Evidence Inconsistent with a Role for the *Bcg* Gene (*Nramp1*) in Resistance of Mice to Infection with Virulent *Mycobacterium tuberculosis*

By Eva Medina and Robert J. North

From the Trudeau Institute, Saranac Lake, New York 12983

Summary

The superior resistance of some strains of mice over others to infection with certain intracellular pathogens, including the vaccine strain of *Mycobacterium bovis*, bacillus Calmette Guerin (BCG), is determined by a gene associated with a small segment of chromosome 1 designated the *Ity/Lsh/Bcg* locus, referred to here as the *Bcg* locus. DBA/2 mice containing the dominant resistant allele of the Bcg gene (Bcg^r), major histocompatibility complex–compatible BALB/c mice containing the recessive susceptible allele (Bcg^s), and congenic C.D2-N20 Bcg^r, which are genetically the same as BALB/c mice except for possessing a small piece of DBA/2 chromosome 1 containing the *Bcg* locus, were used to determine whether the *Bcg* gene determines resistance to infection with the virulent H37Rv strain of *Mycobacterium tuberculosis* (Mtb). According to the survival times of Bcg^r and Bcg^s mice infected via either the intravenous or respiratory route, Bcg^r mice proved much less, rather than more, resistant to Mtb infection than Bcg^s mice. Shorter survival times of Bcg^r mice were associated with an inferior capacity to control Mtb growth in their lungs and to retard the development of Mtb-induced pathology in this organ. Resistance to Mtb infection was a dominant trait in the F1 progeny of Bcg^r and Bcg^s mice. The results show that resistance to Mtb is not determined by the resistance allele of the *Bcg* gene nor by the recently isolated candidate *Bcg* gene *Nramp1*, located in the Bcg locus.

Some strains of mice show a higher level of natural resistance than others to infection with certain pathogens, including *Salmonella typhimurium, Leishmania donovani,* and the attenuated bacillus Calmette Guerin (BCG)[1] strain of *Mycobacterium bovis* (1). The genes responsible for resistance to each of these three pathogens have been mapped independently to a small region of chromosome 1 designated the *Ity/Lsh/Bcg* locus (2–4), and referred to here as the *Bcg* locus. It is generally believed that resistance to all of the pathogens mentioned is under the control of a single dominant gene. According to a number of studies, the gene is expressed in macrophages (5–7), and its dominant resistant allele provides these cells with a superior innate capacity to kill microbial pathogens or restrict their intracellular growth before the generation of specific host immunity.

A knowledge of the genetic and physical intervals defining the *Bcg* locus of mouse chromosome 1 was used recently to identify several candidate transcriptional units for the *Bcg* gene, one of which was choses as the most likely candidate gene because it is expressed exclusively in macrophages. This candidate gene, designated *Nramp1* (for natural resistance–associated macrophage protein), encodes for a polypeptide that is part of the transmembrane domain of a macrophage-specific membrane protein (8). Additional studies with a variety of BCG-resistant (Bcg^r) and -susceptible (Bcg^s) strains of mice showed anti-BCG resistance to be associated with a nonconservative Gly-169–to–Asp-169 substitution within the predicted transmembrane domain 4 of *Nramp1* (9–11). More recently, targeting of the *Nramp1* gene by homologous recombination was used to generate mutant mice bearing a null-resistant allele of the *Nramp1* gene (11). These mice proved to be susceptible to BCG, *S. typhimurium,* and *L. donovani.* An homologous gene has been shown to be expressed in human macrophages (12), thereby opening the way to investigating whether sequence variations in this gene are associated with differences in susceptibility to tuberculosis among families in endemic areas of the disease. Even so, it needs to be remembered that, in spite of the evidence that *Nramp1* is the gene that determines innate resistance of mice to BCG infection, there is little evidence that this gene is responsible for determining resistance to infection with virulent strains of *Mycobacterium tuberculosis* (Mtb).

The purpose of this article is to show, with MHC-compatible Bcg^r and Bcg^s strains of mice, and with a congenic Bcg^r strain, that the *Bcg* gene appears to be of little impor-

[1]*Abbreviations used in this paper:* BCG, bacillus Calmette Guerin; MST, median survival time; Mtb, *Mycobacterium tuberculosis*; *Nramp*, natural resistance–associated macrophage protein.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/96/03/1045/07 $2.00
Volume 183 March 1996 1045–1051

tance in determining resistance to infection with the virulent H37Rv strain of Mtb.

Materials and Methods

Animals. Inbred, pathogen-free, 8-wk-old BALB/c (*Bcg*^{s}), DBA/2 (*Bcg*^{r}), and CD2F1 (BALB/c × DBA/2) mice were purchased from Trudeau Institute Animal Breeding Facility. Congenic C.D2 Idh^b-Ity^r-Pep3^b (C.D2-N20) *Bcg*^{r} breeder mice were kindly provided by Dr. Emil Skamene of the Montreal General Hospital Research Institute (Montreal, Quebec, Canada). These mice differ from BALB/c mice in possessing a small segment of chromosome 1 of DBA/2 mice containing the *Bcg* locus (13). All mice were free of common viral pathogens according to the results of routine testing performed by the Research Animal Diagnostic and Investigative Laboratory, University of Missouri, (Columbia, MO).

