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Joint Effects of Host Genetic Background and Mycobacterial Pathogen on Susceptibility to Infection▽†

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The present study examined the differential contribution of host genetic background and mycobacterial pathogen variability to biological and mechanistic phenotypes of infection. For this purpose, A/J and C57BL/6J mice were infected intravenously with a low dose of *Mycobacterium tuberculosis* H37Rv or the Russia, Japan, and Pasteur substrains of *Mycobacterium bovis* bacille Calmette-Guérin (BCG). The pulmonary bacterial counts (number of CFU) and transcript levels of select cytokines (e.g., *Ifng*, *Il12b*, and *Il4*) at 1, 3, and 6 weeks postinfection were measured as biological and mechanistic phenotypes, respectively. The individual and combined impact of the host and mycobacteria on these phenotypes was assessed using three-way analysis of variance (ANOVA), which partitions phenotypic variation into host, pathogen, time, and interaction effects. All phenotypes, except pulmonary *Il4* transcript levels, displayed evidence for host-mycobacterium specificity by means of significant interaction terms. Pulmonary expression profiles of 34 chemokines and chemokine-related genes were compared across the hosts and mycobacteria. The differences in induction of these immune messenger genes between A/J and C57BL/6J mice were modest and generally failed to reach significance. In contrast, the mycobacteria induced significant variance in a subset of the immune messenger genes, which was more evident in A/J mice relative to that in C57BL/6J mice. Overall, the results demonstrated the importance of considering the joint effects of the mycobacterial and host genetic backgrounds on susceptibility to mycobacterial infections.

Exposure of humans to *Mycobacterium tuberculosis*, the cause of tuberculosis, induces a highly variable response. Among persons exposed to *M. tuberculosis*, only 30 to 50% become infected during an outbreak, and of those infected, only approximately 10% develop clinical disease. There is now clear evidence for the important impact of host genetic factors on this variable response (3, 11, 12). Evidence for an equally important role of *M. tuberculosis* strain variability is emerging (reviewed in references 18 and 35). A univariate view would suggest that those who develop disease either display low resistance to *M. tuberculosis* or are infected with high-virulence strains of *M. tuberculosis*. An alternate multivariate view is that *M. tuberculosis* and its human hosts have coevolved such that host susceptibility and pathogen virulence might better be considered in combination. A specific *M. tuberculosis* isolate may cause disease in certain hosts but not in others, while a specific host may be susceptible to certain *M. tuberculosis* isolates but resistant to others. The extent to which multivariate interactions are decisive for tuberculosis susceptibility has far-reaching consequences for tuberculosis control efforts, including vaccine development. Although studies have examined the impact of host background and mycobacterial type separately, strategies that focus on the simultaneous analysis of host and mycobacteria in disease expression have been lacking. We addressed the question of the interdependence of host genetics and the mycobacterial pathogen on expression of host susceptibility using an animal model. We selected well-characterized biological and mechanistic phenotypes of mycobacterial infection in mice to determine the individual and combined impacts of pathogen and host genetic variability on phenotype variance.

As the mycobacterial pathogens, we employed the H37Rv reference strain of *M. tuberculosis* and the following three strains of *Mycobacterium bovis* bacille Calmette-Guérin (BCG): BCG Russia, BCG Japan, and BCG Pasteur. The most notable genetic differences between *M. tuberculosis* and BCG strains are chromosomal deletions, such as the region of difference 1 (RD1) (29, 42). In addition to RD1, BCG Russia is characterized by the deletion of Rv3698 (RD Russia) (41). BCG Japan contains a 22-bp deletion within the *Rv3405c* gene (4) which renders it incapable of synthesizing phenolic glycolipids (PGLs), cell wall lipids with established roles in virulence (10). In both BCG Japan and BCG Russia, the promoter region of the *phoP* gene contains an IS*6110* element (8) which may also have implications for virulence. BCG Pasteur lacks this insertion, resulting in lower expression of *phoP* (8). Compared to the BCG Russia and Japan strains, BCG Pasteur has deletions of three additional chromosomal segments, RD2, n-RD18, and RD14 (6, 34, 45), and has a number of point mutations that lead to loss-of-function phenotypes (5, 8, 9, 48).

