection in control BALB/c SCID mice (P < 0.001). Results are representative of three separate experiments, and each time group contains five mice and the data are expressed as mean ± SEM. (determined by one-way analysis of variance (ANOVA) with Bonferroni post hoc tests).

**Figure 2.** Effect of reconstitution with normal IFN-γ-activated AMs or J774A.1 macrophages overexpressing IFN-γ on *M. avium* growth. (A). BALB/c SCID mice received I.T. 5 × 10⁵ normal IFN-γ-treated AMs (100 U/mL) from control BALB/c mice or J774A.1 macrophages genetically engineered to express IFN-γ. Following instillation of AMs, mice were infected 10 min later with *M. avium* (1 × 10⁹). After 30 days, the lungs were isolated and the number of *M. avium* was determined as CFU. (A) BALB/c SCID mice infected with *M. avium* alone had more (P < 0.05) *M. avium* growth than normal BALB/c mice. BALB/c SCID mice that received normal AMs showed slight decrease in *M. avium* growth (P > 0.05). However, IFN-γ-activated AMs and macrophages overexpressing IFN-γ showed a significant decrease in *M. avium* growth (*P < 0.05 and **P < 0.001 vs. SCID mice, respectively). Results are representative of three separate experiments, and each time group contains five mice and the data are expressed as mean ± SEM. (B) BALB/c SCID mice received I.T. AMs from normal BALB/c mice treated in vitro with IFN-γ (0–1000 U/mL) followed 10 min later by I.T. *M. avium* (1 × 10⁹). *M. avium* growth was measured by BACTEC assay and expressed as growth index. BALB/c AMs treated with 10 U/mL of IFN-γ administered to SCID mice showed a minimal (statistically not significant) decrease in *M. avium* growth. However, normal AMs activated with either 100, 500, and 1000 U/mL of IFN-γ showed a significant decrease in *M. avium* growth compared with level of infection in control BALB/c SCID mice (P < 0.001). Results are representative of three separate experiments, and each time group contains five mice and the data are expressed as mean ± SEM. (determined by one-way analysis of variance (ANOVA) with Bonferroni post hoc tests).

To determine the impact of the presence of normal AMs in the control of *M. avium* in SCID mice, AMs from BALB/c mice were instilled into the lungs of recipient SCID mice followed 10 min later by airway delivery of *M. avium*. Four weeks later, there was a slight, but not significant, decrease in *M. avium* numbers in the lungs of the AM-treated SCID mice relative to untreated control (Fig. 2A).

To test the hypothesis that IFN-γ activation of AMs was necessary for effective host defense, AMs from normal BALB/c mice with or without treatment with IFN-γ were I.T. administered into the airways of BALB/c or BALB/c SCID mice followed by airway delivery of *M. avium*. BALB/c SCID mice received I.T. AMs from normal BALB/c mice treated in vitro with IFN-γ (0–1000 U/mL) followed 10 min later by I.T. *M. avium* (1 × 10⁹). BALB/c SCID mice that received AMs activated with 100, 500, and 1000 U/mL of IFN-γ exhibited a significant decrease in the number of *M. avium* in their lungs at 4 weeks relative to untreated BALB/c SCID mice (P < 0.05) (Fig. 2B). Administration of AMs from wild-type mice treated in vitro with 10 or 50 U/mL of IFN-γ to BALB/c SCID mice exhibited a trend toward reduced growth of *M. avium*, but it was not significantly (Fig. 2B, P = 0.08). BALB/c SCID AMs administered to BALB/c SCID mice or BALB/c mice did not reduce *M. avium* numbers from the lungs with or without IFN-γ treatment (data not shown). These results suggest that administration of IFN-γ-activated wild-type AMs significantly enhances the ability of SCID mice to resist infection with *M. avium*.

**Effect of IFN-γ overexpressing macrophages on *M. avium* infection**

To test the hypothesis that macrophages actively expressing IFN-γ might be effective in control of *M. avium*,
J774A.1 macrophages overexpressing IFN-γ were I.T. delivered into BALB/c SCID mice followed by airway delivery of *M. avium* as described above. Consistent with our earlier results, administration of normal AMs ex vivo treated with IFN-γ to SCID BALB/c mice resulted in increased resistance to *M. avium* (Fig. 2A). BALB/c SCID mice receiving control J774A.1 macrophages followed by *M. avium* infection exhibited levels of *M. avium* in their lungs that was similar to untreated control animals (Fig. 2A). However, BALB/c SCID mice administered J774A.1 macrophages overexpressing IFN-γ showed a significantly enhanced resistance to *M. avium* growth compared to SCID mice administered with either J774A.1 macrophages or AMs ex vivo treated with IFN-γ (P < 0.05) (Fig. 2A). These results suggest that delivery of IFN-γ-activated normal AMs or J774A.1 macrophages overexpressing IFN-γ to the airway of SCID mice enhances their ability to resist infection by *M. avium* with IFN-γ overexpressing macrophages being the most efficacious.

