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The role of macrophage activation and of *Bcg*-encoded macrophage function(s) in the control of *Mycobacterium avium* infection in mice

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SUMMARY

Following the intraperitoneal inoculation of $2{\cdot}5 \times 10^8$ colony-forming units of *Mycobacterium avium* strain ATCC 25291, there was bacillary growth in the liver, spleen and peritoneal cavity of C57BL/6, C57BL/10, DBA/1 and BALB/c mice whereas DBA/2, C3H/He, CBA/Ca and CD-1 mice controlled the infection showing constant or slightly decreasing numbers of viable bacteria in the liver and spleen and effective clearance of the bacilli from the peritoneal cavities. The acquisition of non-specific resistance (NSR) to *Listeria monocytogenes* during the infection by *M. avium* was high in C57BL/6, BALB/c and C3H/He mice and negligible in DBA/2 and CD-1 mice. The magnitude of the acquisition of NSR was reduced in T cell-deficient mice and was directly proportional to the dose of the inoculum of *M. avium*. The production of hydrogen peroxide by phorbol myristate acetate-stimulated peritoneal macrophages of *M. avium*-infected mice was higher in C57BL/6 and BALB/c mice than in CD-1, DBA/2 and C3H/He animals. BALB/c. Bcg^r (C.D2) mice, unlike their congenic strain BALB/c, restricted bacterial growth following the intravenous inoculation of $2{\cdot}5 \times 10^8$ CFU of *M. avium* as efficiently as DBA/2 mice. C.D2 and BALB/c peritoneal macrophages from infected mice produced similar amounts of H_2O_2 but BALB/c mice developed higher levels of NSR to listeria than C.D2 mice. The production of nitrite by peritoneal macrophages from infected mice was found to be enhanced in DBA/2 and C3H/He but not in BALB/c, C57BL/6, CD-1 and C.D2 mice. Resident peritoneal macrophages from C.D2 mice were more bacteriostatic *in vitro* for *M. avium* than macrophages from BALB/c mice. The same relative differences between the two macrophage populations were observed when the cells were activated with lymphokines. The results show that the resistance to *M. avium* infection in mice is under the control of the *Bcg* gene and that susceptibility may be due to some defect in macrophage antibacterial function not completely overcome by the activation of this phagocyte in the susceptible strains of mice.

Keywords mycobacteria macrophage activation *Bcg* gene immune response

INTRODUCTION

In recent years, the importance of *Mycobacterium avium* has been increasing due to the high incidence of infections by this pathogen in immunocompromised patients, namely those with AIDS (Grange & Yates, 1986). It was found that *M. avium*-infected individuals are anergic to the homologous antigens and that their mononuclear cells do not produce interleukin-2 after antigenic stimulation (Tsuyuguchi *et al.*, 1988). In the mouse, non-specific immunosuppression has been described in heavily infected animals (Watson & Collins, 1980, 1981). On the other hand, it has also been shown that, despite the occurrence of unresponsiveness, a strong immune response to the infection may occur (Appelberg *et al.*, 1989; Orme, 1988; Orme & Collins, 1984).

Different strains of mice vary in their relative susceptibilities to *M. avium* (Orme, Stokes & Collins, 1986; Stokes, Orme & Collins 1986) and to *M. intracellulare*, a closely related species (Goto *et al.*, 1984). A polygenic control of resistance to *M. avium* has been shown by Orme *et al.* (1986). However, with *M. intracellulare*, the resistance to infection has been shown to be associated with the *Bcg* gene. We decided to address this problem further using a *M. avium* strain that had shown a distinct growth behaviour in resistant and susceptible strains of mice (Appelberg *et al.*, 1989). Furthermore, we looked at the acquisition of macrophage activation during the infection in resistant and susceptible strains of mice, since this problem has not been thoroughly studied (Orme *et al.*, 1986). In fact, in that study, only two mouse strains were studied, looking at one parameter of macrophage activation, and the emphasis was given to the study of the survival of *M. avium* in activated macrophages (Orme & Collins, 1983; Stokes *et al.*, 1986; Stokes & Collins 1988). Finally, we assessed the participation of T cells in the activation of the mononuclear phagocytes and its relation to the mycobacterial load. We show here that resistance to infection is related to the expression of the Bcg^r gene and that the

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susceptibility of the Bcg^s mice was not associated with a deficient macrophage activation. On the contrary, although macrophage activation did not strictly correlate with resistance/susceptibility to *M. avium*, it tended to be higher in the susceptible mouse strains. Both T dependent and T independent mechanisms of macrophage activation seemed to occur.