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As hosts, we employed the A/J and C57BL/6J inbred mouse strains. Mice of these strains differ in their responses to mycobacterial infection. Following aerosol or intravenous infection with *M. tuberculosis*, susceptible A/J mice succumb more rapidly than C57BL/6J mice (1, 22). The early death of A/J animals is caused by a progressive interstitial pneumonitis characterized by widespread tissue necrosis and an inability to form cohesive granulomas. In contrast, C57BL/6J mice form functional granulomas and survive for extended periods. Despite these differences, early pulmonary *M. tuberculosis* replication is similar between C57BL/6J and A/J mice (1, 51). A/J and C57BL/6J mice also differ in their innate susceptibility to low-dose infection with BCG Montreal (20, 50). Interestingly, *M. tuberculosis*-resistant C57BL/6J mice are permissive to BCG Montreal and BCG Pasteur replication in the mononuclear phagocyte system organs, whereas *M. tuberculosis*-susceptible A/J mice are resistant to low-dose BCG infection (13, 20). The shift in host susceptibility in response to pathogenic *M. tuberculosis* and avirulent BCG strains has also been observed in other mouse strains (37, 38) and prompted the inclusion of *M. tuberculosis* in this study. Although the host genetic control of *M. tuberculosis* infection is complex, the differential susceptibility in the early growth of BCG Montreal has been shown to be due to two allelic forms of the *Nramp1* gene (50). We have recently demonstrated that bacterial counts of both BCG Pasteur and BCG Russia in the spleen are under the control of a locus indistinguishable from *Nramp1* (14). In our experimental setting, the effect of *Nramp1* on the lung was modest and could not be detected by genetic analysis. We therefore focused our analysis on pulmonary responses, which are less impacted by *Nramp1* than those of the spleen (14).

In the present study, the A/J and C57BL/6J mouse strains were infected with a low dose of BCG Russia, BCG Japan, BCG Pasteur, or *M. tuberculosis* H37Rv, and a set of host response phenotypes was characterized in the lungs of both mouse strains in a time-dependent manner. This design permitted the simultaneous investigation of the effects of the host and pathogen genetic backgrounds on the magnitude and variance of widely used host response phenotypes.

MATERIALS AND METHODS

Animals. A/J and C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in the rodent facility of the Montreal General Hospital. All animal procedures were performed in accordance with the guidelines outlined by the Canadian Council on Animal Care and approved by the Animal Care Committee of McGill University.

Infection of mice. BCG Russia (ATCC 35740) and BCG Pasteur (ATCC 35734) were transformed with a hygromycin resistance vector (7) to allow for growth on hygromycin-containing media and minimize the risk of contamination following isolation from mice. Prior to infection, recombinant BCG Russia, recombinant BCG Pasteur, BCG Japan, and *M. tuberculosis* H37Rv (Pasteur) were grown on a rotating platform at 37°C in Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI) containing 0.05% Tween 80 (Sigma-Aldrich, St. Louis, MO) and 10% albumin-dextrose-catalase (ADC) supplement (Becton Dickinson and Co., Sparks, MD). At early logarithmic phase (optical density at 600 nm [OD_{600}] = 0.4 to 0.5), bacterial cultures were sonicated to disperse clumps and were diluted accordingly. For intravenous infection, mice were injected with $\sim 3 \times 10^3$ CFU of *M. tuberculosis* or BCG in the lateral tail vein. Inoculum doses were verified by serial dilution plating on Middlebrook 7H10 agar (Difco Laboratories, Detroit, MI) supplemented with oleic acid-albumin-dextrose-catalase (OADC) enrichment (Becton Dickinson and Co., Sparks, MD). For aerosol infection, BCG Russia and BCG Pasteur cultures were grown to an OD_{600} of 0.4 and diluted to 3.5×10^7 CFU/ml. Mice were infected for 10 min in an inhalation exposure system (In-Tox Products, Moriarty, NM). At 1 day postinfection, infectious doses of $\sim 2 \times 10^3$ bacilli were confirmed by homogenizing lungs in 2 ml of 0.025% saponin–phosphate-buffered saline (PBS) using large tissue grinders (TYCO Healthcare Group, Mansfield, MA). A total of 200 μl of the homogenate was plated on Middlebrook 7H10 agar (Difco Laboratories, Detroit, MI) supplemented with OADC enrichment (Becton Dickinson and Co., Sparks, MD) and Bactec Panta Plus (Becton Dickinson and Co., Sparks, MD).

Pulmonary BCG growth. Infected mice were sacrificed by CO_2 inhalation at 1, 3, and 6 weeks postinfection. Lungs were aseptically removed and placed in 0.025% saponin-PBS. BCG-infected lungs were homogenized mechanically using a Polytron PT 2100 homogenizer (Brinkman Instruments, Westbury, NY), and lungs infected with H37Rv were disrupted using large tissue grinders (Tyco Healthcare Group, Mansfield, MA). Homogenates were serially diluted 10-fold and plated on Middlebrook 7H10 agar containing OADC enrichment and hygromycin B (Wisent Inc., St.-Bruno, QC, Canada) or Bactec Panta Plus (Becton Dickinson and Co., Sparks, MD), with our modifications. Bacterial enumeration was performed after a 3- or 6-week incubation at 37°C.

