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Mice that carry the resistance allele of the *Bcg* gene (Bcg^r) develop a superior capacity to stabilize bacille Calmette–Guérin (BCG) infection in their lungs and spleen over a protracted period in the absence of specific immunity

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SUMMARY

In mice, natural resistance to infection with BCG is under the influence of an autosomal gene designated *Bcg*. It is shown here in agreement with others that mice that possess the dominant resistant allele of the gene (Bcg^r) are more capable than mice that possess the susceptible recessive allele (Bcg^s) at restricting the growth of BCG in their lungs, as well as in their spleens, during the first 20 days of infection. It is shown, in addition, that in the absence of specific immunity the resistance difference between Bcg^r and Bcg^s mice became much more pronounced as infection progressed beyond day 20. Whereas T cell-depleted Bcg^r mice developed a capacity after day 20 to cause infection in their lungs and spleens to stabilize and plateau for a least 40 days, T cell-depleted Bcg^s mice were unable to prevent infection from progressing in these organs. On the other hand, both types of T cell-depleted mice were capable of causing infection to plateau in their livers and kidneys. Moreover, this T cell-independent mechanism of resistance was essentially abolished in all organs in which it was expressed by treating the mice with hydrocortisone. In the lungs of immunocompetent Bcg^s mice, failure to stabilize infection was associated with heavily infected macrophages and failure to contain BCG at original sites of infection.

Keywords *Bcg* gene natural resistance BCG infection T cell-depleted mice

INTRODUCTION

It has been shown in mice [1–4] that innate resistance, as opposed to acquired specific immunity, to infection with the attenuated strain of *Mycobacterium bovis*, bacille Calmette-Guérin (BCG), is under the influence of a single, dominant autosomal gene designated *Bcg*, which is present in two allelic forms in inbred strains of mice, Bcg^r (dominant resistance) and Bcg^s (recessive susceptible). The *Bcg* locus also influences innate resistance of mice to other intracellular pathogens [4–6], and is believed to be phenotypically expressed by macrophages [7] and to provide these cells with a superior capacity to kill or restrict the growth of the pathogens intracellularly [8]. A candidate gene for the *Bcg* gene was recently cloned [9] and shown to encode for an integral membrane component of certain types of macrophages. However, *in vivo*, the superior anti-BCG resistance of Bcg^r mice appears to have been measured exclusively in the spleen as a slower rate of BCG growth during the first 20 days or so of infection [1]. After this time, acquired specific immunity enables Bcg^r and Bcg^s mice to resolve infection in this organ apparently with equal efficiency. Therefore, on the basis of BCG growth curves, the *Bcg* gene appears to have little or no influence on the acquisition and expression of acquired T cell-mediated immunity.

The study described here employed Bcg^r A/J and Bcg^s B10.A mice to determine whether the difference in the ability of Bcg^r and Bcg^s strains to restrict BCG growth can be amplified by allowing the organism to grow beyond 20 days in the absence of specific immunity. The results show that, whereas in the absence of specific immunity Bcg^r mice develop a capacity to prevent further BCG growth in their lungs and spleens after day 20, Bcg^s mice allowed BCG to grow progressively in these organs. It is also shown that this mechanism of anti-BCG resistance in Bcg^r mice is abolished by treatment with hydrocortisone.

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MATERIALS AND METHODS

Mice

Specific pathogen-free male A/J and B10.A mice, 8–10 weeks old, were purchased from the Trudeau Institute Animal Breeding Facility (Saranac Lake, NY), or from the Jackson Laboratories (Bar Harbor, ME). All animals were provided with sterilized food and water.

Mycobacteria

Mycobacterium bovis, strain BCG Pasteur (TMC no. 1011) 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 (P and B) medium (Difco Labs, 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. Groups of five mice were inoculated via a lateral tail vein with 1–5 $\times$ 10^5 colony-forming units (CFU) of BCG suspended in 0·2 ml PBS. The course of infection was monitored against time in spleens, lungs, livers and kidneys by preparing homogenate of these organs in PBS containing 0·05% Tween, and plating 10-fold serial dilutions of the homogenate on enriched agar (Middlebrook 7H11; Difco Labs). CFU were counted after 2–3 weeks incubation at 37°C.

T cell-deficient mice

Four-week-old A/J and B10.A mice were surgically thymectomized, rested for several weeks, and depleted of $CD4^+$ and $CD8^+$ T cells 2 days before infection by injecting them intravenously with 0·25 mg of anti-CD4 MoAb produced by clone GK1.5 (TIB 207; American Type Culture Collection (ATCC), Rockville, MD) and anti-CD8 MoAb produced by clone 2.43 (TIB 210; ATCC). The mice received an additional injection of 0·125 mg of each MoAb on days 10, 20 and 30 of infection. The extent of T cell depletion was determined by analysing spleen and lymph node cells by flow cytometry with a FACScan cytofluorometer (Becton Dickinson, Mountain View, CA) after labelling the cells with FITC-conjugated $F(ab')_2$ fragments of anti-CD4 or anti-CD8 MoAb, as described previously [10]. Depletion was better than 98%.