### Administration of AMs reduces *M. avium* dissemination in SCID mice

In SCID mice, pulmonary infection with *M. avium* disseminates to other organs (e.g., liver, spleen and kidney). Therefore, *M. avium* CFU in non-pulmonary organs were also determined in homogenates obtained from liver, kidney, and spleen 30 days post AM administration. As expected, there was significant *M. avium* growth in the liver, spleen, and kidneys of control SCID mice (Table 1). SCID mice administered normal AMs did not show any significant difference in *M. avium* in these organs relative to untreated control SCID mice. In contrast, administration of IFN-γ-stimulated AMs to the SCID mice with significantly inhibited the *M. avium* growth (data not shown) and this growth was further decreased in mice administered J774.1 macrophages overexpressing IFN-γ (Table 1). These data suggest that macrophages overexpressing IFN-γ enhanced alveolar host defense during immunosuppression and limited the dissemination of *M. avium* infection.

### Table 1. Effect of reconstitution of IFN-γ overexpressing macrophages on dissemination of *M. avium* in BALB/c scid mice.

| SCID + *M. avium* | Spleen | Liver | Kidney |
|-------------------|--------|-------|--------|
| Control           | *4725 ± 1088 | *3775 ± 757 | *4331 ± 863 |
| J774A.1           | **3825 ± 624 | **3253 ± 710 | **4225 ± 531 |
| J774A.1 + IFN-γ gene | ***420 ± 153 | ***271 ± 50 | ***362 ± 103 |

* M. avium (10^9/50 μL) were administered by airway delivery followed by an administration of macrophages overexpressing IFN-γ into the BALB/c scid mice. Thirty days later, *M. avium* growth was assessed by CFU from liver, kidney, and spleen homogenates. The *M. avium* growth slightly decreased in BALB/c scid mice alone or mice administered with control macrophages. However, *M. avium* growth was significantly decreased in BALB/c scid mice mice administered with macrophages overexpressing IFN-γ (*P < 0.05, **P < 0.05, ***P < 0.001). The results are expressed as the mean ± SEM (n = 3) of three experiments performed in triplicate (determined by one-way analysis of variance (ANOVA) with Bonferroni post hoc tests).
Attachment/phagocytosis of \textit{M. avium} by reconstituted AMs

To determine the functionality of the AMs possible mechanisms by which adoptive transfer of AMs from normal mice to SCID mice decreases \textit{M. avium} growth, we first compared the extent to which \textit{M. avium} are phagocytized by normal AMs and SCID AMs. AMs were isolated from BALB/c and BALB/c SCID mice and then incubated with \textit{M. avium}. After 2 h, significantly more \textit{M. avium} were attached/phagocytosed by the BALB/c AMs (197 ± 28.6 bacilli/100 AMs) compared to AMs from BALB/c SCID mice (74 ± 9.2 bacilli/100 AMs, \(P < 0.05\)). These results suggest that normal BALB/c AMs exhibit greater ability for attachment/phagocytosis of the \textit{M. avium} than the resident SCID AMs (\(P < 0.05\)).

We next determined if this enhanced ability of normal AMs to phagocytize \textit{M. avium} in vitro could be demonstrated in vivo in the lungs of SCID mice. To differentiate normal AMs instilled into the airway of SCID mice from endogenous SCID AMs, normal AMs were Dil-labeled prior to transfer to the SCID mouse airway. As shown in Figure 3, Dil-labeled AMs from normal BALB/c mice demonstrated significantly better attachment/phagocytosis of FITC-labeled \textit{M. avium} in SCID mice than the resident SCID AMs (\(P < 0.05\), all comparisons). There was no difference between donor SCID AMs and resident SCID AMs in the ability to attach and phagocytose \textit{M. avium} in SCID mice (Fig. 3) or between donor BALB/c AMs and resident AMs in BALB/c mice. A time course of attachment/phagocytosis of \textit{M. avium} in vivo at 1, 7, 14, and 28 days post infection with \textit{M. avium} demonstrated that donor BALB/c AMs delivered to SCID mice had significantly better attachment/phagocytosis of \textit{M. avium} compared to resident BALB/c SCID AMs at each time point (Fig. 3).

