I/St mice hyper-susceptible to *M. tuberculosis* are resistant to *M. avium*


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ABSTRACT

We previously demonstrated that mice of the I/St strain are extremely susceptible to *Mycobacterium tuberculosis*, as well as to taxonomically distant intracellular bacteria, *Chlamydia pneumoniae* and *Salmonella enterica*. To broaden our knowledge about the control of susceptibility to intracellular pathogens, we studied the infection caused by virulent *Mycobacterium avium* strain 724 in a panel of inbred mouse strains, and found that I/St mice are resistant to *M. avium*. By comparing I/St mice with B6 mice, we demonstrated that: (i) B6 mice are much more susceptible to infection caused by *M. avium* in terms of bacterial multiplication in the lung tissue and severity of lung pathology; (ii) in B6 but not in I/St mice infection leads to a prolonged leukocyte infiltration of the lung tissue, development of necrotic lung granuloma and lethality; (iii) unfavorable infectious course in B6 mice is accompanied by the elevated production of IFN-γ, TNF-α and, especially, IL-12 in the lungs. Importantly, *M. avium*-resistant I/St mice carry a functional *r* allele of the *Slc11a1* (former – *Nramp1*) gene, while B6 mice have *Slc11a1*<sup>s</sup> genotype. Segregation genetic analysis in (I/St x B6) F2 hybrids demonstrated that susceptibility/resistance to infection caused by *M. avium* largely depended upon *Slc11a1* genotype, with a relatively weak influence of other genetic traits. This close-to-monogenic pattern differs sharply from the host control of many other intracellular bacterial infections, for which the involvement of numerous quantitative trait loci (QTL) was ubiquitously observed.
INTRODUCTION

Organisms of the *Mycobacterium avium* complex, widespread environmental mycobacteria, are intracellular human pathogens in the absence of the normal T cell immunity [14, 15]. They are present in approximately 70% of patients with advanced untreated AIDS and considered the major killer in this cohort [25]. On the background of less severely impaired immunity, e.g., in older persons and in children, *M. avium* may cause chronic lung disease [4, 13, 27]. In the model systems based upon infection of C57BL/6 (B6) inbred mice and their genetic derivatives bearing knock-out mutations in genes important for immunity it was demonstrated that T cell immune response to *M. avium* plays both protective and pathological roles in the course of infection. Thus, IFN-γ production by CD4+ T lymphocytes is critically required for protection against rapid death, but also causes degenerative events, e.g., granuloma necrosis, in lung tissue [6]. Similarly, the presence of intact TNF-α receptors is required to maintain integrity of the *M. avium*-induced granuloma, but granuloma necrosis is likely triggered by TNF-α [3, 7]. These features of *M. avium* infection are very similar to those that reflect the balance between immune defense and pathogenesis in tuberculosis [35, 37]. Given the apparent similarities between TB granulomas in patients and *M. avium*-induced granulomas in B6 mice [3], the underlying immune and genetic mechanisms are worthy of investigation.

Initial studies on the genetics of susceptibility to *M. avium*-induced infection were based on traditional comparisons of bacterial loads in organs following intravenous challenge between mice of various inbred strains [1, 29, 40]. It was shown that susceptible and resistant phenotypes of individual strains followed the distribution pattern of susceptible (s) and resistant (r) alleles of the *Bcg* (later – *Nramp1*; presently – *Slc11a1*) Chromosome 1 gene [38, 41], which encodes a proton-coupled divalent metal ion transporter associated with bacterial phagosomes in macrophages [12]. Subsequently, dependence of susceptibility to infection on the expression of *r* and *s* alleles of this gene was confirmed using *Bcg*-congenic mouse strains and the model of in
vitro infection of macrophages with *M. avium* [5, 22]. However, it remains unknown whether *Slc11a1* is a major gene predominantly determining the outcome of *M. avium*-caused disease, or it is accountable for only a small to moderate proportion of the total genetic variation. *A priori*, the latter assumption seems more realistic, given the polygenic nature of the control of infections caused by *M. tuberculosis* and other intracellular parasites in mouse models [16, 21, 33, 34, 36; see also 19 for review], and marginal to no role of *Slc11a1* in TB susceptibility [20, 24]. However, direct segregation genetic analysis to prove or refute this hypothesis has not been performed so far. Also, a “natural” model of *M. avium* infection via the respiratory tract was characterized in considerable detail with respect to the host immunity [6], but has never been applied to genetic studies.