Mycobacteria. M. tuberculosis H37Rv (TMC #102) was obtained from the Trudeau Mycobacterial Culture (TMC) collection. It was supplied in vials as a frozen (−70°C) log phase dispersed culture in Proskauer and Beck medium (Difco Laboratories Inc., Detroit, MI) containing 0.01% Tween 80. For each experiment, a vial was thawed, subjected to 5 s of ultrasound to break up aggregates, and diluted appropriately in PBS containing 0.01% Tween 80. Mice were inoculated with ~10^5 CFU of bacilli in 0.2 ml of PBS via a lateral tail vein, or with ~5×10^2 CFU by aerosol. Infection by aerosol was performed in a Middlebrook airborne infection apparatus (Tri Instruments, Jamaica, NY). Bacilli were enumerated at progressive times in the lungs, kidneys, livers, and spleens of infected mice by preparing homogenates of these organs in PBS containing 0.05% Tween 80 and plating 10-fold serial dilutions of the homogenates on enriched agar (Middlebrook 7H11; Difco Laboratories Inc.). Colonies were enumerated after 3–4 wk of incubation at 37°C.

Histology. Pieces of lungs were taken at day 70 of infection and fixed in 10% phosphate-buffered formalin. The tissues were then dehydrated and wax embedded according to routine procedures. Sections 4–6 μm thick were stained with Ziehl Nielsen stain for acid-fast bacilli and counterstained with methylene blue. Photomicrographs were taken with a microscope (Microphot-Fx; Nikon Inc., Melville, NY).

Results

Bcg^r *Mice Are Much More Susceptible than Bcg*^{s} *Mice to Mtb Infection Initiated Intravenously or by Aerosol.* In the first experiments, DBA/2 (*Bcg*^{r}) and MHC-compatible BALB/c (*Bcg*^{s}) mice were infected with 10^5 Mtb intravenously or with 5×10^2 Mtb by aerosol, and growth of the organism in lungs, livers, spleens, and kidneys was followed over a 100-d period. Additional mice were infected to obtain survival data. The results in Fig. 1 show that, in the case of infection via the intravenous route, *Bcg*^{r} mice were substantially less, rather than more, capable than *Bcg*^{s} mice of controlling the growth of Mtb in their lungs, in that the former mice had ~100 times more bacilli in this organ than the latter on day 100 of infection. Both strains of mice were similar, on the other hand, in terms of their ability to stabilize infection in other organs. It can be seen with both strains of mice that, after a brief period of bacillary growth in the liver, spleen, and kidneys, infection was stabilized and caused to plateau until day 100. This superior resistance of BALB/c mice was independent of the size of inoculum. Thus, DBA/2 mice inoculated intravenously with 10^3 CFU of Mtb H37Rv contained 2.5 logs more bacilli in their lungs than BALB/c mice inoculated with the same number of bacteria (7.1 ± 0.3 log for DBA/2; 4.6 ± 0.2 log for BALB/c) at day 80 of infection.

In the case of infection initiated via the respiratory route (Fig. 2), *Bcg*^{r} mice were likewise less capable than *Bcg*^{s} mice of controlling Mtb growth in their lungs. However, *Bcg*^{r} mice allowed much less initial seeding of Mtb from the lungs to the liver and spleen. In spite of this, however, Mtb grew more extensively in the livers of these mice than in the livers of *Bcg*^{s} mice after day 75 of infection. The smaller numbers of Mtb in the livers of DBA/2 mice on day 30 of the aerosol infection indicates that fewer organisms disseminated from the lungs to implant in the livers at this stage of infection. Presumably, similar levels of resistance were expressed in the livers of DBA/2 and BALB/c mice between days 30 and 70. The large increase in the number of Mtb in the livers of DBA/2 mice after day 70 might have resulted, at least in part, from continuous seeding of bacilli from the lungs, because of a massive increase in the number of bacilli in this organ from day 70 on. It could also have resulted from the expression of less resistance in the livers of DBA/2 mice after day 70.