Morphologic assessment of the lungs from the SCID mice demonstrated that normal BALB/c AMs effectively phagocytized FITC-\textit{M. avium} compared to either donor SCID AMs or resident SCID AMs (\(P < 0.05\)) (Fig. 4). These data provide further evidence that normal AMs delivered into the lungs of SCID mice can effectively function better and viable in the immunodeficient lung environment within the time constraints of the experiment.

Effect of airway delivery of AMs on IFN-\(\gamma\) and TNF levels

IFN-\(\gamma\) and TNF-\(\alpha\) play an important role in host defense against mycobacterial infection. We analyzed IFN-\(\gamma\) and TNF-\(\alpha\) levels in the lungs of SCID mice and control BALB/c mice following instillation of \textit{M. avium} and/or AMs. IFN-\(\gamma\) levels were significantly increased in wild-type BALB/c mice 4 weeks following \textit{M. avium} challenge. In contrast, SCID mice had very low levels of IFN-\(\gamma\) before \textit{M. avium} and this did not increase substantially with \textit{M. avium} infection (Fig. 5A). IFN-\(\gamma\) levels in SCID mice that had received normal BALB/c AMs or IFN-\(\gamma\)-activated AMs, were significantly higher at day 30 (\(P < 0.05\), all comparisons, Fig. 5A). However, mice receiving either IFN-\(\gamma\) primed AMs or IFN-\(\gamma\)-transgene expressing AMs exhibited significantly higher levels of IFN-\(\gamma\) relative to those administered normal AMs.

![Figure 4](image-url). Morphological evidence that Dil-labeled AMs reconstituted into SCID mice attach/phagocytose \textit{M. avium}. BALB/c SCID mice received \(5 \times 10^5\) Dil labeled normal BALB/c AMs or BALB/c SCID AMs and 24 h later FITC-labeled \textit{M. avium} (\(2 \times 10^7\)). One hour later, the animals were killed, lungs were perfused, and the lung sections were examined by confocal microscopy. In Figure 4A, the Dil labeled donor SCID AMs and free unattached FITC-labeled \textit{M. avium} are visible. SCID AMs do not reveal internalization of \textit{M. avium} (insert). In 4B, the donor normal BALB/c AMs reveal more phagocytosed \textit{M. avium} (insert) as indicated by arrows.
As expected, SCID mice receiving SCID AMs had low IFN-γ levels (Fig. 5A).

We next examined TNF-α levels from the BALs to determine if the increase in IFN-γ levels correlated with TNF-α levels. SCID mice had low TNF-α levels in the BAL with or without M. avium infection compared to control BALB/c mice (Fig. 5B). After receiving normal AMs, SCID mice demonstrated an increase in TNF-α levels on day 30 (Fig. 5B) and these TNF-α levels were increased to even greater levels in SCID mice that received IFN-γ–activated AMs (P < 0.05) (Fig. 5B). SCID mice that received M. avium alone or SCID mice reconstituted with SCID AMs maintained low TNF-α levels at all time points [data not shown].

IFN-γ or TNF-α mRNA expression was measured by semiquantitative PCR from murine lungs that were obtained 30 days following macrophage reconstitution and challenged with M. avium infection. SCID mice reconstituted with J774A.1 cells with and without M. avium had low detectable IFN-γ or TNF-α expression. SCID mice reconstituted with J774A.1 macrophages overexpressing IFN-γ exhibited significantly increased IFN-γ and TNF-α expression (Fig. 5C). These levels were further increased in response to M. avium infection (Fig. 5C).

There was a direct correlation between TNF-α and IFN-γ levels on day 1, 7, 14, and 30 (r² = 0.8). Furthermore, the lung burden of M. avium in SCID mice was inversely correlated with the levels of TNF-α and IFN-γ (r² = 0.65, P value < 0.05). Thus, the increase in IFN-γ and TNF-α levels in the lungs of SCID mice reconstituted with activated AMs inversely correlated with the levels of M. avium organisms, suggesting that these cytokines are involved in the conferred resistance to M. avium infection in SCID mice.