Hydrocortisone acetate

Hydrocortisone acetate (2·5 mg; United Research Laboratories; Philadelphia, PA) was given subcutaneously at 7 day intervals, starting at day 15 of infection.

RESULTS

Bcg^r *mice permit less preimmunity BCG growth in their spleens and lungs than* Bcg^s *mice*

Figure 1 shows the results of an experiment that followed the growth of BCG in the lungs, livers, spleens and kidneys of *Bcg*^{r} (A/J) and *Bcg*^{s} (B10.A) mice inoculated with approximately 5×10^5 BCG intravenously. It shows, in agreement with the findings of others [1,3,4], that BCG grew faster in the spleens of *Bcg*^{s} mice over the first 20 days or so of infection, to the extent that there was about 1 log more BCG in this organ in *Bcg*^{s} than in *Bcg*^{r} mice. Figure 1 also reveals that BCG grew about 1 log more in the lungs of *Bcg*^{s} mice over the first 20 days of infection, thereby showing that this organ, as well as the spleen, needs to be taken into account when analysing the function of the *Bcg* gene. On the other hand, BCG grew at the same rate in the livers and kidneys of both strains of mice until day 10. Both *Bcg*^{s} and *Bcg*^{r} strains were equally capable of causing immunologically mediated resolution of infection in their spleens and lungs after day 20, and in their livers and kidneys after day 10.

Bcg^r *mice are more capable than* Bcg^s *mice of controlling infection in their lungs and spleens in the absence of specific immunity*

The foregoing results show that the advantage that *Bcg*^{r} have over *Bcg*^{s} mice in controlling BCG growth is limited to their lungs and spleens, and is only evident in these organs during the first 20 days of infection, before immunologically mediated resolution of infection begins. It was considered likely, therefore, that this difference in BCG resistance could be amplified by allowing BCG to grow for more than 20 days in resistant and susceptible strains rendered incapable of generating specific immunity. This prediction proved correct, as is shown by the results in Fig. 2. It can be seen that, whereas BCG grew progressively in the lungs of T cell-depleted *Bcg*^{s} mice beyond day 20, its growth was essentially stabilized and caused to plateau after day 20 in the lungs of T cell-depleted *Bcg*^{r} mice. This resulted in almost 2 logs more BCG growth in the lungs of the former over the latter mice by day 55. The absence of specific immunity also allowed BCG to reach much higher numbers in the spleens of *Bcg*^{s} mice by day 55 of infection. However, in the livers and kidneys of T cell-depleted *Bcg*^{r} and *Bcg*^{s} mice the situation was different, in that neither strain was significantly more resistant to BCG growth than the other, except perhaps for a brief period in the liver. In both organs in both strains, BCG growth was essentially stabilized after an initial period of growth. This resulted in approximately the same number of BCG in these organs in both strains, when the experiment was terminated on day 55.

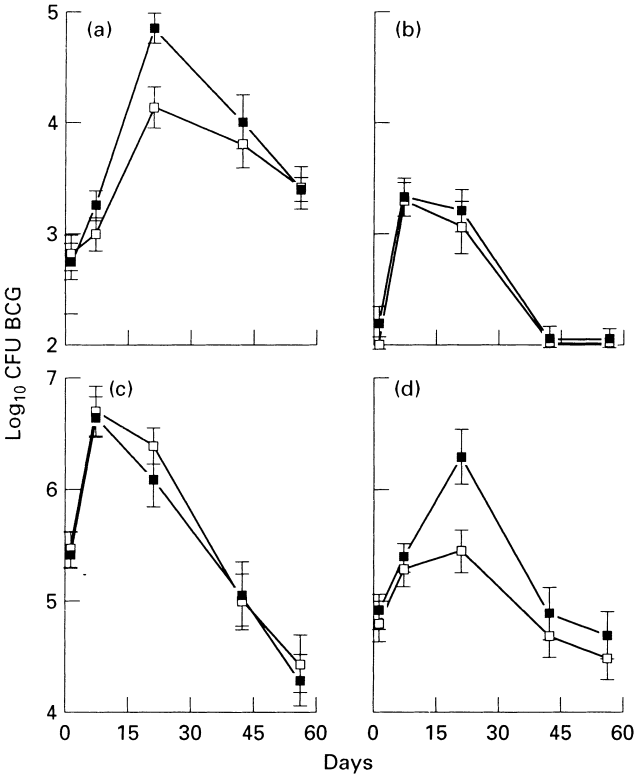

Fig. 1. Growth of *Mycobacterium bovis* BCG in the lungs (a), kidneys (b), livers (c) and spleens (d) of A/J (*Bcg*^{r}) (□) and B10.A (*Bcg*^{s}) (■) mice inoculated with 5×10^5 bacilli intravenously. Data are expressed as geometric means of groups of five mice ± s.d. CFU, Colony-forming units.