RNA isolation. For the isolation of total cellular RNA, lungs were harvested from control and BCG Pasteur-, BCG Japan-, BCG Russia-, or H37Rv-infected mice at 1, 3, or 6 weeks postinfection and stored in RNAlater (Qiagen, Mississauga, ON, Canada). Lungs were mechanically disrupted, and RNA was purified using the RNeasy minikit (Qiagen, Mississauga, ON, Canada), according to the manufacturer's instructions. Genomic DNA was removed during the RNA extraction process using the RNase-free, DNase kit (Qiagen, Mississauga, ON, Canada). The concentration and integrity of all RNA samples were assessed using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

Gene quantification studies. Synthesis of first-strand cDNA was performed with 0.5 μg of total RNA using the QuantiTect reverse transcription kit (Qiagen, Mississauga, ON, Canada), as recommended by the manufacturer. QuantiTect gene expression assays for *Ifng*, *Il12b*, *Il4*, and the *Gapdh* endogenous control gene were purchased from Qiagen (Qiagen, Mississauga, ON, Canada). cDNA was amplified using QuantiTect reagents on the Rotor-Gene 3000 (Corbett Research, Sydney, Australia), as specified. All real-time experiments were repeated twice, with similar results.

For multigene expression profiling, samples were reverse transcribed with the RT^2 first-strand kit (SuperArray Bioscience Corporation, Frederick, MD). cDNA was amplified on ABI Prism 7500 (Applied Biosystems, Foster City, CA) using either the mouse inflammatory cytokine and receptor RT^2 Profiler PCR array (product no. APMM-011A) or the mouse chemokine and receptor PCR array (product no. PAMM-022A) in combination with the RT^2 real-time PCR SYBR green/ROX master mix (SuperArray Bioscience Corporation, Frederick, MD). Of the 84 genes tested, 34 met our inclusion criteria. These genes were included in both sets of arrays and dissociation assays did not show evidence of cross-hybridization. Using the NormFinder (2) and geNorm (49) algorithms, *Gusb*, *Hprt*, *Hspcb*, *Gapdh*, and *Actb* were determined to be a suitable set of endogenous control genes for normalization.

Statistical methods. A three-way analysis of variance (ANOVA) was performed to investigate differences in replication across bacteria, mouse strains, and time following intravenous infection. An analysis of residuals for this model detected violations of the assumptions of normality and heterogeneity of variance of errors across groups for ANOVA. Thus, CFU values were log transformed (natural logarithm), and an ANOVA model assuming unequal variances, with residual variances specified for each combination of bacterium, mouse strain, and time, was considered (30). We used the procedure PROC MIXED, available in SAS, version 9.2 (SAS Institute Inc., Cary, NC), to fit the model. Degrees of freedom were adjusted using the Kenward-Roger correction (23) because the groups were unbalanced in the sense that groups did not have the same number of subjects. Pairwise post hoc comparisons of means between bacterial strains at each time were performed, when warranted, and *P* values were adjusted for multiple testing with the simulation-based adjustment for multiple testing implemented in the SAS %SimTests macro (52) to ensure an overall family-wise error rate (FWER). Also, for each bacterial strain, we performed a two-way ANOVA to investigate differences in bacterial replication across the mouse strain and time. Differences in the log-transformed pulmonary loads following aerosol infection were statistically examined with the Student *t* test for unequal variances. *P* values of <0.05 were used to indicate statistical significance.

Fold changes in transcript levels between infected and control samples were determined by the threshold cycle ($\Delta\Delta CT$) calculation (32). Data are presented as the mean fold differences ± standard errors of the means (SEM) on a