Effect of AMs on M. avium infection in IFN-γ KO mice

IFN-γ KO mice and individuals with a defect in IFN-γ signaling also exhibit enhanced susceptibility to mycobacterial infection (Borges et al. 2012). Given the above...
results, we determined whether the transfer of normal or IFN-γ-activated macrophages altered the susceptibility of IFN-γ KO mice to M. avium. We administered normal or IFN-γ-activated AMs to IFN-γ KO mice and subsequently challenged the recipient mice with M. avium. IFN-γ KO mice and the background control mice that received normal AMs did not show any significant change in their susceptibility to M. avium growth as measured by determining lung CFU at 30 days post AM administration (data not shown). In contrast, IFN-γ KO mice administered IFN-γ overexpressing J774.1 cells had significantly fewer lung M. avium than those that were untreated (Fig. 6). These results show that the IFN-γ KO mice are highly susceptible to pulmonary M. avium infection and that administration of IFN-γ overexpressing macrophages diminishes M. avium susceptibility in these mice.

We also examined BAL samples from the same mice for the presence of IFN-γ and TNF-α. As expected, IFN-γ KO mice had no detectable IFN-γ in the BAL with or without challenge with M. avium, nor did IFN-γ KO mice that received M. avium alone or IFN-γ KO mice reconstituted with AMs from IFN-γ KO mice (data not shown). In contrast, IFN-γ KO mice that received macrophages overexpressing IFN-γ, demonstrated a significant increase in IFN-γ and TNF-α levels [Fig. 6B and C (P < 0.001)]. These data suggest that IFN-γ is one of the critical mediators in clearance of M. avium and that reconstitution of macrophages overexpressing IFN-γ effectively restores the level of this critical cytokine in the IFN-γ KO mice, as well as that of TNF-α.

Discussion

M. avium is an important opportunistic pathogen. It leads to progressive chronic pulmonary infection in patients with underlying lung disease, as well as in a subpopulation of older women (Field and Cowie 2006; Kim et al. 2008; Kikuchi et al. 2009). In immunocompromised hosts, such as those with advanced HIV infection, M. avium causes severe disseminated disease (Horsburgh et al. 2001; Griffith et al. 2007; Orme and Ordway 2014). Susceptibility to M. avium infection has been linked to impaired AM function (Bermudez et al. 1994; Nepal et al. 2006). In this study, we demonstrated that transfer of IFN-γ-activated AMs or an IFN-γ overexpressing murine macrophage cell line into the lungs of two different types of immunosuppressed mice conferred enhanced resistance to lung infection and subsequent dissemination of M. avium following acquisition of the organism via a pulmonary route.

We have previously shown that AMs from normal mice can be transferred to the airways of SCID mice and that these reconstituted AMs exhibit enhanced phagocytic activity compared with resident SCID AMs for up to 28 days following airway delivery (Wu et al. 2001; Pasula et al. 2002a). Therefore, we assessed whether the delivery of AMs from immunocompetent mice to the airways of SCID mice would enhance resistance of these animals to M. avium growth in vivo despite the presence of persistent systemic immunosuppression. We found that SCID mice alone and SCID mice reconstituted with wild-type...
AMs or SCID AMs became heavily infected with *M. avium*, suggesting that the simple transfer of normal AMs to SCID mice was ineffective in enhancing their resistance to the *M. avium* infection.

Optimal host response against many invading respiratory pathogens is known to depend on T-cells and on the production of IFN-γ. IFN-γ is important in the control of *M. avium* infection and may act in part by enhancing the antimicrobial activity of local macrophage populations (Rose et al. 1991; Jouanguy et al. 1999; Ehrt et al. 2001). IFN-γ-deficient mice are more susceptible to *M. avium* infection (Cooper et al. 1993; Flynn et al. 1993; Kamijo et al. 1993; Newport et al. 1996), as are humans with defects in IFN-γ-mediated signal transduction. In this study, airway delivery of AMs exogenously treated with IFN-γ or the use of J774A.1 murine macrophages genetically modified to overexpress IFN-γ conferred significant resistance to *M. avium* infection in SCID mice and IFN-γ KO mice. This effect was not limited to the *M. avium* burden in the lung, but these therapies also decreased the number of organisms recovered from distant tissue sites. Interestingly, instillation of fibroblasts constitutively expressing IFN-γ into the peritoneal cavity of BALB/c mice has been previously reported to enhance resistance to pulmonary infection with *M. avium* (Kim et al. 2000). Aerosol delivery of IFN-γ to human patients has also been used effectively in the treatment of pulmonary *M. avium* infection (Hallstrand et al. 2004). Our results suggest that immunocompetent AMs can partially restore alveolar host defense against *M. avium* by a process that is associated with the production of IFN-γ.