MATERIALS AND METHODS

Mice

CD-1 and C57BL/6 mice were purchased from Instituto Gulbenkian de Ciência (Oeiras, Portugal); BALB/c, C3H/He, DBA/2, CBA/Ca and C57BL/10 were from Bantin and Kingman (Hull, UK) and DBA/1 from Harlan Olac (Bicester, UK). BALB/c. *Bcg*^{r} (C.D2) mice were bred at our facilities from parent mice generously given by Dr E. Skamene. The animals were kept under standard hygiene conditions, fed commercial chow and given acidified drinking water *ad libitum*. They were used within 6–8 weeks of age. Athymic nude mice (C57BL/6 nu/nu) and euthymic C57BL/6 nu/+ mice were purchased from Bommia (Ry, Denmark) and kept in sterilized housing and given sterile food and water. T cell depleted mice were obtained from adult BALB/c mice which were thymectomized at 8 weeks of age, irradiated (750 rad of *X*-radiation) and given 2×10^6 bone marrow cells that were pre-treated *in vitro* with anti Thy 1·2 monoclonal antibody (1/100 dilution of culture supernatant from hybridoma HO-13-14) plus 1:10 dilution of guinea-pig serum.

Bacteria

M. avium ATCC 25291 was grown in Middlebrook 7H9 medium (DIFCO, Detroit, MI) for two weeks. The bacteria were collected by centrifugation and were suspended in a small volume of saline with 0·04% Tween 80 (T80) (Sigma, St Louis, MO), briefly sonicated (15 sec, at 50 W) with a Branson sonifier to disrupt bacterial clumps and diluted in the same medium to an OD_{600} of 0·48 being then frozen at −70°C. When needed, aliquots were thawed at 37°C, diluted to the desired concentration and inoculated. *Listeria monocytogenes* CCM 5576 was cultured in antibiotic 3 medium (DIFCO) for 36 h and aliquots were frozen at −70°C until use being then diluted in saline to the appropriate concentration before inoculation.

Evaluation of bacillary growth

The number of CFU of *M. avium* in the spleen and liver of infected mice was determined by serial diluting and plating the tissue homogenates onto 7H10 agar medium (DIFCO). The CFU numbers in the peritoneal cavities were determined in the same way as with the tissue homogenates using an aliquot of the collected lavage fluid which was treated with 0·1% saponin (Sigma) to lyse the cells.

Study of the non-specific resistance to Listeria monocytogenes

At different times of *M. avium* infection, mice were intravenously inoculated with $1{\cdot}5 \times 10^6$ CFU of *L. monocytogenes* and the index of immunity (II) was calculated at 48 h as described previously (Appelberg *et al.*, 1989). It is equal to the difference between the $\log_{10}$ of the Listeria CFU in the control (*M. avium*-non-infected) mice and the $\log_{10}$ of the CFU of *Listeria* in the *M. avium*-infected mice. Since we did not find any significant differences in the CFU numbers of *Listeria* in controls of the six strains infected for 48 h with the same inoculum dose used to assess NSR (data not shown), we assume that no other loci in addition to the *Bcg* gene (e.g. the *Hc* locus) are affecting the acquisition of such parameters of macrophage activation. The results are expressed as a difference between two means. Therefore, no data are presented for a s.d. value. However, the s.d. of the geometric mean of the CFU for each group was smaller than 5% of the mean.