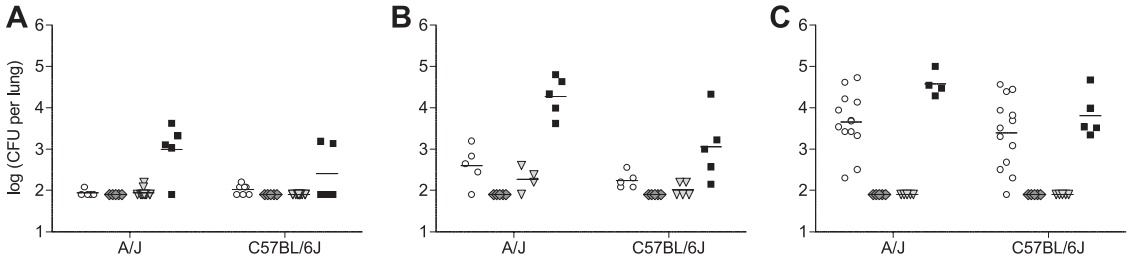

FIG. 1. Pulmonary bacterial counts of BCG and *M. tuberculosis* in the A/J and C57BL/6J mouse strains. The number of bacteria (in $\log_{10}$CFU) in the lungs of individual A/J and C57BL/6J mice was quantified at 1 week (A), 3 weeks (B), and 6 weeks (C) following intravenous infection with a low dose ($\sim 3 \times 10^3$ bacilli) of BCG Russia, BCG Japan, BCG Pasteur, or *M. tuberculosis*. The number of mice used per time point varied from 4 to 13. Mycobacterial counts in A/J and C57BL/6J mice were compared across time by three-way ANOVA. BCG Japan was excluded from the analysis because bacteria were unrecoverable in all mice tested and the lack of variance precluded the use of ANOVA. Three-way ANOVA showed significant effects of bacteria [$F(2, 30.9) = 118.3$; $P < 0.001$], mouse strain [$F(1, 34.3) = 18.6$; $P < 0.001$], and time [$F(2, 23) = 49.2$; $P < 0.001$] on bacterial counts. Significant interaction effects between the bacteria and mouse strain [$F(2, 30.9) = 5.5$; $P = 0.01$] were detected, providing evidence of host-pathogen specificity. A significant interaction was also detected between the bacteria and time [$F(4, 21.6) = 31.1$; $P < 0.001$] but not between the mouse strain and time [$F(2, 23) = 1.8$; $P = 0.2$]. A triple interaction between the effects was not detected [$F(4, 21.6) = 0.6$; $P = 0.7$]. The limit of detectability was 80 bacilli/lung. Bars indicate the mean numbers of $\log_{10}$CFU for the group. BCG Russia, white circle; BCG Japan, gray diamond; BCG Pasteur, light gray triangle; *M. tuberculosis*, black square.

logarithmus dualis ($\log_2$) scale, which assumes an optimum PCR efficiency (E) of 2. When applicable, 95% confidence intervals (CI) were computed with a lower limit of $2^{-\Delta\Delta CT - Z_{1.960}\mathrm{SEM}}$ and an upper limit of $2^{-\Delta\Delta CT + Z_{1.960}\mathrm{SEM}}$, where $-\Delta\Delta CT \pm Z_{1.960}$SEM denotes the upper and lower confidence limits of the change in $-\Delta\Delta CT$, SEM is the standard error of the mean change $-\Delta\Delta CT$, and $Z_{1.960}$ is the 95th percentile of the standard normal distribution (16). Differences in *Ifng*, *Il12b*, and *Il4* expression levels were analyzed by a three-way ANOVA model with unequal variances. Residual variances for each combination of mouse strain, bacterial strain, and time were considered (30). We used the procedure PROC MIXED, available in SAS, version 9.2 (SAS Institute Inc., Cary, NC), to fit the model. Real-time SuperArray data were statistically examined using ANOVA for unequal variances with a Benjamini and Hochberg multiple-test correction (GeneSpring GX software; Agilent Technologies, Palo Alto, CA).

RESULTS

To analyze the extent to which the mycobacterial and host genetic backgrounds contribute to the variance of common measures of susceptibility, we used C57BL/6J and A/J mice and *M. tuberculosis*, BCG Russia, BCG Japan, and BCG Pasteur as host and mycobacterial variants, respectively. Mice were intravenously infected with a low dose of bacteria ($\sim 3 \times 10^3$ bacilli), and phenotypic traits of infection were measured at 1, 3, and 6 weeks, which are time points classically used to decipher susceptibility to BCG infection. Since the ability of mycobacteria to replicate in the lungs is a critical predictor of morbidity and mortality, pulmonary bacterial loads in mice of both strains were compared by three-way ANOVA. This type of analysis partitions phenotypic variation into host-, pathogen-, and time-related effects. Importantly, it provides a measure of host-pathogen specificity through its interaction term (26). The kinetics of lung CFU were significantly different for *M. tuberculosis*, BCG Russia, BCG Pasteur, and BCG Japan in both A/J and C57BL/6J mice (Fig. 1). As expected, *M. tuberculosis* had the largest bacillary burdens across all time points and in both mouse strains. The highest bacterial count among the BCG substrains was observed for BCG Russia, which increased progressively in mice of both strains. At the 6-week time point, BCG Russia counts were similar, on average, to those of *M. tuberculosis* bacilli in the C57BL/6J strain, although they were lower, on average, than counts of *M. tuberculosis* bacilli in the A/J strain. BCG Japan was unrecoverable from the lung, and counts of BCG Pasteur remained low throughout the experiment. As would be expected, these striking differences in bacterial growth resulted in a very strong impact of the mycobacterial variant on the CFU phenotype, with differences in pulmonary burdens across the mycobacteria more pronounced in A/J mice than in C57BL/6J mice (Fig. 1). When the host component of the contribution to the number of lung CFU was analyzed, the strongest effect was observed from *M. tuberculosis*, where the pulmonary loads of *M. tuberculosis* were significantly larger in A/J mice (least-squares mean [LSM] of $\log_{10}$CFU = 3.96; standard error [SE] = 0.14) than in C57BL/6J animals (LSM of $\log_{10}$CFU = 3.13; SE = 0.20) ($P = 0.002$). These results indicated that although the host genetic background impacted bacterial replication, mycobacteria had a dominant effect (Fig. 1).