TNF-α production is also closely linked to resistance to mycobacterial infections (Flynn et al. 1995). In fact, the ability of IFN-γ to enhance resistance to mycobacterial infection may in part be mediated by an increase in macrophage production of TNF-α (Appelberg et al. 1994). The critical importance of TNF-α in control of mycobacteria is underscored by the reactivation of dormant mycobacterial infection with use of TNF-α-deficient mice. These results support an important role for IFN-γ and TNF-α in control of *M. avium* infection and restoration of IFN-γ and TNF production by administered AMs markedly enhances the effector function of AMs in response to *M. avium*. In this model of AM reconstitution, TNF-α release by IFN-γ-activated AMs is the key to an effective AM-mediated response to mycobacteria in vivo. We do not know if TNF-α production is released from the administered AMs or due to effect of IFN-γ on resident AMs or both. Further the protective effect may be the result of activation in T-cells or other resident cells. In future studies, the use of TNF-α −/− mice or use of TNF-α antibodies in vivo to inhibit TNF-α activity will permit direct in vivo assessment if TNF-α is a critical mediator in the AM response to mycobacterial infection. Similar to this study, use of reconstituted TNF-α +/+ AMs into TNF-α-deficient mice may allow us to confirm or refute this hypothesis in vivo.

In summary, we have shown that airway administration of IFN-γ-activated AMs or J774A.1 macrophages overexpressing IFN-γ to SCID mice markedly enhances resistance to infection with *M. avium*. Furthermore, the inability of IFN-γ KO mice to control the *M. avium* infection could also be rectified by the administration of macrophages overexpressing IFN-γ. Our studies may provide a basis for developing a complementary and alternative approach to the prevention of *M. avium* infection in individuals with local or systemic immunodeficiency. Our results cannot yet be extrapolated to the efficacy of this approach for the treatment of active *M. avium* infection. Future studies are planned to determine the effect of administration of IFN-γ-producing AM in mice with established *M. avium* infection.

**Conflict of Interest**

None declared.

**References**

Appelberg, R., and I. M. Orme. 1993. Effector mechanisms involved in cytokine-mediated bacteriostasis of *Mycobacterium avium* infections in murine macrophages. Immunology 80:352–359.

Appelberg, R., A. G. Castro, J. Pedrosa, R. A. Silva, I. M. Orme, and P. Minóprio. 1994. Role of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of Mycobacterium avium infection. Infect. Immun. 69:3962–3971.

Armstrong, M. Y., and M. T. Cushion. 1994. Animal models. The Pneumocystis Workshop:181-222.

Bartralot, R., R. M. Pujol, V. García-Patos, D. Sitjas, N. Martin-Casabona, P. Coll, et al. 2000. Cutaneous infections due to nontuberculous mycobacteria: histopathological review of 28 cases. Comparative study between lesions observed in immunosuppressed patients and normal hosts. J. Cutan. Pathol. 27:124–129.

Beltan, E., L. Horgen, and N. Rastogi. 2000. Secretion of cytokines by human macrophages upon infection by...
pathogenic and non-pathogenic mycobacteria. Microb. Pathog. 28:313–318.

Bermudez, L. E., C. A. Kemper, and S. C. Deresinski. 1994. Dysfunctional monocytes from a patient with disseminated Mycobacterium kansasii infection are activated in vitro and in vivo by GM-CSF. Biotherapy 8:135–142.

Bonay, M., F. Bouchonnet, V. Pelicic, B. Lagier, M. Grandsaigne, D. Lecossier, et al. 1999. Effect of stimulation of human macrophages on intracellular survival of Mycobacterium bovis Bacillus Calmette-Guerin. Evaluation with a mycobacterial reporter strain. Am. J. Respir. Crit. Care Med. 159(5 Pt 1):1629–1637.

Borges, M., P. Barreira-Silva, M. Flórido, M. B. Jordan, M. Correia-Neves, and R. Appelberg. 2012. Molecular and cellular mechanisms of mycobacterium avium-induced thymic atrophy. J. Immunol. 189:3600–3608.

Chan, H. T., K. Kedzierska, J. O’Mullane, S. M. Crowe, and A. Jaworowski. 2001. Quantifying complement-mediated phagocytosis by human monocyte-derived macrophages. Immunol. Cell Biol. 79:429–435.

Chen, W., E. A. Havell, and A. G. Harmsen. 1992. Importance of endogenous tumor necrosis factor alpha and gamma interferon in host resistance against Pneumocystis carinii infection. Infect. Immun. 60:1279–1284.

Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme. 1993. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J. Exp. Med. 178:2243–2247.