Study of hydrogen peroxide production by peritoneal macrophages

The peritoneal exudate of intraperitoneally infected mice was collected with Hanks' balanced salt solution (HBSS) (Gibco, Grand Island, NY) and centrifuged over 55% Percoll (Sigma). The overlying ring of cells devoid of granulocytes was collected, extensively washed with cold HBSS and the cell suspension was counted; 1×10^6 cells in RPMI 1640 medium (Gibco) were added to each well of 24-well tissue culture plates (Nunc, Roskilde, Denmark) and incubated for 1·5–2 h at 37°C in humidified atmosphere with 5% CO_2. Non-adherent cells were removed by washing with warm phenol red-free HBSS and H_2O_2 production was evaluated as described by Ruch, Copper & Baggiolini (1983). After the reaction, the supernatant was collected for fluorimetric analysis and the protein content of the cell monolayer was determined. Results are expressed as nmol H_2O_2 μg of protein/60 min.

Study of nitrite production by peritoneal cells

Peritoneal cells were collected as described above and plated (1×10^6 cells/well) in 24-well plates in 0·4 ml of RPMI 1640 medium. After removing the non-adherent cells, the monolayer was incubated in RPMI 1640 medium supplemented with 10% of fetal calf serum (FCS) (Gibco) at 37°C in 5% CO_2 as above, either in the absence or presence of 10 ng of lipopolysaccharide from *Escherichia coli* (LPS) (Sigma)/ml. The nitrite content of the supernatant was determined at 24 h of culture using the Griess reagent (Green *et al.*, 1982) as described by Stuehr & Marletta (1985).

In vitro infection of macrophage monolayers

Resident peritoneal cells were collected with HBSS, washed, counted and plated in 24-well plates (3×10^6 cells per well). After removal of non-adherent cells, 200 μl of RPMI 1640 medium with 5×10^6 CFU of *M. avium* were added per well and incubated for 4 h. The non-phagocytozed bacilli were removed by extensive washing of the wells with warm HBSS. A group of wells were then lysed with saponin 0·1% (Sigma) and serial dilutions were seeded onto Middlebrook 7H10 medium with OADC supplement for CFU counts ($t = 0$). The other wells were added 400 μl of RPMI 1640 medium with 10% FCS and incubated for different periods of time. Cells were then lysed as described and CFU counts were made. To some wells, 100 μl of lymphokine-containing medium were added daily. This medium was obtained from supernatants of spleen cell cultures (50×10^6 cells in 20 ml RPMI medium with 10% FCS/10 cm diameter tissue culture dishes) stimulated for 48 h with 4 μg of concanavalin A/ml, after coincubation with Sephadex G50 (Pharmacia, Uppsala, Sweden) (1:1).