In contrast to BCG Russia and *M. tuberculosis*, BCG Pasteur and BCG Japan were consistently associated with low bacillary counts in the lungs of both strains of mice. Unlike BCG Japan, which failed to grow in the spleen (data not shown), BCG Pasteur thrives in the spleen (14, 25). This raised the question of if the low pulmonary burden of BCG Pasteur reflected the inability of BCG Pasteur to home to the lung following intravenous infection or if BCG Pasteur had a general limited capacity to multiply in lungs. To test the ability of Pasteur to replicate in the lung, bacilli were directly implanted into the lung by aerosol. Using a high-dose infection model ($\sim 2 \times 10^3$ bacilli), the counts of BCG Pasteur and BCG Russia at the 6-week time point were compared. BCG Pasteur still had smaller pulmonary burdens in both the A/J mice ($P = 0.001$) and the C57BL/6J mice ($P = 5.7 \times 10^{-5}$) (Fig. 2), suggesting that the observed differences were not due to spread to the lung but rather to a decreased capacity of BCG Pasteur to replicate in the lung. In addition, a difference in BCG Russia pulmonary loads between the mouse strains was observed ($P = 0.009$), with C57BL/6J mice displaying higher numbers of CFU. This was in contrast to *M. tuberculosis*, which reached higher bacterial counts in the lungs of A/J mice following

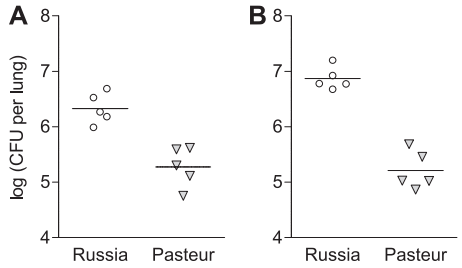

FIG. 2. Pulmonary load of BCG Pasteur and BCG Russia in A/J and C57BL/6J mice following a high-dose aerosol infection. The bacterial counts (number of $\log_{10}$CFU) of BCG Russia (white circle) or BCG Pasteur (gray triangle) were determined in the lungs of A/J (A) and C57BL/6J (B) mice at 6 weeks following a high-dose ($\sim 2 \times 10^3$ bacilli) aerosol infection. Experiments were repeated twice, with similar results. Bars are representative of the mean numbers of $\log_{10}$CFU for the group.

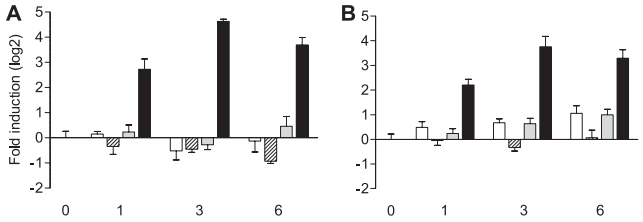

FIG. 4. Pulmonary transcription of *Il12b* in A/J and C57BL/6J mice infected with BCG or *M. tuberculosis*. The levels of induction of *Il12b* during a low-dose intravenous infection with BCG Russia, BCG Japan, BCG Pasteur, or *M. tuberculosis* were compared in A/J (A) and C57BL/6J (B) mice at 1, 3, and 6 weeks. Data shown represent the fold changes ($\log_2$) in *Il12b* transcription in infected mice ($n = 4$) relative to those in uninfected mice ($n = 4$) ± SEM. BCG Russia, white; BCG Japan, hatched; BCG Pasteur, gray; *M. tuberculosis*, black.

intravenous infection (Fig. 1). These results demonstrated that differences in pulmonary bacterial counts reflect different abilities of the three mycobacteria to grow in lungs, and this difference depended on the mouse strain used.