Damsker, B., and E. J. Bottone. 1985. Mycobacterium avium- Mycobacterium intracellulare from the intestinal tracts of patients with the acquired immunodeficiency syndrome: concepts regarding acquisition and pathogenesis. J. Infect. Dis. 151:179–181.

Denis, M., D. N. Wedlock, and B. M. Budde. 2005. IFN-g enhances bovine macrophage responsiveness to Mycobacterium bovis: impact on bacterial replication, cytokine release and macrophage apoptosis. Immunol. Cell Biol. 83:643–650.

Dhople, A., and M. Ibannez. 1995. In vitro activities of 2,2’-bipyridyl analogues against Mycobacterium avium and Mycobacterium tuberculosis. Tuber. Lung Dis. 76:136–140.

van Duin, D., J. Goldfarb, S. K. Schmitt, J. W. Tomford, M. J. Tuohy, and G. S. Hall. 2010. Nontuberculous mycobacterial blood stream and cardiac infections in patients without HIV infection. Diagn. Microbiol. Infect. Dis. 67:286–290.

Ehr, S., D. Schnappinger, S. Bekiranov, J. Drenkow, S. Shi, T. R. Gingeras, et al. 2001. Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nicotinic oxide synthase-2 and phagocyte oxidase. J. Exp. Med. 194:1123–1140.

Eymard, J. C., M. Lopez, A. Cattan, O. Bouche, J. C. Adzijian, and J. Bernard. 1996. Phase I/II trial of autologous activated macrophages in advanced colorectal cancer. Eur. J. Cancer 32A:1905–1911.

Ezekowitz, A. B., D. Williams, H. Koziel, M. Armstrong, A. Warner, F. Richards, et al. 1991. Uptake of Pneumocystis carinii mediated by the macrophage mannose receptor. Nature 351:155–158.

Fenton, M. J., and M. W. Vermeulen. 1996. Immunopathology of tuberculosis: roles of macrophages and monocytes. Infect. Immun. 64:683–690.

Field, S. K., and R. L. Cowie. 2003. Treatment of Mycobacterium avium intracellulare complex lung disease with a macrolide, ethambutol, and clofazimine. Chest 124:1482–1486.

Field, S. K., and R. L. Cowie. 2006. Lung disease due to the more common nontuberculous mycobacteria. Chest 129:1653–1672.

Flynn, J. L., and J. Chan. 2003. Immune evasion by Mycobacterium tuberculosis: living with the enemy. Curr. Opin. Immunol. 15:450–455.

Flynn, J. L., J. Chan, K. J. Triebold, D. K. Dalton, T. A. Stewart, and B. R. Bloom. 1993. An essential role for interferon-g in resistance to Mycobacterium tuberculosis infection. J. Exp. Med. 178:2249–2254.

Flynn, J. L., M. Goldstein, J. Chan, K. Triebold, K. Pfeffer, C. Lowenstein, et al. 1995. Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. Immunity 2:561–572.

Giacomini, E., E. Iona, L. Ferroni, M. Miettinen, L. Fattorini, G. Orefici, et al. 2001. Infection of human macrophages and dendritic cells with Mycobacterium tuberculosis induces a differential cytokine gene expression that modulates T cell response. J. Immunol. 166:7033–7041.

Glassroth, J. 2008. Pulmonary disease due to nontuberculous mycobacteria. Chest 133:243–251.

Griffith, D. E., T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, et al. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am. J. Respir. Crit. Care Med. 175:367–416.

Hallerstrand, T. S., H. D. Ochs, Q. Zhu, and W. C. Liles. 2004. Inhaled IFN-gamma for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-gamma deficiency. Eur. Respir. J. 24:367–370.

Hartmann, P., R. Becker, C. Franzén, E. Schell-Frederick, J. Römer, M. Jacobs, et al. 2001. Phagocytosis and killing of Mycobacterium avium complex by human neutrophils. J. Leukoc. Biol. 69:397–404.

Haverkamp, M. H., J. T. van Dissel, and S. M. Holland. 2006. Human host genetic factors in nontuberculous mycobacterial infection: lessons from single gene disorders affecting innate and adaptive immunity and lessons from molecular defects in interferon-gamma-dependent signaling. Microbes Infect. 8:1157–1166.
Honig, M. G., and R. I. Hume. 1989. Dil and diO: versatile staining tools for neuronal labelling and pathway tracing. Trends Neurosci. 12:333–335, 340–331.