The greater susceptibility of *Bcg*^{r} mice to Mtb infection is

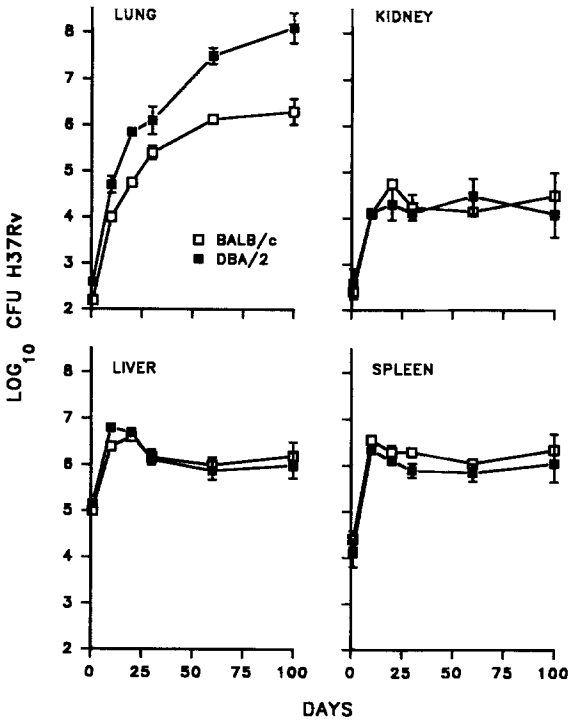

Figure 1. Growth of Mtb H37Rv in lungs, livers, spleens, and kidneys of *Bcg*^{r} (DBA/2) and *Bcg*^{s} (BALB/c) mice inoculated intravenously with 2×10^5 organisms. *Bcg*^{r} mice were substantially less resistant to Mtb infection, although only in the lungs. Means are for five mice per time point ± SD.

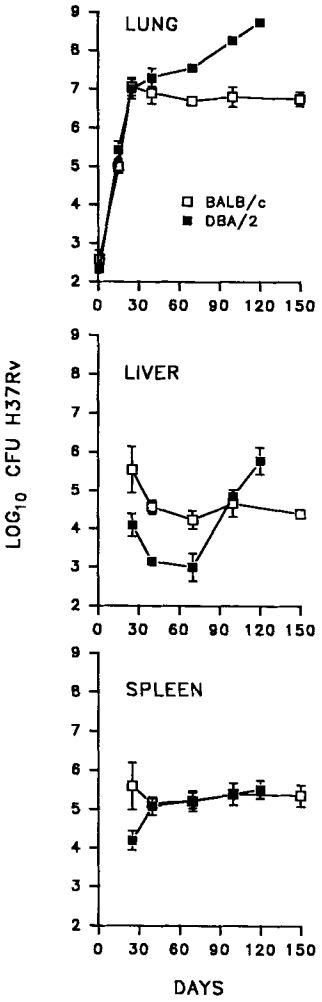

Figure 2. Growth of Mtb in lungs, livers and spleens of *Bcg*^{r} (DBA/2) and *Bcg*^{s} (BALB/c) mice given 5 × 10^2 Mtb via the respiratory route. *Bcg*^{r} mice were much less resistant than *Bcg*^{s} mice in their lungs after day 20. In addition, *Bcg*^{r} mice showed a smaller number of Mtb in their livers and spleens on day 20, although their livers were less capable than those of *Bcg*^{s} of controlling infection from day 70 on. Means are for five mice per time point ± SD.

even more convincingly demonstrated by the survival data shown in Figs. 3 and 4. It can be seen that after initiating infection via either route, the median survival time (MST) of *Bcg*^{r} mice was substantially shorter, being less than half that of *Bcg*^{s} mice.

Resistance to Mtb Infection Is a Dominant Trait in F1 Mice. To determine whether superior anti-Mtb resistance of *Bcg*^{s}

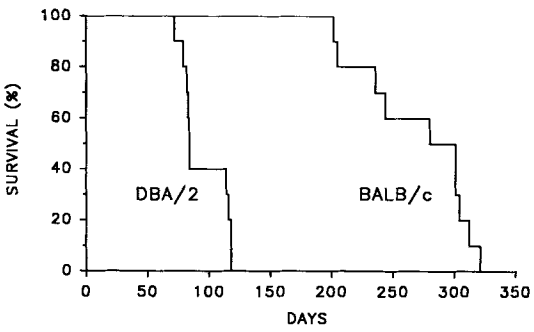

Figure 3. Survival times of *Bcg*^{r} (DBA/2) and *Bcg*^{s} (BALB/c) mice infected intravenously with Mtb as described for Fig. 1. *Bcg*^{r} mice died much sooner (MST = 84 d) than *Bcg*^{s} mice (MST = 290 d). There were 10 mice in each group.

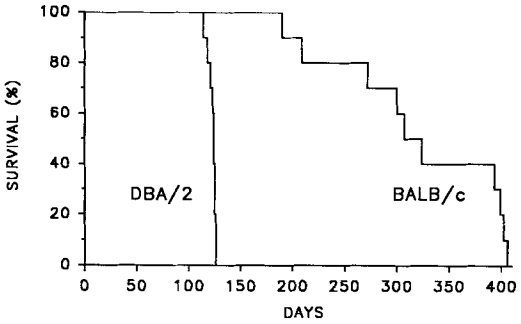

Figure 4. Survival times of mice infected with Mtb aerosol as described for Fig. 3. *Bcg*^{r} (DBA/2) mice died much sooner (MST = 124 d) than *Bcg*^{s} (BALB/c) mice (MST = 303 d). There were 10 mice per group.