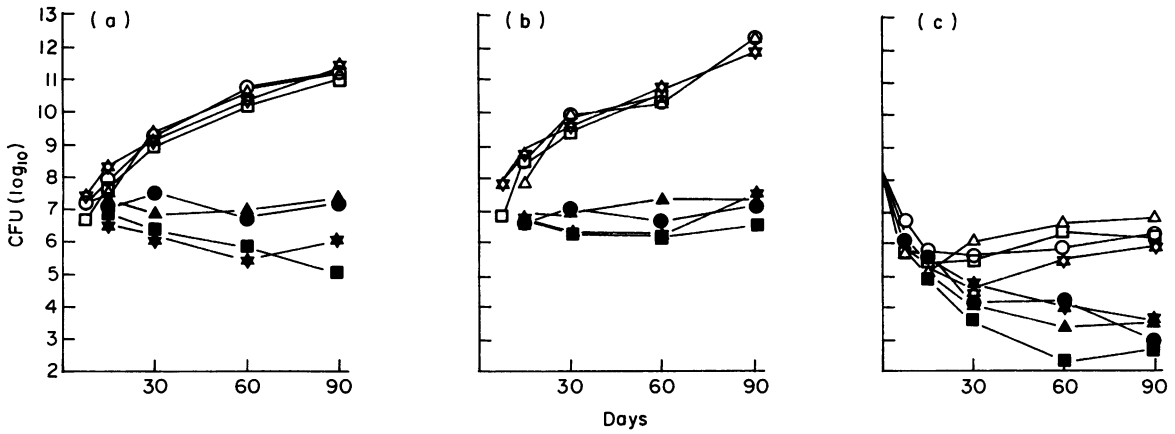

Fig. 1. Colony-forming units (CFU) numbers in the spleen (a), liver (b), and peritoneal cavity (c) of mice inoculated intraperitoneally with $2{\cdot}5 \times 10^8$ CFU of *M. avium*. CFU counts were performed at different times of infection up to 90 days. Each value represents the mean of four to eight mice of the following strains: C57BL/6 (○), C57BL/10 (□), DBA/1 (△), BALB/c (☆), CD-1 (●), DBA/2 (▲), C3H/He (★) and CBA/Ca (■). The s.d. of the geometric mean of CFU was smaller than 5% of the mean for any time point and was not depicted for sake of clarity.

RESULTS

A roughly constant number of CFU was detected in the spleen and liver of CD-1, C3H/He, DBA/2 and CBA/Ca mice inoculated IP with $2{\cdot}5 \times 10^8$ CFU of *M. avium* throughout the 3 months of the study (Fig. 1a, b). These resistant strains showed a progressive clearance of the inoculum in the peritoneal cavity along the time of the infection (Fig. 1c). They did not show any detectable signs of disease. On the contrary, C57BL/6, C57BL/10, DBA/1 and BALB/c mice showed progressive bacterial proliferation in the spleen and liver (Fig. 1a, b) while the initial clearance of the peritoneal inoculum was substituted after the first 15 to 30 days post-inoculation by a progressive bacillary multiplication plateauing at about 10^6 to 10^7 CFU per peritoneal cavity (Fig. 1c). These susceptible mice died from the infection in 3–4 months showing signs of cachexia (weight loss, muscle and subcutaneous tissue atrophy, ruffled fur) and massive hepatomegaly and splenomegaly with loss of the normal red colour of these organs which appeared pale and upon histologic examination exhibited extensive infiltration by heavily infected foamy macrophages (not shown).

The development of NSR to a heterologous bacterial challenge was studied. This parameter is an *in vivo* measure of systemic macrophage activation (North, 1974; North & Spitalny, 1981).

M. avium-resistant CD-1 and DBA/2 mice did not develop any significant levels of NSR to a *Listeria* challenge during 90 days of infection by $2{\cdot}5 \times 10^8$ CFU of *M. avium* (Fig. 2). On the other hand, *M. avium*-susceptible C57BL/6 and BALB/c mice and *M. avium*-resistant C3H/He mice developed high levels of NSR peaking at 15 days of *M. avium* infection (Fig. 2). The development of NSR during *M. avium* infection was reduced in nu/nu as compared with nu/+ C57BL/6 mice (Fig. 3a). Similarly, T cell depletion of adult BALB/c mice led to a reduction in their ability to develop NSR during *M. avium* infection (Fig. 3b). The levels of NSR acquired at 15 days of *M. avium* infection varied with the mycobacterial inoculum dose and between the different mouse strains (Fig. 4). Thus, C57BL/6 and BALB/c mice showed enhanced levels of NSR for the lower inocula tested (6×10^6 and 6×10^7 CFU) while NSR

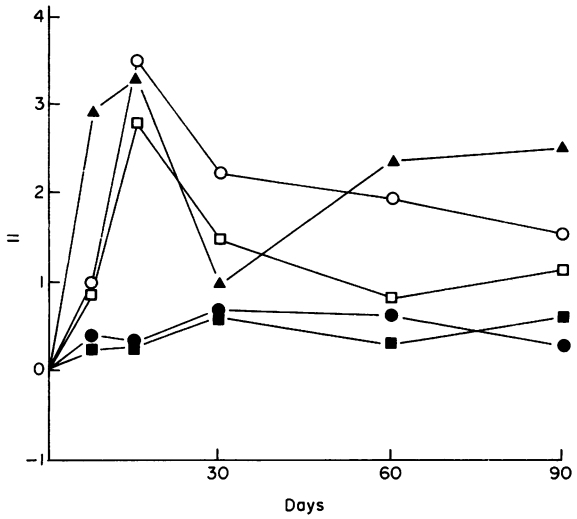

Fig. 2. Development of non-specific resistance to *Listeria monocytogenes* expressed as the Index of Immunity (II) in mice infected with $2{\cdot}5 \times 10^8$ CFU of *M. avium* up to 90 days of infection. Each point represents the mean of three or four mice of the following strains: CD-1 (■), DBA/2 (●), C3H/He (▲), C57BL/6 (□) and BALB/c (○).