Abolition of BCG resistance in T cell-depleted mice by treatment with hydrocortisone acetate

A previous publication from this laboratory showed [11] that mycobacteria infections of T cell-depleted, as well as of immunocompetent mice, can be severely exacerbated by treatment with corticosteroids. It was considered likely, therefore, that the

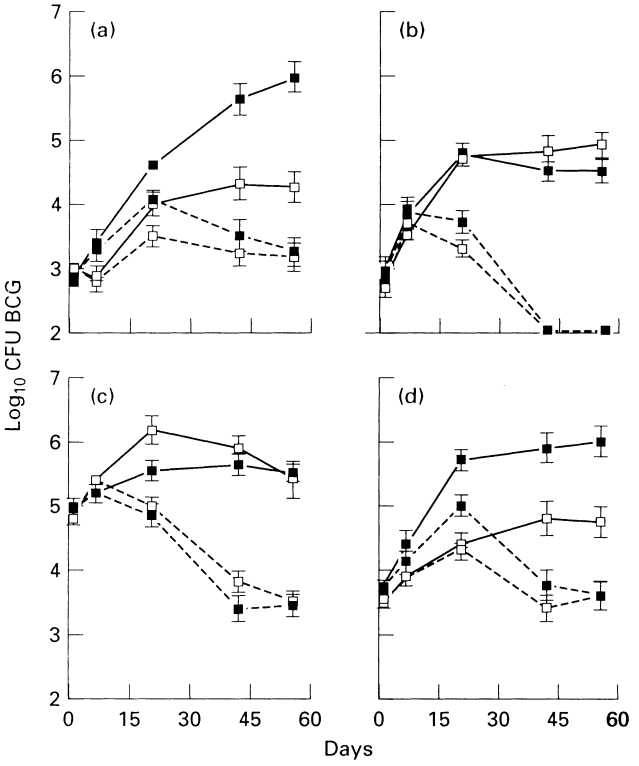

Fig. 2. Growth of *Mycobacterium bovis* BCG in the lungs (a), kidneys (b), livers (c) and spleens (d) of T cell-depleted A/J (Bcg^r) (□–□) and B10.A (Bcg^s) (■–■) mice, and in immunocompetent A/J (□- - -□) and B10.A (■- - -■) mice. Infection was initiated with 10^5 bacilli intravenously. Data are expressed as geometric means of groups of five mice ± s.d. CFU, Colony-forming units.

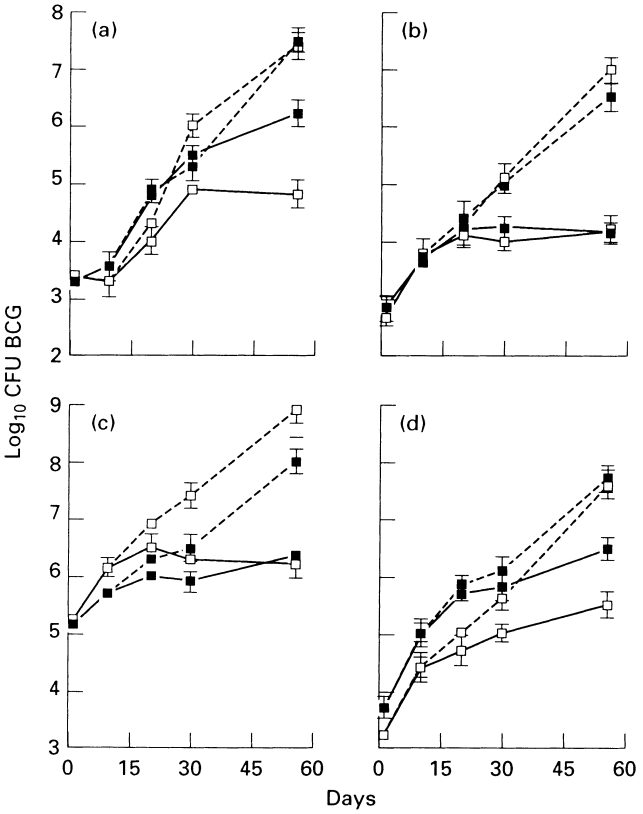

Fig. 3. Exacerbation of *Mycobacterium bovis* BCG infection in the lungs (a), kidneys (b), livers (c) and spleens (d) of T cell-depleted A/J (Bcg^r) (□- - -□) and T cell-depleted B10.A (Bcg^s) (■- - -■) mice given weekly subcutaneous injections of 2·5 mg of hydrocortisone (HC) starting at day 15 during infection. T cell-depleted control mice (□–□ and ■–■) received equivalent volumes of PBS. Infection was initiated with 2×10^5 bacilli intravenously. Data are expressed as geometric means of groups of five mice ± s.d. CFU, Colony-forming units.