BALB/c mice over *Bcg*^{r} DBA/2 mice is a dominant trait, growth of Mtb in the organs of these strains was compared with its growth in their F1 progeny over a 100-d period. As can be seen in Fig. 5, F1 mice were the same as BALB/c mice in displaying a superior ability over DBA/2 mice to resist the growth of Mtb in their lungs after inoculation with 10^5 bacilli via the intravenous route. These results are supported by the survival data in Fig. 6 showing that F1 and BALB/c mice survived more than twice as long as DBA/2 mice from the 10^5-bacilli intravenous inoculum.

Resistance to Mtb Infection Is Not Determined by the Bcg^s *Gene.* The foregoing results show that mice that possess

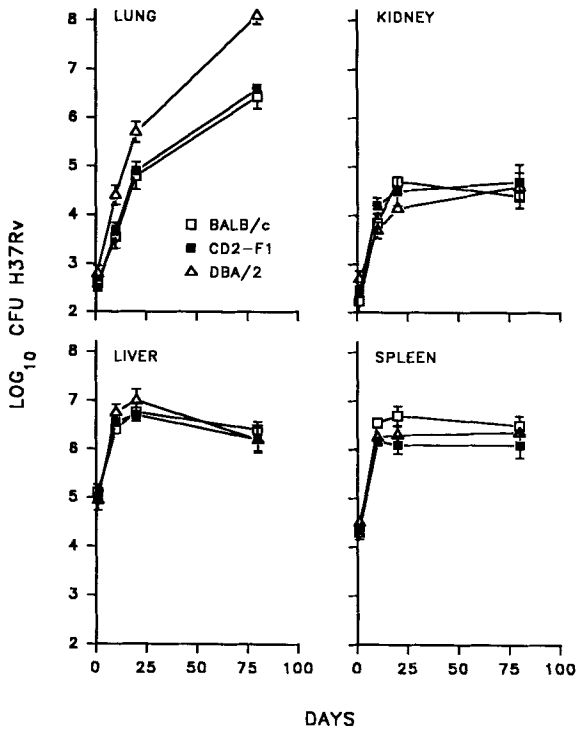

Figure 5. Growth of Mtb in lungs, livers, spleens, and kidneys of *Bcg*^{s} (BALB/c), *Bcg*^{r} (DBA/2) mice, and their F1 progeny (CD2-F1) after intravenous inoculation of 10^5 bacilli. CD2-F1 mice, like BALB/c mice, were substantially more resistant to Mtb growth in the lungs. Means are for five mice per time point ± SD.

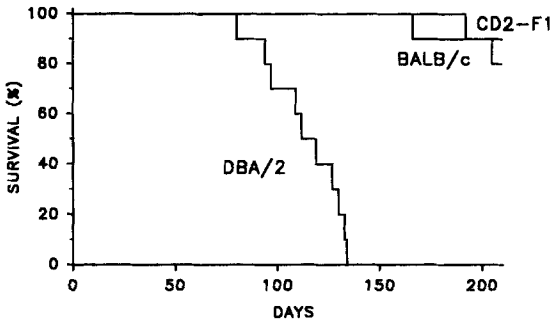

Figure 6. Evidence that CD2-F1 progeny of *Bcg*^{s} (BALB/c) and *Bcg*^{r} (DBA/2) mice, like BALB/c, were much more capable of surviving infection with Mtb. In this experiment, 10 mice per group were infected intravenously, with 10^5 Mtb as described for Fig. 5.

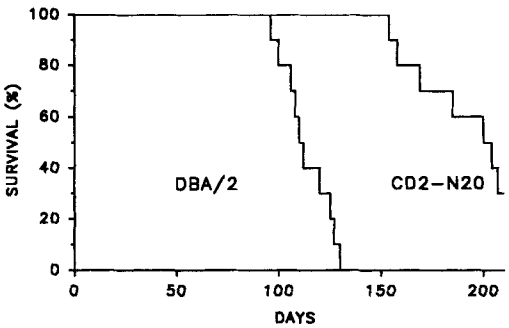

Figure 8. Survival data of mice infected as described for Fig. 7. *Bcg*^{r} C.D2-N20-congenic mice were more capable than *Bcg*^{r} DBA/2 mice of surviving infection. Results were obtained with 10 mice per group.

the resistance allele of the *Bcg* gene are less, rather than more, resistant to infection with virulent Mtb, and that the F1 progeny of *Bcg*^{r} and *Bcg*^{s} mice are resistant like *Bcg*^{s} mice. This could mean that the recessive *Bcg*^{s} allele is responsible for resistance to Mtb and that it is dominant when measured against Mtb in the F1. If so, it would follow that *Bcg*^{s} BALB/c mice should be more resistant to Mtb infection than congenic C.D2-N20 mice that differ from BALB/c mice in possessing a small piece of chromosome 1 of DBA/2 mice containing the *Bcg*^{r} gene. The results in Figs. 7 and 8 show this not to be the case, in that *Bcg*^{r}-congenic C.D2-N20 mice, like BALB/c mice, were much more resistant than DBA/2 mice to Mtb infection, as measured by superior capacities to control Mtb growth in their lungs and to survive infection. It can be concluded from these results, therefore that the presence of the *Bcg*^{s} allele of the *Bcg* gene is not needed for BALB/c mice to be more resistant to Mtb infection than *Bcg*^{r} DBA/2 mice. In other words, resistance to Mtb infection is determined by genes other than the *Bcg* gene.