Previously, we demonstrated that mice of the I/St inbred strain are exceptionally susceptible to *M. tuberculosis* infection and develop severe pulmonary disease in various experimental models of acute, chronic and reactivation TB [9, 26, 32]. Susceptibility did not depend upon the *Slc11a1* gene, since I/St mice carry its resistant allele [18], but was controlled by a few interacting quantitative trait loci mapped to the Chromosomes 3, 9, 17 and X [18, 36]. Interestingly, when TB-susceptible I/St mice were infected with virulent strains of taxonomically distant intracellular bacteria, *Chlamydia pneumoniae* and *Salmonella enterica* serovar Typhimurium, they also displayed a susceptible phenotype characterized by a significantly shortened survival time, higher levels of pro-inflammatory IL-6 and TNF-α, and rapid development of lung pathology after *C. pneumoniae* infection [23]. Since in other model systems severity of infection caused by *S. enterica* substantially depended upon the expression of *r* and *s* alleles of *Slc11a1* [42], it was reasonable to assume that I/St mice represent an interesting example of *Slc11a1*-independent susceptibility to a wide spectrum of intracellular infections. Previously these mice have not been studied regarding their immune response to *M. avium* infection, or as a partner for segregation genetic analysis of this infection in a polygenic setting.
In the present study we compared susceptibility to the *M. avium* 724 virulent chicken isolate [31] between several inbred mouse strains. After establishing that I/St mice expressed a surprisingly resistant phenotype with respect to the bacterial loads in organs, the degree of lung pathology and mortality, we performed segregation genetic and comparative immunologic analyses using B6 mice as a susceptible partner.

**MATERIALS AND METHOD**

**Animals.** Mice of inbred strains A/SnEgYCit (A/Sn), BALB/cJCit (BALB/c), C3H/SnYCit (C3H), I/StSnEgYCit (I/St), C57BL/6YCit (B6), and (I/St x B6)F1 and F2 hybrids were bred and maintained under conventional, non-SPF conditions at the Animal Facilities of the Central Institute for Tuberculosis (CIT, Moscow, Russia) in accordance with guidelines from the Russian Ministry of Health # 755, and the NIH Office of Laboratory Animal Welfare (OLAW) Assurance #A5502-06. Water and food were provided *ad libitum*. Female mice of 2.5-3.0 mo of age in the beginning of experiments were used. All experimental procedures were approved by the CIT institutional animal care committee.

**Infection.** Mice were infected with *M. avium* strain 724 characterized earlier [31], a kind gift of T. Ulrichs, Max Planck Institute for Infectious Biology, Berlin. Following 3 weeks of growth in Dubos broth at 37°C, mycobacteria were suspended in sterile saline containing 0.05% Tween 20 and kept at -80°C until used. For intravenous (i. v.) and intratracheal (i. t.) infections, 10^7 and 10^4 CFU were delivered, respectively, into the tail lateral vein or cannulated trachea as previously described for *M. tuberculosis* infection [9, 26]. Mice were infected also by the respiratory route with 2.5 x 10^3 viable CFU using an Inhalation Exposure System (Glas-Col, Terre Haute, IN). Animals were exposed for 40 min to an aerosol produced by nebulizing 8 ml of a bacterial suspension in PBS solution with 0.05% Tween-80 at concentration of 1 x 10^7 bacilli/ml.
**CFU counts.** At indicated time points following infection, lungs from individual mice were homogenized in 2.0 ml of sterile saline, and 0.2 ml samples of 10-fold serial dilutions were plated on Dubos agar (Difco) and incubated at 37°C for 20-22 days before *M. avium* CFU were counted.