Horsburgh, C. R. Jr, J. Gettings, L. N. Alexander, and J. L. Lennox. 2001. Disseminated Mycobacterium avium complex disease among patients infected with human immunodeficiency virus, 1985–2000. Clin. Infect. Dis. 33:1938–1943.

Johnson, M. M., and J. A. Odell. 2014. Nontuberculous mycobacterial pulmonary infections. J. Thorac. Dis. 6:210–220.

Jouanguy, E., R. Doffinger, S. Dupuis, A. Pallier, F. Altare, and J. L. Casanova. 1999. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. Curr. Opin. Immunol. 11:346–351.

Kamijo, R., J. Le, and D. Shapiro. 1993. Mice that lack the interferon-gamma receptor have profoundly altered responses to infection with Bacillus Calmette-Guerin and subsequent challenge with lipopolysaccharide. J. Exp. Med. 178:1435–1440.

Karakousis, P. C., R. D. Moore, and R. E. Chaisson. 2004. Mycobacterium avium complex in patients with HIV infection in the era of highly active antiretroviral therapy. Lancet. Infect. Dis. 4:557–565.

Karcher, E. L., C. S. Johnson, D. C. Beitz, and J. R. Stabel. 2001. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N. Engl. J. Med. 345:1098–1104.

Kituchi, T., A. Watanabe, K. Gomi, T. Sakakibara, K. Nishimori, H. Daito, et al. 2009. Association between mycobacterial genotypes and disease progression in Mycobacterium avium pulmonary infection. Thorax 64:901–907.

Kim, T. S., S. W. Chung, B. Y. Kang, Y. Y. Choe, and S. Y. Hwang. 2000. Induction of in vivo persistent antimycobacterial activity by interferon-gamma-secreting fibroblasts. Vaccine 18:1067–1073.

Kim, R. D., D. E. Greenberg, M. E. Ehrmantraut, S. V. Guide, L. Ding, Y. Shea, et al. 2008. Pulmonary nontuberculous mycobacterial disease: prospective study of a distinct preexisting syndrome. Am. J. Respir. Crit. Care Med. 178:1066–1074.

Kuebler, R. R., and H. Smith. 1976. Statistics. John Wiley and Sons, New York.

Kuroda, E., T. Kito, and U. Yamashita. 2002. Reduced expression of STAT4 and IFN-gamma in macrophages from BALB/c mice. J. Immunol. 168:5477–5482.

Labro, M. T. 2000. Interference of antibacterial agents with phagocyte functions: immunomodulation or “Immuno-Fairy Tales”? Clin. Microbiol. Rev. 13:615–650.

Leemans, J. C., J. P. Juffermans, S. Florquin, N. V. Rooijen, M. J. Vervoordeldonk, A. Verbon, et al. 2001. Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J. Immunol. 166:4604–4611.

Li, Y.-J., L. Danelishvili, D. Wagner, M. Petrofsky, and L. E. Bermudez. 2010. Identification of virulence determinants of Mycobacterium avium that impact on the ability to resist host killing mechanisms. J. Med. Microbiol. 59:8–16.

Manca, C., L. Tsenova, A. Bergtold, S. Freeman, M. Tovey, J. M. Musser, et al. 2001. Virulence of a Mycobacterium tuberculosis clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-alpha/beta. Proc. Natl. Acad. Sci. U. S. A. 98:5752–5757.

Martin, W. J. 2nd, M. Wu, and R. Pasula. 2005. A novel approach to restore lung immunity during systemic immunosuppression. Trans. Am. Clin. Climatol. Assoc. 116:221–226; discussion 226–227.

Miwa, S., M. Shirai, M. Toyoshima, T. Shirai, K. Yasuda, K. Yokomura, et al. 2014. Efficacy of clarithromycin and ethambutol for Mycobacterium avium complex pulmonary disease. A preliminary study. Ann. Am. Thorac. Soc. 11:23–29.

Monnet, L., J. L. Breau, D. Moro, H. Lena, J. C. Eymard, O. Menard, et al. 2002. Intraperitoneal infusion of activated macrophages and gamma-interferon in malignant pleural mesothelioma: a phase II study. Chest 121:1921–1927.

Motamedi, N., L. Danelishvili, and L. E. Bermudez. 2014. Identification of Mycobacterium avium genes associated with resistance to host antimicrobial peptides. J. Med. Microbiol. 63(Pt 7):923–930.

Murray, H. W., and C. F. Nathan. 1999. Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. J. Exp. Med. 189:741–746.
Adoptive Transfer of IFN-γ Macrophages Inhibits Mycobacterial Growth in Mice  R. Pasula et al.