superior ability of T cell-depleted Bcg^r mice over T cell-depleted Bcg^s mice to restrict BCG growth in their lungs and spleens after day 20 could be abolished by treating the mice with hydrocortisone. Figure 3 shows that 2·5 mg of hydrocortisone given subcutaneously at 7 day intervals, starting on day 15 post-inoculation of BCG, resulted in exacerbation of BCG infection in all organs of T cell-depleted Bcg^r and Bcg^s mice. However, infection in the lung and spleens of Bcg^r mice was exacerbated much more than in these organs in Bcg^s mice, thereby resulting in both types of mice having about the same level of infection in these organs at day 55. Thus in the lungs of Bcg^r mice hydrocortisone treatment caused 1000-fold increase in BCG growth, whereas it caused only a 10-fold increased BCG growth in the lungs of Bcg^s mice. In the liver and kidney, however, hydrocortisone treatment caused somewhat more exacerbation of infection in Bcg^r mice.

DISCUSSION

This study shows, in agreement with the findings of others [1–4], that mice that possess the dominant resistance allele of the *Bcg* gene (Bcg^r) are more capable than those that possess the recessive susceptible allele (Bcg^s) of restricting the growth of BCG in their spleens during the first 20 days or so of infection initiated by inoculating the organism intravenously. Also in agreement with the findings of others [1] is the result showing that this resistance advantage was not maintained after the onset of specific immunity, in that after 20 days of progressive BCG growth, Bcg^s mice were just as capable as Bcg^r mice of resolving infection in their spleens. An important new piece of information revealed by the present study is that the superior anti-BCG resistance of Bcg^r mice during the first 20 days of infection is also expressed in their lungs, the organs most susceptible to infection with virulent *Myco. tuberculosis* [12]. On the other hand, Bcg^r mice showed no preimmunity resistance advantage over Bcg^s mice in their livers and kidneys. Presumably, therefore, liver and kidney macrophages of Bcg^s mice are just as restrictive of BCG growth as those of Bcg^r mice. This information needs to be considered in light of the recent elegant isolation of a candidate gene for the *Bcg* gene [9], which encodes for a macrophage transmembrane protein, and which is expressed in the spleens of Bcg^r mice, but apparently not in their livers and lungs.

A key additional finding revealed by the present study is that the difference between the amount of BCG growth that can take place in Bcg^r *versus* Bcg^s mice can be increased considerably by allowing BCG infection to proceed beyond 20 days in the absence of specific immunity. It was shown that T cell-depleted Bcg^r mice were capable, after day 20 of infection, of acquiring a capacity to prevent further BCG in their lungs, spleens, livers and kidneys, and to cause infection to plateau in these organs for at least 35 days. In

contrast, T cell-depleted Bcg^s mice, although capable of preventing further BCG growth in their livers and kidneys after day 20, were not capable of doing so in their lungs and spleens. In these latter organs, infection continued to progress until the experiment was terminated. Thus in terms of both restricting BCG growth during the first 20 days of infection and preventing further growth of BCG after that time in the absence of specific immunity, Bcg^s mice were as capable as Bcg^r mice in their livers and kidneys, but not in their lungs and spleens. The additional findings, that this T cell-independent ability of Bcg^r and Bcg^s mice to stabilize infection after day 20 can be abolished in the organs in which it is expressed by treatment with hydrocortisone, indicate that it is based on the same mechanism in both strains of mice. These findings are difficult to reconcile with the notion that only Bcg^r mice possess the resistance allele of the *Bcg* gene, without invoking the idea that T cell-independent resistance expressed in the livers and kidneys of Bcg^r and Bcg^s mice is not determined by the *Bcg* gene, even though the resistance is corticosteroid-sensitive, like that in the lungs and spleen.

Given that BCG appears to be exclusively located in macrophages in the lungs of both strains of mice, it follows that resistance to BCG after day 20 in the lungs of T cell-depleted Bcg^r mice is dependent on a macrophage-based, corticosteroid-sensitive mechanism that is essentially absent from the lungs of Bcg^s mice. Presumably, the same mechanism exists in the spleens of Bcg^r mice. However, it remains to be determined whether the same mechanism exists in the livers and kidneys of both strains of mice. This question is currently under investigation in this laboratory.

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