Next, we determined the pulmonary transcript levels of the gamma interferon (*Ifng*) cytokine in A/J or C57BL/6J mice intravenously infected with the mycobacterial pathogens (Fig. 3). *M. tuberculosis* consistently induced the largest transcript levels of *Ifng*, followed by BCG Pasteur. Induction of *Ifng* by BCG Russia was delayed, particularly in the A/J mouse strain. Three-way ANOVA showed significant effects of mycobacteria [$F(3, 20.6) = 123.7$; $P < 0.001$] and of time [$F(2, 26.7) = 74.1$; $P < 0.001$] on *Ifng* transcription, with a significant interaction between the two effects [$F(6, 16) = 16.2$; $P < 0.001$]. The interaction between the host strain and time was not significant [$F(2, 26.7) = 2.6$; $P = 0.1$], and a triple interaction between host, pathogen, and time was not detected [$F(6, 16) = 1.3$; $P = 0.3$]. Although the effect of the host strain was not significant [$F(1, 40.7) = 2.9$; $P = 0.1$], there was a significant interaction between the host and bacteria [$F(3, 20.6) = 10.7$; $P < 0.001$], reflective of significant host-pathogen specificity in *Ifng* induction.

The pulmonary transcript levels of interleukin 12b (*Il12b*) and interleukin 4 (*Il4*) were measured next. Bacterium-related differences in *Il12b* induction were observed in both A/J and C57BL/6J mice (Fig. 4), with *M. tuberculosis* inducing significantly more *Il12b* than the three BCG strains. Three-way ANOVA testing identified significant effects of the mycobacteria [$F(3, 25.8) = 190.5$; $P < 0.001$] and mouse strain [$F(1, 46.8) = 8.8$; $P = 0.005$] on *Il12b* transcription. There was a significant interaction between the two effects [$F(3, 25.8) = 6.1$; $P = 0.003$], indicating specificity of the host-pathogen interaction on *Il12b* induction. The effect of time was also significant [$F(2, 30.5) = 4.1$; $P = 0.03$], and there was a significant interaction between the bacterial strain and time [$F(6, 19) = 4.9$; $P = 0.004$] but not between the mouse strain and time [$F(2, 30.5) = 1.9$; $P = 0.2$]. A triple interaction between the effects was not detected [$F(6, 19) = 1.1$; $P = 0.4$]. Unlike *Il12b*, pulmonary *Il4* expression negatively correlated with infection with all four mycobacteria (Fig. 5). Three-way ANOVA testing showed significant effects of the mycobacteria [$F(3, 24.8) = 8.7$; $P < 0.001$], mouse strain [$F(1, 33.3) = 8.3$; $P = 0.01$], and time [$F(2, 24) = 6.7$; $P = 0.005$] on *Il4* transcription. The interaction between the host and bacteria was not significant [$F(3, 24.8) = 2.7$; $P = 0.07$], indicating that mycobacterial variation and host genetics independently act on *Il4* induction. There was significant interaction between the effects of bacteria and time [$F(6, 20.1) = 3.0$; $P = 0.03$] and between the host strain and time [$F(2, 24) = 6.8$; $P = 0.005$]. A triple interaction was not observed [$F(6, 20.1) = 0.8$; $P = 0.6$]. Thus, our analysis detected evidence for host-pathogen specificity in the induction of *Ifng* and *Il12b* but not in the induction of *Il4*.

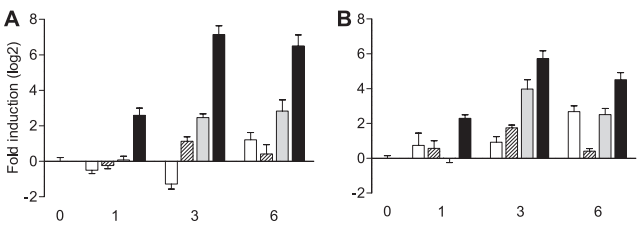

FIG. 3. Pulmonary *Ifng* transcript levels in the A/J and C57BL/6J mouse strains following mycobacterial infection. The levels of induction of *Ifng* transcription by BCG Russia (white), BCG Japan (hatched), BCG Pasteur (gray), and *M. tuberculosis* (black) were compared in the A/J (A) and C57BL/6J (B) mouse strains at 1, 3, and 6 weeks following a low-dose intravenous infection. Fold induction ($\log_2$) is the ratio of *Ifng* expression in infected mice ($n = 4$) relative to that in uninfected mice ($n = 4$). Error bars represent SEM.