**Lung cell immunophenotyping by flow cytometry.** Lung cell suspensions obtained by enzymatic disruption of lung tissue as described previously [8, 9] were washed twice in phosphate-buffered saline containing 0.01% NaN₃ and 0.5% BSA and incubated for 15 min at 4°C in the presence of CD16/CD32 mAB (clone 2.2G2, PharmMingen, San Diego, CA) to block Fc receptors. Cells were then stained with the following directly conjugated antibodies according to the manufacturer’s instructions: FITC-anti-CD4 (clone H129.19, PharMingen), FITC-anti-CD8a (clone 53-6.7, PharMingen), PE-anti-CD19 (clone 1D3, PharMingen), FITC-MAC-3 (clone M3/84, PharMingen), and FITS-anti-Ly6G (clone RB6-8C5, Caltag, Burlingame, CA). Stained cells (10⁴ per sample) were washed twice, fixed with 1% paraformaldehyde and analyzed by flow cytometry, using a FACSCalibur cytometer (BD, San Diego, CA) and BD-CellQuestPro (Beckton Dickinson) and FlowJo 4.5.9 (Tree Star, Inc., San Carlos, CA) software.

**Histopathology.** Three, eight and sixteen weeks post infection with *M. avium*, lung tissue was examined for pathology. Mice were euthanized by a thiopental overdose. Lung tissue (the middle right lobe) was frozen using a –60°C to –20°C temperature gradient in an electronic Cryotome® (ThermoShandon, UK), and serial 6-8µm-thick sections were made across the widest area of the lobe. Sections were stained with hematoxylin and eosin, and examined by an experienced pathologist without knowledge of the experimental group.

**Cytokine assays.** At weeks 4 and 8 post-infection, whole lungs from individual mice were homogenized in 2 ml of sterile saline, and suspensions frozen at -30°C until used. Immediately
before assaying, thawed samples were centrifuged at 800g to remove debris, and IFN-γ, IL-6, IL-10, IL-12, and TNF-α were measured in supernatants using sandwich ELISA assays. The following ELISA kits were purchased from PharMingen and used according to the manufacturer’s instructions: OptEIA mouse IFN-γ Set (sensitivity - 21pg/ml), OptEIA mouse TNF-α Set (31pg/ml), OptEIA mouse IL-12 Set (31pg/ml), OptEIA mouse IL-10 Set (63pg/ml) and OptEIA mouse IL-6 Set (15.6 pg/ml).

**Slc11a1 genotyping.** *Slc11a1* alleles in (I/St x B6) F2 mice were determined by PCR-RFLP using *BcmFI* restriction nuclease (NEB, Beverly, MA). The PCR product of a portion of *Slc11a1* gene containing G169D mutation in the *s* allelic variant was amplified from genomic DNA using native Pfu polymerase (Stratagene, La Jolla, CA) under the following conditions: primers 5´-CCCACCCCATCTATGTTATCA-3´ forward, 3´-CCCTGCCTACTTTTATCCCCCAA-5´ reverse, 2 min denaturation at 94°C, followed by 30 cycles at: 94°C – 15 sec, 66°C – 20 sec; 72°C – 45 sec. Length variants were resolved on 4% agarose gels.

**Statistical analysis.** The differences between experimental groups were considered statistically significant at $P < 0.05$. The one way ANOVA, Gohan’s survival curves and Fisher’s processing of the data was performed using BIOSTAT (Practica, Moscow, Russia) and Graph Pad Prism 4 software.

**RESULTS AND DISCUSSION**

**Susceptibility to infection with M. avium follows the Slc11a1 allelic pattern irrespective of the dose and route of challenge.** In early studies it has been demonstrated that the capacity of mice to control multiplication of *M. avium* in livers and spleens follows the distribution pattern of *r* and *s* alleles of the *Slc11a1* (then – *Bcg*) gene [1, 29]. However, it was worthwhile to perform a small-scale screening experiment in order to: (i) include lung CFU counts in the analysis; (ii) confirm that non-SPF mice follow the *Slc11a1* pattern of susceptibility; (iii) add
TB-supersusceptible I/St strain to the panel; (iv) apply the “natural”, respiratory route of infection.