Nau, G. J., J. F. Richmond, A. Schlesinger, E. G. Jennings, E. S. Lander, and R. A. Young. 2002. Human macrophage activation programs induced by bacterial pathogens. Proc. Natl. Acad. Sci. U. S. A. 99:1503–1508.

Nepal, R. M., S. Mampe, B. Shaffer, A. H. Erickson, and P. Bryant. 2006. Cathepsin L maturation and activity is impaired in macrophages harboring M. avium and M. tuberculosis. Int. Immunol. 18:931–939.

Newport, M., C. Huxley, S. Huston, C. Hawrylowicz, B. Ragnarson, B., L. Bengtsson, and A. Haegerstrand. 1992. Host response to tuberculosis. N. Engl. J. Med. 335:1941–1949.

Orme, I. M., and D. J. Ordway. 2014. Host response to nontuberculous mycobacterial infections of current clinical importance. Infect. Immun. 82:3516–3522.

Pasula, R., J. F. Downing, J. R. Wright, D. L. Kachel, T. E. Davis Jr, and W. J. Martin II. 1997. Surfactant protein A (SP-A) mediates attachment of Mycobacterium tuberculosis to murine alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 17:209–217.

Pasula, R., J. R. Wright, D. L. Kachel, and W. J. Martin. 1999. Surfactant protein A suppresses reactive nitrogen intermediates by alveolar macrophages in response to Mycobacterium tuberculosis. J. Clin. Invest. 103:483–490.

Pasula, R., T. Weaver, M. A. Martinez, and W. J. Martin II. 2002a. Morphologic detection and functional assessment of reconstituted normal alveolar macrophages in the lungs of SCID mice. J. Immunol. 169:4504–4510.

Pasula, R., P. Wisniowski, and W. J. Martin II. 2002b. Fibronectin facilitates Mycobacterium tuberculosis attachment to murine alveolar macrophages. Infect. Immun. 70:1287–1292.

Pasula, R., B. E. Britigan, J. Turner, and W. J. Martin 2nd. 2009. Airway delivery of silica increases susceptibility to mycobacterial infection in mice: potential role of repopulating macrophages. J. Immunol. 182:7102–7109.

Pieters, J. 2001. Entry and survival of pathogenic mycobacteria in macrophages. Microbes Infect. 3:249–255.

Qiao, Y., S. Prabhakar, E. M. Coccia, M. Weiden, A. Canova, E. Giacomini, et al. 2002. Host defense responses to infection by Mycobacterium tuberculosis. Induction of IRF-1 and a serine protease inhibitor. J. Biol. Chem. 277:22377–22385.

Ragnarson, B., L. Bengtsson, and A. Haegerstrand. 1992. Labeling with fluorescent carbocyanine dyes of cultured endothelial and smooth muscle cells by growth in dye-containing medium. Histochemistry 97:329–333.

Rose, R., J. Fuglestad, and L. Remington. 1991. Growth inhibition of Mycobacterium avium complex in human alveolar macrophages by the combination of recombinant macrophage colony-stimulating factor and interferon-gamma. Am. J. Respir. Cell Mol. Biol. 4:248–254.

Sangari, F. J., A. Parker, and L. E. Bermudez. 1999. Mycobacterium avium interaction with macrophages and intestinal epithelial cells. Front Biosci. 4:D582–D588.

Sato, K., T. Akaki, and H. Tomioka. 1998. Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). Clin. Exp. Immunol. 112:63–68.

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 component C3. J. Immunol. 144:2771–2780.

Sugawara, I., H. Yamada, Y. Kazumi, N. Doit, K. Otomo, T. Aoki, et al. 1998. Induction of granulomas in interferon-γ gene disrupted mice by avirulent but not by virulent strains of Mycobacterium tuberculosis. J. Med. Microbiol. 47:871–877.

Suzuki, T., P. Arumugam, T. Sakagami, N. Lachmann, C. Chalk, A. Sallese, et al. 2014. Pulmonary macrophage transplantation therapy. Nature 514:450–454.

Theus, S. A., R. P. Andrews, C. Steel, and P. Walzer. 1995. Adoptive transfer of lymphocytes sensitized to the major surface glycoprotein of Pneumocystis carinii confers protection in the rat. J. Clin. Invest. 95:2587–2593.

Weaver, T., C. L. Hall, D. L. Kachel, R. P. Ward, M. D. Williams, D. G. Perry, et al. 1996. Assessment of in vivo attachment/phagocytosis by alveolar macrophages. J. Immunol. Methods 193:149–164.