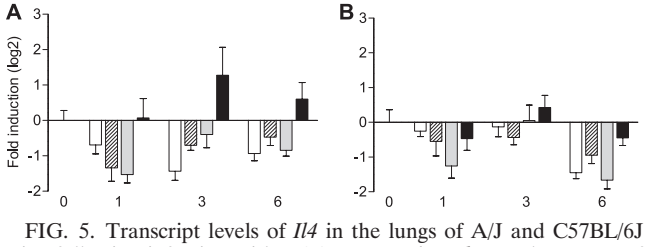

FIG. 5. Transcript levels of *Il4* in the lungs of A/J and C57BL/6J mice following infection with BCG or *M. tuberculosis*. The extent of pulmonary *Il4* transcription in A/J (A) and C57BL/6J (B) mice was determined at 1, 3, and 6 weeks following a low-dose intravenous infection with BCG Russia, BCG Japan, BCG Pasteur, or *M. tuberculosis.* Data shown are presented as fold changes in *Il4* transcription in infected mice ($n = 4$) relative to those in uninfected mice ($n = 4$) ± SEM. BCG Russia, white; BCG Japan, hatched; BCG Pasteur, gray; *M. tuberculosis*, black.

TABLE 1. Genes differentially expressed across mycobacteria at week 3

Gene[b]	Adjusted *P* value	
	A/J mice	C57BL/6J mice
Identified in A/J and C57BL/6J mice		
Ccl20	0.037	0.048
Ccl4	0.006	0.021
Ccl5	0.006	0.021
Cxcl10	0.006	0.021
Specific to A/J mice		
Ccl1	0.013	—[a]
Ccl12	0.006	—
Ccl2	0.033	—
Ccl7	0.020	—
Ccl8	0.009	—
Ccr5	0.020	—
Cx3cl1	0.007	—
Cxcl11	0.029	—
Cxcl9	0.006	—
Cxcr3	0.011	—
Tnf	0.011	—
Specific to C57BL/6J mice		
Ccl11	—	0.048
Ccl19	—	0.032
Ccr9	—	0.048
Cxcl5	—	0.048
Il18	—	0.039

[a] —, nonsignificant.
[b] *Ccl*, chemokine (C-C motif) ligand; *Ccr*, chemokine (C-C motif) receptor; *Cx3cl1*, chemokine (C-X3-C motif) ligand 1; *Cxcl*, chemokine (C-X-C motif) ligand; *Cxcr*, chemokine (C-X-C motif) receptor; *Tnf*, tumor necrosis factor; *Il18*, interleukin 18.

TABLE 2. Genes differentially expressed across mycobacteria at week 6

Gene[b]	Adjusted *P* value	
	A/J mice	C57BL/6J mice
Identified in A/J and C57BL/6J mice		
Ccr1	0.037	0.007
Ccr2	0.034	0.023
Specific to A/J mice		
Ccl5	0.048	—[a]
Ccl8	0.040	—
Ccr5	0.040	—
Ccr9	0.034	—
Cxcl10	0.040	—
Cxcl15	0.034	—
Cxcl5	0.048	—
Cxcl9	0.040	—
Cxcr3	0.008	—
Specific to C57BL/6J mice		
Ccl19	—	0.018

[a] —, nonsignificant.
[b] *Ccl*, chemokine (C-C motif) ligand; *Ccr*, chemokine (C-C motif) receptor; *Cxcl*, chemokine (C-X-C motif) ligand; *Cxcr*, chemokine (C-X-C motif) receptor.

Transcriptional levels of 34 cytokines, chemokines, and chemokine-related genes were profiled by real-time PCR in response to *M. tuberculosis*, BCG Russia, and BCG Pasteur in A/J and C57BL/6J mice at 3 and 6 weeks postinfection. Due to the low variability of BCG Japan-induced host responses, this strain was not included in this analysis. Moreover, given the large number of chemokines studied, a three-way ANOVA analysis was not performed. Instead, one-way ANOVA analyses comparing the two mouse strains were performed for each mycobacterium and at each time point. Unexpectedly, significant differences in cytokine and chemokine transcript levels were not detected between A/J and C57BL/6J mice. Hence, subsequent ANOVA analyses were focused on the extent of differential gene expression across the three species of mycobacteria in mice of each strain at each time point. The magnitude of the transcriptional response was shown to be the largest during *M. tuberculosis* infection, while BCG Russia had lagged expression for most of the genes tested. Expression differences across the three mycobacteria were more pronounced in the A/J mouse strain. Among the genes that were significantly up- or downregulated, 15 and 11 genes were differentially expressed across bacteria at 3 and 6 weeks, respectively (Tables 1 and 2; see also Tables S1 and S2 in the supplemental material). In C57BL/6J mice, differences in induction related to the mycobacteria for 9 genes at 3 weeks and for 3 genes at 6 weeks were observed (Tables 1 and 2; see also Tables S3 and S4 in the supplemental material). These results demonstrated that the magnitude of differences in cytokine and chemokine levels following mycobacterial infection was critically dependent on the host background.