was not detected for such inoculum doses in infected CD-1, C3H/He and DBA/2 mice (Fig. 4). With infections by 6×10^8 CFU of *M. avium*, C3H/He already exhibited high levels of NSR, similar to those found in C57BL/6 and BALB/c mice. Only with the higher inocula (6×10^9 CFU) were the levels of NSR high in the CD-1 and DBA/2 mice.

Since immunologically activated macrophages produce enhanced levels of hydrogen peroxide (Adams & Hamilton, 1984), this parameter was also studied for comparison with the previous results. The amount of H_2O_2 produced by peritoneal macrophages from *M. avium*-infected mice (15 and 30 days of intraperitoneal infection) was higher in the susceptible C57BL/6 and BALB/c strains than in the resistant CD-1, C3H/He and DBA/2 strains (Fig. 5).

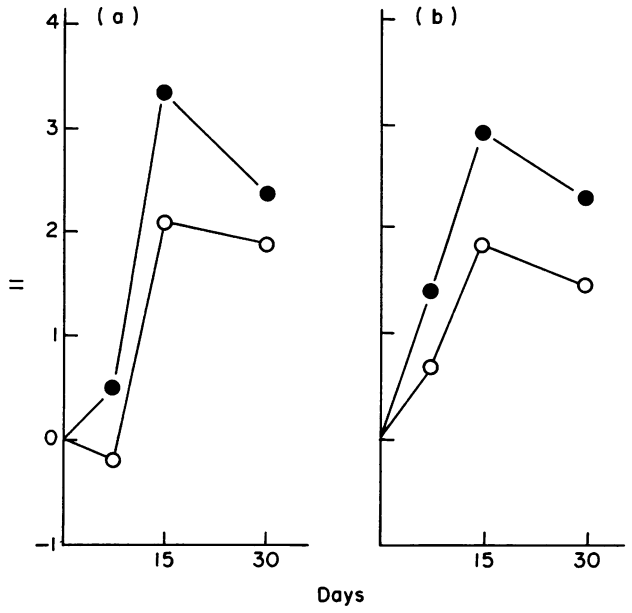

Fig. 3. Development of non-specific resistance to Listeria in C57BL/6 nu/nu (○) or nu/+ (●) mice (a) and in T-cell depleted (○) or control (●) BALB/c mice (b) intravenously infected for up to 30 days with $2{\cdot}5 \times 10^8$ CFU of *M. avium*.

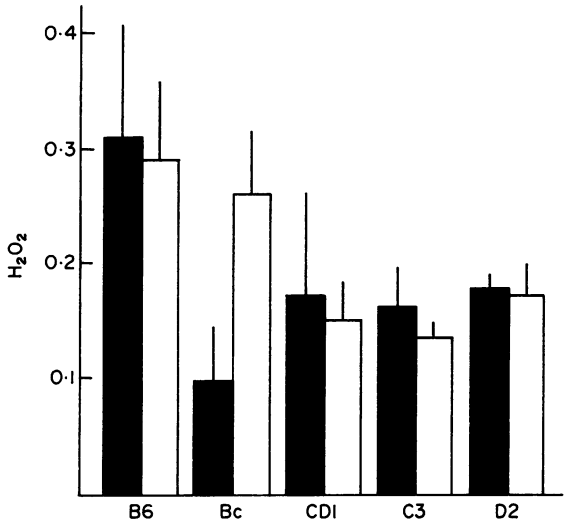

Fig. 5. Production of H_2O_2 by peritoneal macrophages of C57BL/6 (B6), BALB/c (Bc), CD-1, C3H/He (C3) and DBA/2 (D2) mice intraperitoneally infected with $2{\cdot}5 \times 10^8$ CFU of *M. avium* for 15 (closed columns) or 30 (open columns) days. Each value represents the mean of four pools of two mice. Data are expressed as nmol of $H_2O_2/\mu g$ of adherent cell protein/60 min.