We first infected mice of B6 (\textit{Slc11a1}^s), BALB/c (\textit{Slc11a1}^s), C3H (\textit{Slc11a1}^r), A/Sn (\textit{Slc11a1}^r) and I/St (\textit{Slc11a1}^r) strains in groups of 10 with 10^7 \textit{M. avium} strain 724 CFU/mouse i. v. and evaluated mycobacterial loads in their lungs at weeks 3 and 8 post-challenge. In agreement with earlier findings, approximately 1.5-2 log differences between \textit{Slc11a1}^r and \textit{Slc11a1}^s strains were universally observed (data not shown). To our surprise, I/St mice were resistant to \textit{M. avium} infection despite their high susceptibility not only to \textit{M. tuberculosis} but also to taxonomically distant intracellular bacteria (see above). We then performed experiments in which infection was initiated by introducing \textit{M. avium} via the respiratory tract and multiplication of mycobacteria was followed for a substantially longer period. Delivery of mycobacteria by either intratracheal instillation (Fig. 1A) or aerosol exposure (Fig. 1B) in B6 and I/St mice provided phenotypes consistent with those observed after i. v. challenge, and the interstrain differences increased up to 4 logs along the course of the disease. Following dissemination at week 3 post-infection, the difference in mycobacterial multiplication in spleens of B6 and I/St mice reached approximately 3 logs by week 16 (data not shown). Moreover, resistant and susceptible phenotypes of I/St and B6 mice, respectively, were readily confirmed in a survival experiment using the aerosol route of infection: all B6 mice died by 7 post-infection, whereas all I/St mice survived at least 11 months when the experiment was terminated (Fig. 1C). Thus, the disease caused by \textit{M. avium} infection is effectively controlled by I/St mice, which differs profoundly from infections caused by \textit{M. tuberculosis} and other intracellular bacteria [23].

**Lung pathology.** \textit{M. avium} 724 infection in mice was suggested as a model of TB lung pathology in humans [3, 6]. Therefore, we determined if the differences in mycobacterial multiplication in the lungs and survival time were reflected by the dynamics of lung pathology.
After 3 weeks of infection, lung tissue from mice of both B6 and I/St strains showed slight hyperemia, enlarged thickness of alveolar septae and a low-grade mononuclear infiltration (data not shown). By week 8, lungs of B6 mice displayed numerous granulomata of different size, infiltration of peribrocheal and perivascular zones, severe hyperemia and very thick alveolar septae walls (Fig. 2A). Granulomata contained macrophages and epithelioid cells surrounded by lymphocytes. Lung pathology in I/St mice was substantially milder, represented mainly by moderate infiltration and an increased thickness of alveolar septae walls in the absence of condensed inflammatory foci (Fig. 2B). By week 16 of infection, the differences between B6 and I/St lungs became striking. B6 mice developed severe lung pathology characterized by large atelectasis, hyperemia, edema and necrotic foci. Both diffuse and compact cell infiltrations of the lung, as well as large foci with central caseous necrosis surrounded by a well-established zone of degenerating neutrophils, demarcated from remaining breathing tissue by a wide layer of mononuclear cells, were readily observed (Fig. 2C). This is in full agreement with the results obtained by Ehlers et al. [6] after aerogenic challenge of B6 mice with a 10-fold higher dose of \( M. avium \) 724. Lung pathology in I/St mice was significantly less prominent, represented by small and medium-size granulomata with central zones containing live macrophages and epithelioid cells without signs of necrosis (Fig. 2D). Interstrain differences in the degree of lung pathology were reflected by the lung weights which were many fold higher in B6 compared to I/St mice at weeks 8 and 16 post infection (Fig. 2E).