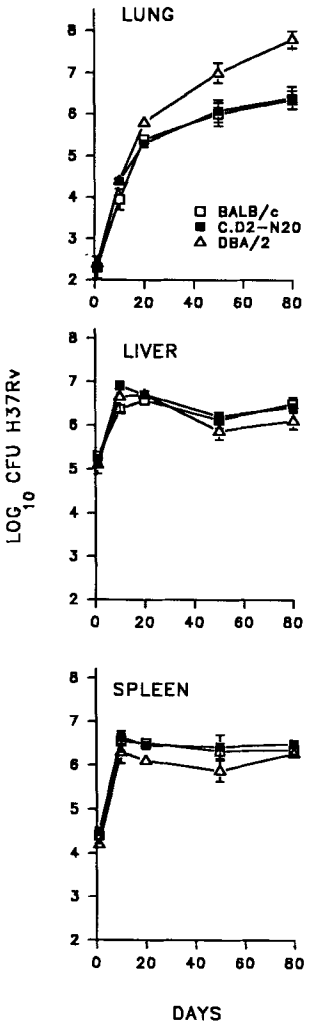

Figure 7. Evidence that the superior anti-Mtb resistance of *Bcg*^{s} (BALB/c) mice over *Bcg*^{r} (DBA/2) mice was not dependent on the presence of the *Bcg*^{s} allele. Congenic C.D2-N20 BALB/c mice possessing a small segment of DBA/2 chromosome 1 containing the *Bcg*^{r} locus were, like BALB/c mice, much more capable than DBA/2 mice of controlling Mtb infection in the lungs following intravenous inoculation of 2×10^5 bacilli. Therefore, superior resistance of BALB/c mice depends on genes other than those in the *Bcg* locus. Means are for five mice per time point ± SD.

The Superior Anti-Mtb Resistance of BALB/c Mice Is Associated with Slower Development of Lung Pathology. It has been shown in preceding sections that, in spite of their possession of the susceptibility allele of the *Bcg* gene, BALB/c mice are much more resistant to Mtb infection than DBA/2 mice that contain the dominant resistance allele of the gene, as measured by the ability of the former mice to restrict the growth of Mtb in their lungs and survive infection. Because the difference in anti-Mtb resistance was seen only in the lungs, it was anticipated that the longer survival time of *Bcg*^{s} mice would be associated with the development of much less lung pathology. This prediction proved correct, as evidenced by the photos of 20-μm sections of lungs of BALB/c and DBA/2 mice (Fig. 9), which show that on day 70 of infection the lungs of BALB/c mice displayed fewer and smaller Mtb-induced lesions than the lungs of DBA/2 mice. At higher magnification, the lung lesions in each strain consisted of areas of intense alveolitis in which contiguous air sacs were filled with leukocytes, most of which were mononuclear. Within each lesion, acid-fast bacilli were located in collections of epithelioid macrophages occupying contiguous air sacs. These sites of infection were larger and more numerous in lung lesions of DBA/2 mice (Fig. 9) and contained larger numbers of bacilli. A histological examination of the livers and spleens of both strains of mice (not shown) revealed that Mtb infection did not cause progressive pathology. Instead, stabilization of infection in these organs was associated with small compact granulomas that did not change in size or composition over time. This is in keeping with the results of another study in this laboratory (14).