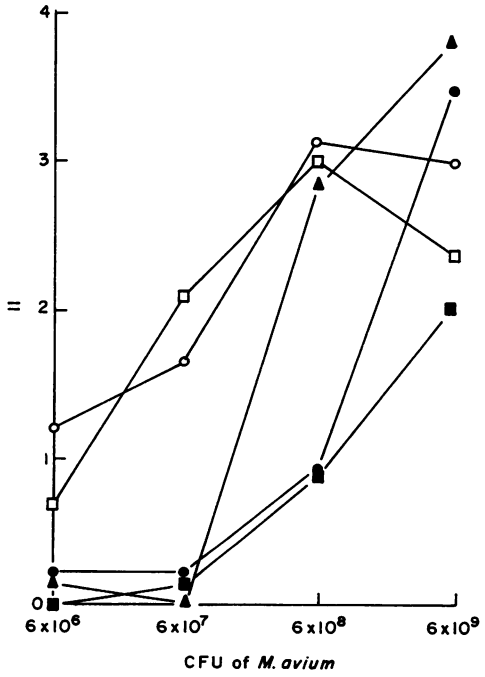

Fig. 4. Development of non-specific resistance to Listeria in C57BL/6 (□), BALB/c (○), CD-1 (●), DBA/2 (■) and C3H/He (▲) mice intravenously infected for 15 days with various inoculum doses of *M. avium*.

The study of the susceptibility to *M. avium* infection among the seven inbred strains of mice shown above suggested a role for the *Bcg* gene in the regulation of such susceptibility. This was further tested by studying the susceptibility of a BALB/c congenic strain that has received a segment of chromosome 1 from DBA/2 mice that contains the Bcg^r allele. This mouse strain, BALB/c.Bcg^r (C.D2), did not allow the proliferation of *M. avium* in their spleens and liver unlike the congenic BALB/c which exhibited extensive bacterial multiplication in those organs following the intravenous inoculation with 2×10^8 CFU of *M. avium* (Fig. 6). In fact, C.D2 mice were as resistant as the donor strain DBA/2. The peritoneal macrophages from infected C.D2 and BALB/c mice produced similar amounts of H_2O_2 at 15 and 30 days of intraperitoneal infection (macrophages from C.D2 mice produced $52{\cdot}3 \pm 19{\cdot}5$ and $98{\cdot}6 \pm 27{\cdot}4$ nmol H_2O_2/mg protein at days 15 and 30, respectively and macrophages from BALB/c mice produced $38{\cdot}2 \pm 22{\cdot}1$ and $126{\cdot}8 \pm 20{\cdot}1$ nmol H_2O_2/mg protein at the same times of infection). The development of NSR was higher in BALB/c than in C.D2 mice (Fig. 7).

Immunological activation of macrophages has been shown to lead to enhanced secretion of reactive nitrogen intermediates including nitrite (Ding, Nathan & Stuehr, 1988; Stuehr & Marletta, 1985). Peritoneal macrophages from infected mice were cultured *in vitro* for 24 h and the nitrite content of the culture supernatants was evaluated. Only the cells from infected DBA/2 and C3H/He mice produced nitrite amounts that were significantly higher than those produced by resident peritoneal macrophages from uninfected mice (Fig. 8a). The presence of LPS in the culture medium led to a general increase in the amounts of nitrite produced including those secreted by resident peritoneal cells (from $0{\cdot}070 \pm 0{\cdot}004$ to $0{\cdot}112 \pm 0{\cdot}028$ nmol/μg of protein/60 min.). Again, DBA/2 and C3H/He cells produced enhanced levels of nitrite as compared with resident cells (Fig. 8b). C57BL/6 mice at 15 days of infection also showed