Thus, the conclusion that lung pathology triggered by \( M. avium \) in mice and by \( M. tuberculosis \) in humans displays similar features [3, 6] is correct, as far as susceptible B6 mice are concerned, but not universal. In resistant I/St mice, the hallmark of “human-type TB lung pathology”, i.e., central caseous necrosis of granuloma, never developed following \( M. avium \) infection, whereas the opposite was true for the \( M. tuberculosis \)-triggered disease (Apt, Kondratieva and Averbakh, in preparation). Furthermore, in our model system there was an obvious correlation between two
parameters of susceptibility to infection: *M. avium* grew significantly better in the lungs and the degree of lung pathology was significantly greater in susceptible B6 than in resistant I/St mice (Figs. 1 and 2). In contrast, it was reported recently that granuloma necrosis developed in the lungs of B6 but not of BALB/c and DBA/1 mice despite similar levels of *M. avium* multiplication in all three strains [10]. The most likely explanation of this discrepancy is that in our system interstrain phenotypic differences were determined largely by the alleles of *Slc11a1* gene (see below), whereas in the study of Florido and Appelberg [10] all mouse strains were of *Slc11a1* genotype, and other genetic polymorphism(s) were apparently operating.

**Dynamics of cellular infiltration of the lung.** To characterize the dynamics of lung inflammation in more detail, we performed FACS analysis of the enzyme-digested lung tissue at different stages of the disease (Fig. 3). In agreement with histopathology data, there was little difference between B6 and I/St lungs at week 3 of infection regarding lung content of CD4$^+$ and CD8$^+$ T cells, CD19$^+$ B cells, Mac-3$^+$ macrophages and Ly-6G$^+$ neutrophil granulocytes. However, by week 8 post-challenge the numbers of T cells, B cells and neutrophils increased 10 to 20-fold in B6 mice (Fig. 3A-D), reflecting the rapid development of lung inflammation. The increase in macrophage content was less prominent (~2.5-fold) but also statistically significant ($P < 0.01$, Fig 3E). In contrast, no statistically significant changes in lung cell content between weeks 3 and 8 of infection were observed in I/St mice. By week 16 of infection, the numbers of T lymphocytes in the lungs of B6 mice dropped dramatically (Fig. 3A, B), whereas abundant infiltration with macrophages, neutrophils and, to a somewhat lesser extent, B lymphocytes continued (Fig. 3C-E). In I/St mice, the size of lymphocyte and macrophage populations in the lungs slowly increased between weeks 8 and 16 of infection, which is in a good agreement with slowly progressing lung pathology. Importantly, in sharp contrast to the inflammatory response induced by *M. tuberculosis* infection [8], following *M. avium* challenge lung neutrophil influx in I/St mice remained very low throughout the infectious course. The lack of necrosis and a low
level of lung neutrophil influx seem to be characteristic features of mycobacterial infections efficiently controlled by a resistant host, as demonstrated earlier for *M. tuberculosis* [8, 30] and here for *M. avium* infections.

**Cytokine response in the lungs.** The balance between different cytokines produced locally by cells of immune system is thought to be critical in determining protection vs. immune pathology in the course of infections caused by *M. avium* [6] and *M. tuberculosis* [28]. Thus, we compared mice of the two strains regarding their capacity to produce key effector and inflammatory cytokines in the lungs shortly after initiation (week 4) and at the peak (week 8) of the inflammatory response, using whole-organ homogenates for the analysis. As shown in Table 1, the levels of IFN-γ, TNF-α and IL-12 were strikingly higher in the lungs of B6 compared to I/St infected mice. At week 8 post-infection inter-strain differences in cytokine contents may be explained by underlying differences in the numbers of cytokine-producing cells per organ (Table 1 and Fig. 3). However, at the earlier stage of the disease, when the cellularity of I/St and B6 lungs was similar, different cytokine production may be due rather to the different size of the mycobacterial load, i.e., antigenic stimulus, in the two mouse strains (Fig. 1), or to the genetically distinct capacity to produce pro-inflammatory cytokines in response to *M. avium*, or both. Whatever the reason, these differences may have a prominent influence on lung pathology. Whereas an intact IL-12 – IFN-γ cytokine axis is requisite for protective immunity against *M. avium* [2, 10], hyperproduction of these mediators by B6 mice observed in our experiments (Table 1) likely leads to severe lung inflammation and necrosis (Fig. 2), which is in agreement with the results and concept provided by Ehlers et al. [6].