DISCUSSION

Analysis of functional host phenotypes in the mouse has demonstrated that bacteria from different *M. tuberculosis* lineages cause a range of immunological and pathological effects (15, 33). Specifically, the Euro-American group of strains is unable to produce a PGL molecule that has been associated with subversion of innate immune responses and increased mortality in mice (43). Differences in the magnitude of the inflammatory response to infection with BCG substrains have also been observed (21). Moreover, different strains of BCG induce different levels of protective tuberculosis immunity in mice and vary in incidences of adverse effects in human studies (28, 31). These studies show an association between mycobacterial genetic variation and host immune responses. On the other hand, it has been clearly established that genetically distinct inbred mice and humans differ dramatically in their susceptibility to mycobacterial infections. In humans, it has recently been shown that susceptibility to infection with *M. tuberculosis* is under genetic control and strongly impacted by a locus on chromosome 11 (11, 12). A number of additional genes modulate the risk of developing clinical tuberculosis in infected persons, including the *NRAMP1* gene (19, 36). The mouse orthologue of the latter gene was previously identified as the major determinant of innate susceptibility to infection with several BCG strains in the spleen (14, 50). In addition, variable susceptibility of inbred strains of mice to *M. tuberculosis* has been linked to a number of loci (24, 27, 39, 40, 46, 47, 53). Taken together, these data establish that genetic variability of both the mycobacterial pathogen and murine or human host strongly impacts different aspects of the host susceptibility to mycobacterial infection. However, there is a lack of studies

that have systematically analyzed, in parallel, the joint contribution of both the host and mycobacteria on host responses.

A consistent finding of our experiments was that all infectious phenotypes were more sensitive to variation of the mycobacterial pathogen than to that of the host background. Given the very small number of mycobacteria and host strains, this may be at least partly a result of the larger genetic variability among the four types of mycobacteria than that among the more closely related A/J and C57BL/6J mice. By including wild-derived mice or mice with known genetic defects in such comparisons, by studying a later phase of infection, or by changing the mode and dose of infection, the relative impact of the host genetic background versus that of the bacterial pathogen may well have been different. Despite such limitations, our data are consistent with the view that the major effect on host responses is due to the type of mycobacteria, while the host genetic background is the modulator of responsiveness. Yet, even in our study with a dominant impact of the mycobacterial pathogen on the studied phenotypes, it became clear that a comparison of the mycobacteria for both pulmonary CFU burden and mechanistic phenotypes (*Ifng* and chemokine transcript levels) had more discriminatory power in A/J mice than in C57BL/6J mice. This highlights problems that can be encountered when studies employing the same mycobacterial strain are compared in different hosts. Above all, the analysis of common host responses to mycobacteria in this model system provided proof of principle for host-pathogen joint effects on phenotype variance. This is an important extension of our previous observation of BCG strain-specific genetic control of BCG Russia and BCG Pasteur replication (14).

The detection of significant interactions of host and pathogen on phenotype expression by necessity invokes differences in the pathogenesis, either quantitative or qualitative, between different host-pathogen strain combinations. Given that both the number of lung CFU and the wide array of mechanistic phenotypes studied showed evidence for significant joint mycobacterium-host effects, it seems likely that similar effects are also seen in human populations. This opens the question of if the search for protective correlates of tuberculosis should be focused on mechanistic phenotypes that show little sensitivity to host-pathogen variability or if different host-pathogen combinations invoke different protective correlates. Similarly, if a large number of persons are exposed to an array of *M. tuberculosis* strains, it is possible that among the persons who develop tuberculosis, some are susceptible to all strains while others are susceptible to specific subsets of *M. tuberculosis* strains. Such a hypothetical scenario would provide a straightforward biological explanation as to why *M. tuberculosis* strains are preferentially associated with distinct human populations (17, 44). To judge the relevance of the above-described considerations will require a more accurate estimate of the relative importance of pathogen, host, and joint effects on phenotype expression. This in turn will require much larger comparative studies covering a larger range of phenotypes as well as hosts and pathogens.

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