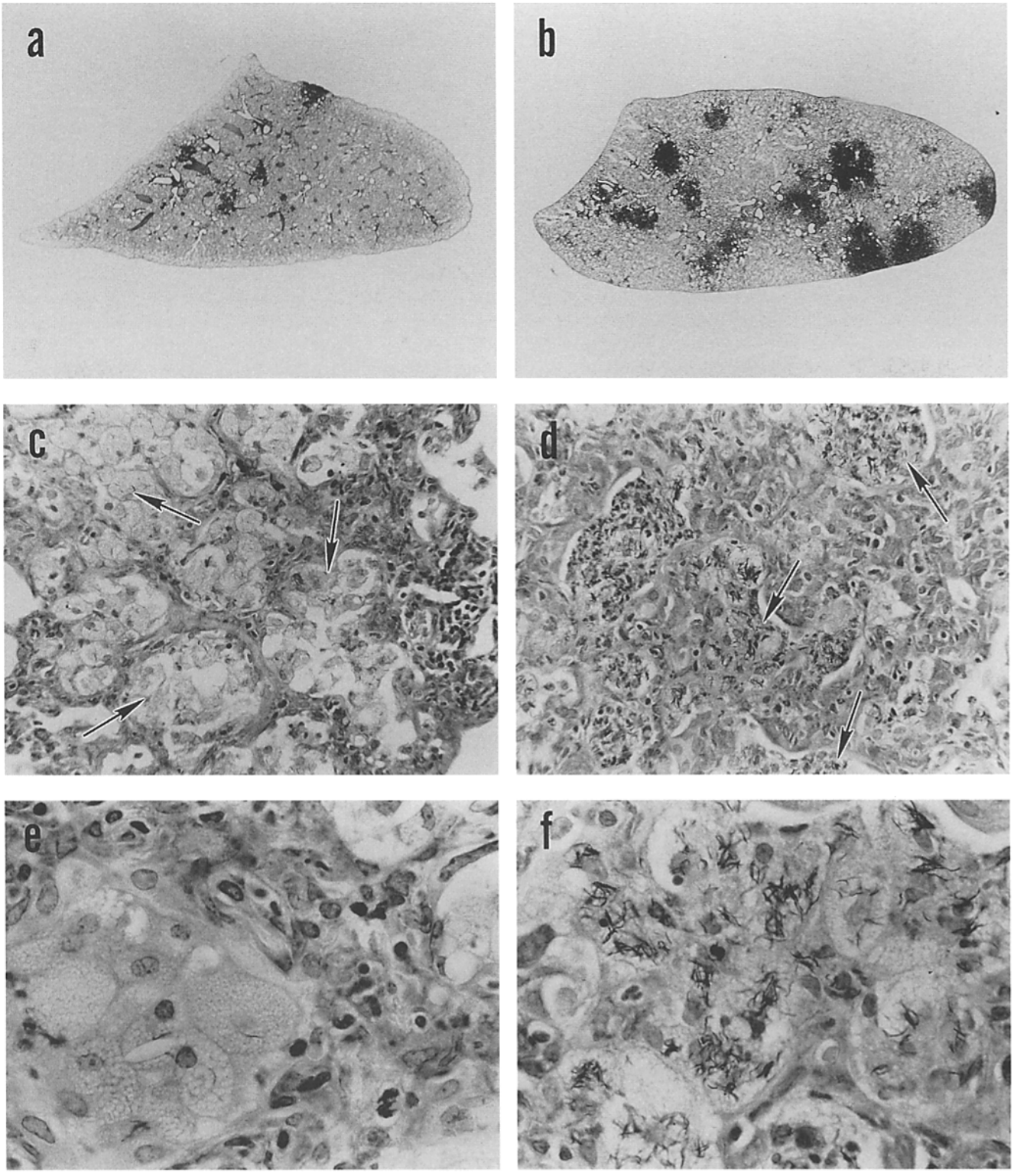

Figure 9. Evidence that the superior ability of *Bcg*^{s} (BALB/c) mice over *Bcg*^{r} (DBA/2) mice for controlling Mtb infection in their lungs was associated with substantially less pathology in this organ. On day 70 of infection initiated with 10^5 Mtb via the intravenous route, there were fewer and smaller lesions in 20-μm crystal violet–stained sections of lungs of BALB/c mice (*a*) than in the lungs of DBA/2 mice (*b*). In both cases, Mtb was confined to collections of epithelioid macrophages (*arrows*) that were more degenerate in lung lesions of DBA/2 mice (*d*) than in lung lesions of BALB/c mice (*c*). At higher power, these sites of infection in DBA/2 mice (*f*) contained many more acid-fast bacilli than those in BALB/c mice (*e*). Samples were stained with Ziehl Nielsen counterstained with methylene blue. ×8 (*a* and *b*); ×300 (*c* and *d*); ×700 (*e* and *f*).

Discussion

This study leaves little doubt that the dominant resistant allele of the *Bcg* gene (*Bcg*^{r}), which is responsible for the ability of certain strains of mice to better resist the growth of BCG and certain other pathogens during the preimmunity stage of infection, has little or no influence on the ability of these strains to resist infection with virulent Mtb. The results show that *Bcg*^{r} DBA/2 mice are considerably less, rather than more, resistant to Mtb infection than *Bcg*^{s} BALB/c mice. This was the case with mice infected either via the respiratory or intravenous route. Superior anti-Mtb resistance of *Bcg*^{s} mice was measured as (*a*) a longer survival time, (*b*) a superior ability to control infection in the lung, and (*c*) slower development of lung pathology. Resistance to Mtb infection was a dominant trait in the F1 progeny of BALB/c and DBA/2 mice, which suggested the possibility that the *Bcg*^{s} rather than the *Bcg*^{r} allele of the *Bcg* gene was responsible for resistance to Mtb. However, this proved not to be the case, as evidenced by the finding that *Bcg*^{r}-congenic C.D2-N20 BALB/c mice were like *Bcg*^{s} BALB/c mice in being more resistant than *Bcg*^{r} DBA/2 mice to infection with Mtb. Because the recently isolated candidate gene for the *Bcg* gene, *Nramp1*, is contained within the *Bcg* locus (8), it follows that *Nramp1* also has little, if any, influence on resistance to Mtb infection, at least according to the way that anti-Mtb resistance was measured in this study.