**Slc11a1 is a major gene determining susceptibility to M. avium.** Despite their *Slc11a1* genotype, I/St mice proved to be extremely susceptible to three different intracellular bacteria whose interactions with the mouse host are under polygenic control [23]. In order to directly
assess the role of *Slc11a1* gene in *M. avium* infection, we performed segregation genetic analysis in (*B6*<sup>s/s</sup> x *I/St*<sup>r/r</sup>) F2 mice, using restriction polymorphism of a *Slc11a1* PCR product for genotyping and lung CFU counts on week 5 of infection as a reliable parameter of susceptibility. We anticipated that segregation of numerous genetic loci involved in the control of infection would provide a continuum of phenotypes without obvious linkage with *Slc11a1* alleles, at least in small-size samplings, especially since F1 hybrids between the two mouse strains expressed an intermediate susceptibility phenotype, although biased towards resistance (data not shown). To our surprise, a simple segregation picture in F2 mice was obtained, in that the lung CFU counts were significantly (*P* < 0.01 - 0.05) higher in animals bearing *Slc11a1*<sup>s/s</sup> genotype (Fig. 4). Susceptibility to *M. avium* infection was inherited as a single recessive Mendelian trait (*χ*<sup>2</sup> = 1.39; *n*<sup>'</sup> = 2). No difference between groups of resistant mice bearing *Slc11a1*<sup>th</sup> and *Slc11a1*<sup>rs</sup> genotypes was found, which confirms the dominant nature of *r* allele [38]. High deviation values in both resistant groups suggest that “resistance” might be a more complex trait than “susceptibility”, and non-*Slc11a1* loci may modify the expression level of the former. With this regard, recent finding of Appelberg and colleagues [10] that the *Hc* locus encoding complement component C5 is involved in the control of *M. avium* granuloma necrosis is of particular interest. *I/St* mice carry *Hc*<sup>0</sup> allele and have non-detectable level of complement [39], which opens a possibility that *Hc* segregation on *Slc11a1*-resistant background may modify lung pathology and thus the conditions of *M. avium* growth, leading to variable CFU counts, while on the background of “major” defect in *Slc11a1* gene these minor variations remain invisible.

Overall, our results indicate that, unlike many other intracellular bacterial infections including TB, infection caused by *M. avium* is predominantly controlled by the *Slc11a1* gene. A putative functional role of this gene as an endosome efflux pump that sequesters iron and, possibly, other divalent cations from the endosomal system [12, 17] suggests a difference between *M. avium* and *M. tuberculosis* in intraendosomal, 2<sup>+</sup>-cation-dependent metabolism. Recent studies by Wagner
et al. [43] in an *in vitro* macrophage-infection system demonstrated similar ability of the two species to accumulate and retain iron in the phagosome using transferrin receptor and siderophore production. Although nickel and zinc concentrations were significantly higher in *M. avium*- compared to *M. tuberculosis*-infected phagosomes at 1 h post-infection, these differences disappeared shortly thereafter, leaving open the question about possible physiological consequences of these temporary shifts. Presently, it remains unclear whether or not a functional r allele deprives *M. avium* of the host sources of 2⁺-cations more efficiently than *M. tuberculosis*.

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References


FIGURE LEGENDS

**Fig. 1.** I/St mice are resistant and B6 mice are susceptible to *M. avium* infection administered via respiratory tract. I/St and B6 mice were infected with either $10^4$ CFU given as an i. t. inoculum (A), or $\sim2 \times 10^3$ CFU given in aerosol (B, C). Estimation of the initially inhaled mycobacterial dose was performed by plating lung homogenates from mice of the two strains at the 24h post-infection time point in groups of 3 in 2 independent experiments. No statistical difference in a mean size of the challenging dose between I/St ($2.5 \times 10^3$ per lung) and B6 ($2.2 \times 10^3$ per lung) mice was observed ($P = 0.24, N = 6$). At indicated time points, lung CFU were estimated in 4 individual mice as described in Materials and Methods (A, B). Results of one of 4 similar experiments are displayed as mean ± SD ($n = 4$ for each point). In (C), mortality of B6 and I/St mice in groups of 8 was monitored until all B6 mice died. All I/St mice survived > 10 months.

**Fig. 2.** B6 mice develop substantially more severe lung pathology compared to I/St mice following infection with *M. avium*. At weeks 8 (A, B) and 16 (C, D) post aerosol challenge, cryosections of lung tissue from B6 (A, C) and I/St (B, D) mice were stained with hematoxilin-eosin and examined microscopically (magnification = x 125). Results of one of two similar experiments are displayed. At week 8, numerous granulomata were present in the lungs of B6 mice (A), whereas lung pathology in I/St mice was represented by moderate infiltration and an increased thickness of alveolar septae (B). At week 16, B6 mice developed severe lung pathology (C) with necrotic foci losing their vascular appearance (arrow). Lung pathology in I/St mice was significantly less prominent, represented by small and medium-size granulomata (D). The weight of lungs estimated in two independent experiments in 4 individual mice each ($n = 8$) was significantly ($P < 0.001$) higher in B6 compared to I/St mice at weeks 8 and 16 of infection (E).
**Fig. 3.** Differences in the cell content in the lungs of B6 and I/St mice during infection with *M. avium*. By week 8 post-challenge, the numbers of CD4 T cells (A), CD8 T cells (B), B cells (C), neutrophils (D) and macrophages (E) increased significantly (*P* < 0.01) in B6 mice compared to week 3, but did not change in I/St mice. At week 16, the numbers of T lymphocytes in the lungs of B6 mice dropped, whereas abundant infiltration with macrophages and neutrophils continued. In I/St mice, the size of lymphocyte and macrophage populations in the lungs slowly increased between weeks 8 and 16 of infection. The results of one of two similar experiments obtained in 3 individual mice per time point are represented as mean ± SEM.

**Fig. 4.** Susceptibility to infection is largely determined by the *Sc11a1* gene. Individual (I/St x B6) F2 mice were genotyped for the *r* and *s* alleles of the *Sc11a1* gene, infected aerogenically with ~2 x 10^3 *M. avium*, and lung CFU were assessed at week 5 post infection. Significance of the differences between groups of mice bearing *s/s* and *r/~* genotypes are indicated.
Table 1. Cytokine production by lung cells from B6 and I/St mice infected with *M. avium*<sup>a</sup>

<table>
<thead>
<tr>
<th>Mouse strain/cytokine (pg/ml)</th>
<th>4 wk post-infection</th>
<th>8 wk post-infection</th>
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<tr>
<td></td>
<td>B6</td>
<td>I/St</td>
</tr>
<tr>
<td>Total lung cellularity (x 10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>26 ± 4</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1688 ± 162&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25± 4</td>
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<tr>
<td>TNF-α</td>
<td>432 ± 58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;31*</td>
</tr>
<tr>
<td>IL-12</td>
<td>6,481 ±550&lt;sup&gt;*&lt;/sup&gt;</td>
<td>278 ± 31</td>
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<tr>
<td>IL-6</td>
<td>128 ± 22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;16</td>
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<sup>a</sup> Mice were infected aerogenically with ~ 2 x 10<sup>3</sup> CFU of *M. avium* as described in Materials and Methods, and at indicated time points lungs were removed, homogenized in 2 ml of sterile saline and used for both immediate plating to estimate CFU counts and for evaluation of the cytokine content. To this end, homogenates were frozen at -30ºC until used. After thawing, tubes were centrifuged to remove cell debris, and supernatant used for evaluation of cytokines in ELISA format as described in Materials and Methods. Results obtained in one of two similar experiments, each including 3 mice evaluated individually, are displayed as mean ± SD (pg/ml). IL-10 levels were always below sensitivity of the test.

<sup>b</sup> P < 0.001 compared to I/St counterparts.

* Below sensitivity of corresponding kit.

** Above the plateau level of calibration curve.
Fig. 1
Fig. 3
Fig. 4

- Scl11a1 genotype

- CFU/lung, 10^6

- P<0.05

- P<0.01

- NS