It needs to be brought to mind, in this connection, that the superior resistance of *Bcg*^{r} mice to BCG infection has been measured almost exclusively in the spleen as a superior ability to restrict the growth of BCG during the preimmunity first 20 d of infection (15, 16). On the other hand, it has been shown (17) that *Bcg*^{r} and *Bcg*^{s} strains of mice are similar in terms of their capacity to restrict preimmunity growth of BCG in other organs. Moreover, both types of mice seem equally capable of resolving BCG infection in all organs from 20 d of infection on, after specific immunity is acquired. Indeed, being an attenuated organism, BCG does not cause progressive disease in any organ of susceptible or resistant mice. In contrast, Mtb causes progressive disease and kills both susceptible and resistant mice. However, as shown here and elsewhere (14, 18), progressive disease in mice is confined to the lungs. Therefore, tuberculosis in mice is similar to tuberculosis in most humans, in whom the disease is also confined to the lung (19), in spite of hematogenous dissemination of bacilli to other organs. The results presented here indicating the existence of a gene(s) that makes some strains of mice more capable than others of controlling the growth of virulent Mtb in their lungs and consequently of enabling the mice to survive longer from tuberculosis would seem more relevant to the human situation than a gene that influences the level of resistance against an attenuated organism only in the spleen.

The gene(s) responsible for the superior resistance of BALB/c mice to tuberculosis appears to have little relationship to the recessive *Tbc-1* gene described some years ago (20) that makes I/St mice more susceptible to Mtb infection than A/Sn mice. It is almost certain, on the other hand, that MHC genes play a role in determining resistance of different mouse strains to tuberculosis, although published papers dealing with this subject are contradictory (21, 22). MHC genes were not a factor in the results presented here, because the mouse strains used are MHC compatible. In fact, the strains included in this study have been used in most published studies of the genetic basis of anti-BCG resistance and for producing congenic strains possessing the resistant DBA/2 *Bcg* locus of chromosome 1 (13).

According to a number of in vitro studies (5, 7) the *Bcg*^{r} allele of the *Bcg* gene makes macrophages of resistant mice innately more capable of restricting the growth of mycobacteria and of inactivating other microbial pathogens. Presumably, in the case of resistance to BCG, however, this would not apply to liver and lung macrophages, because resistance to BCG infection in these organs is the same in *Bcg*^{r} and *Bcg*^{s} mice. Moreover, it is apparent, even in the spleen, that the *Bcg* gene has a limited influence on the ultimate outcome of infection, in that innate differences in antimicrobial resistance mechanisms between *Bcg*^{r} and *Bcg*^{s} macrophages are eventually overridden by additional antimicrobial mechanisms acquired by these cells in response to specific immunity. Indeed, the capacity to generate specific immunity to BCG seems essentially the same in *Bcg*^{r} and *Bcg*^{s} mice, so far as the ability of mice to resolve infection is concerned. However, this appears not to be the case for resistance to infection with Mtb, as described here. According to our results with BALB/c and DBA/2 mice infected with the same number of Mtb via the respiratory route (Fig. 3), the superior resistance of the former mice over the latter was seen in the lung after, rather than before, the onset of expression of specific immunity, as evidenced by a substantial reduction in the rate of Mtb growth in this organ from day 20 on. We are aware, however, that immunity to Mtb infection eventually is expressed by macrophages, and that the superior resistance of BALB/c over DBA/2 mice to this pathogen may depend on a superior ability of BALB/c macrophages to acquire increased mycobactericidal function in response to cytokines secreted by Mtb-specific Tcells.

Lastly, it needs to be mentioned that virulence of the H37Rv strain of Mtb for mice is similar to that of the recently isolated C strain that is responsible for a larger number of tuberculosis cases throughout the New York metropolitan area (14). On the basis of this evidence, it would seem reasonable to assume that H37Rv is still virulent for humans, in spite of its having been isolated >60 yr ago.

We acknowledge the expert technical assistance of Lynn Ryan, Debra Duso, and Ron LaCourse.

This work was supported by grants HL-51960 and AI-37844 from the National Institutes of Health, and a general tuberculosis support grant from The G. Harold and Leila Y. Mathers Charitable Foundation.

Address correspondence to Dr. Eva Medina, Trudeau Institute, P.O. Box 59, Saranac Lake, NY 12983.

Received for publication 7 September 1995 and in revised form 1 November 1995.

References

1. Skamene, E., P. Gros, A. Gorget, P.A.L. Kongshavn, C.S. Charles, and B.A. Taylor. 1982. Genetic regulation of resistance to intracellular pathogens. *Nature (Lond.).* 297:506–509.
2. Plant, J., and A.A. Glynn. 1979. Locating salmonella resistance gene on mouse chromosome 1. *Clin. Exp. Immunol.* 37:1–6.
3. Bradley, D.J., B.A. Taylor, J. Blackwell, E.P. Evans, and J. Freeman. 1979. Regulation of *Leishmania* populations within the host. III. Mapping of the locus controlling susceptibility to visceral leishmaniasis in the mouse. *Clin. Exp. Immunol.* 37:7–14.
4. Schurr, E., E. Skamene, A. Forget, and P. Gros. 1989. Linkage analysis of the Bcg gene on mouse chromosome 1: identification of a tightly linked marker. *J. Immunol.* 142:4507–4513.
5. Stach, J.L., P. Gros, A. Forget, and E. Skamene. 1984. Phenotypic expression of genetically controlled natural resistance to *Mycobacterium bovis* (BCG). *J. Immunol.* 132:888–892.
6. Lissner, C.R., R.N. Swanson, and A.D. O'Brien. 1983. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: expression of the *Ity* gene in peritoneal and splenic macrophages isolated *in vitro*. *J. Immunol.* 131:3006–3013.
7. Olivier, M., and C.E. Tanner. 1987. Susceptibilities of macrophage populations to infection *in vitro* by *Leishmania donovani*. *Infect. Immun.* 55:467–471.
8. Vidal, S.M., D. Malo, K. Vogan, E. Skamene, and P. Gros. 1993. Natural resistance to infection with intracellular parasites: identification of a candidate gene for *Bcg*. *Cell.* 73:469–485.
9. Gruenheid, S., M. Cellier, S. Vidal, and P. Gros. 1995. Identification and characterization of a second mouse N*ramp* gene. *Genomics.* 25:514–525.
10. Malo, D., K. Vogan, S. Vidal, J. Hu, M. Cellier, E. Schurr, A. Fuks, N. Bumstead, K. Morgan, and P. Gros. 1994. Haplotype mapping and sequence analysis of the mouse N*ramp* gene predicts susceptibility to infection with intracellular parasites. *Genomics.* 23:51–61.
11. Vidal, S., M.L. Tremblay, G. Govoni, S. Gauthier, G. Sebastiani, D. Malo, E. Skamene, M. Olivier, S. Jothy, and P. Gros. 1995. The *Ity/Lsh/Bcg* locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the *Nramp1* gene. *J. Exp. Med.* 182:655–666.
12. Cellier, M., G. Govoni, S. Vidal, T. Kwan, N. Groulx, J. Liu, F. Sanchez, E. Skamene, E. Schurr, and P. Gros. 1994. Human natural resistance–associated macrophage protein: cDNA cloning, chromosomal mapping, genomic organization, and tissue-specific expression. *J. Exp. Med.* 180:1741–1752.
13. Mock, B.A., D.L. Holiday, D.P. Cerretti, S.C. Darnell, A.D. O'Brien, and M. Potter. 1994. Construction of a series of congenic mice with recombinant chromosome 1 regions surrounding the genetic loci for resistance to intracellular parasites (*Ity, Lsh,* and *Bcg*), DNA repair responses (Rep-1), and the cytoskeletal protein villin (Vil). *Infect. Immun.* 62:325–328.
14. Dunn, P.L., and R.J. North. 1995. Virulence ranking of some *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains according to their ability to multiply in the lungs, induce lung pathology, and cause mortality in mice. *Infect. Immun.* 63:3428–3437.
15. Skamene, E. 1989. Genetic control of susceptibility to mycobacterial infections. *Rev. Infect. Dis.* 11:394–399.
16. Buschman, E., A.S. Apt, B.V. Nickonenko, A.M. Moroz, M.H. Averbakh, and E. Skamene. 1988. Genetic aspects of innate resistance and acquired immunity to mycobacteria in inbred mice. *Springer Semin. Immunopathol.* 10:319–336.
17. Orme, I.M., and F.M. Collins. 1984. Demonstration of acquired resistance in *Bcg*^{r} inbred mouse strains infected with a low dose of BCG Montreal. *Clin. Exp. Immunol.* 56:81–88.
18. North, R.J., and A.A. Izzo. 1993. Mycobacterial virulence. virulent strains of *Mycobacteria tuberculosis* have faster in vivo doubling times and are better equipped to resistant growth-inhibiting functions of macrophages in the presence and absence of specific immunity. *J. Exp. Med.* 177:1723–1733.
19. Hopewell, P.C. 1994. Overview of clinical tuberculosis. *In* Tuberculosis: Pathogenesis, Protection and Control. B.R. Bloom, editor. ASM Press, Washington, DC. 25–46.
20. Buschman, E., A.S. Apt, B.V. Nickonenko, A.M. Moroz, M.H. Averbakh, and E. Skamene. 1988. Genetic aspects of innate resistance and acquired immunity to *Mycobacteria* in inbred mice. *Springer Semin. Immunopathol.* 10:319–336.
21. Brett, S., J.M. Orrell, J. Swanson Beck, and J. Ivanyi. 1992. Influence of H-2 genes on growth of *Mycobacterium tuberculosis* in the lungs of chronically infected mice. *Immunology.* 76: 129–132.
22. Apt, A.S., V.G. Avdienko, B.V. Nikonenko, I.B. Kramnik, A.M. Moroz, and E. Skamene. 1993. Distinct H-2 complex control of mortality, and immune responses to tuberculosis infection in virgin and BCG-vaccinated mice. *Clin. Exp. Immunol.* 94:322–329.