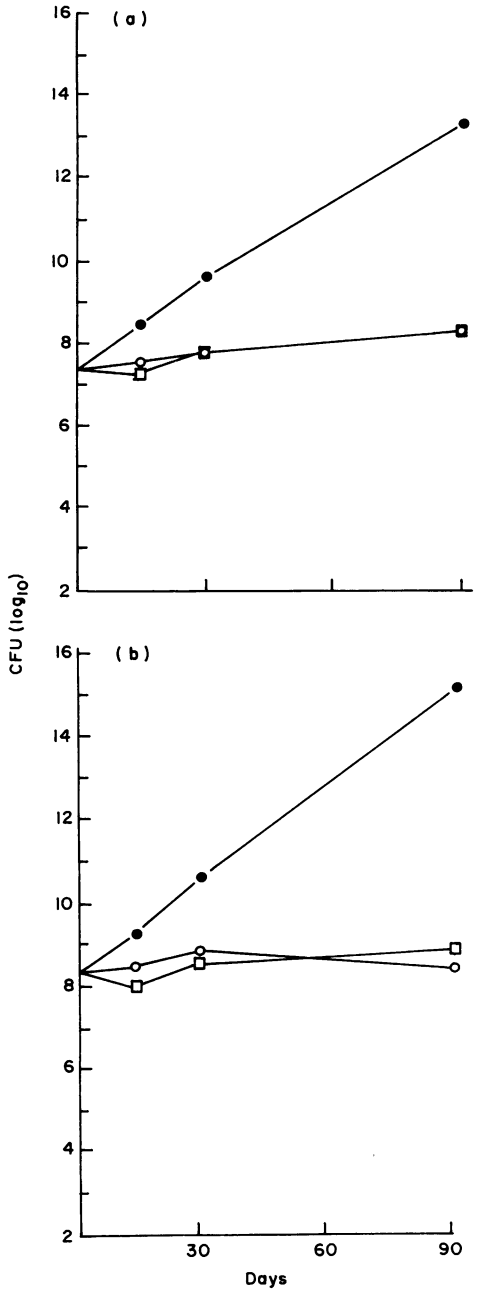

Fig. 6. CFU counts in the spleen (a) and liver (b) of BALB/c (●), BALB/c.*Bcg*^{r} (C.D2) (○), and DBA/2 (□) mice intravenously infected with 2×10^8 CFU of *M. avium*. Each point represents the mean of four mice. The s.d. of the geometric mean of CFU was smaller than 5% of the mean for any time point and was not depicted for sake of clarity.

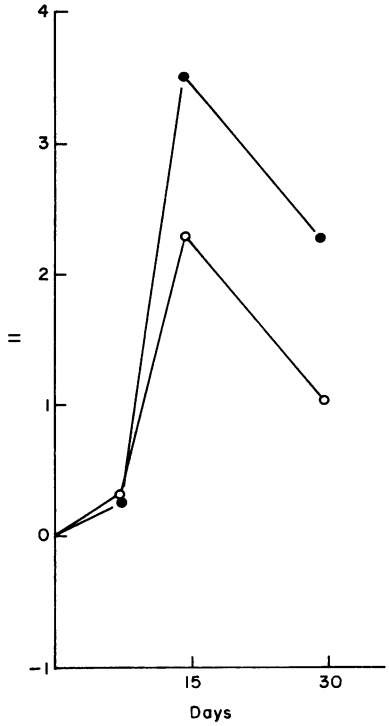

Fig. 7. Development of non-specific resistance to listeria in BALB/c (●) and C.D2 (○) mice intravenously infected with $2{\cdot}5 \times 10^8$ CFU of *M. avium*.

such enhancement in nitrite production by their peritoneal macrophges in the presence of LPS (Fig. 8b).

Resident peritoneal macrophages from uninfected BALB/c and C.D2 mice were cultured *in vitro* at high density of adherent cells (resulting in confluent macrophage monolayers) and infected with *M. avium* giving an infection ratio of about 1 bacterium:2 macrophages. This protocol avoided the problem of extracellular growth of the bacilli which did not occur in the 96 h of the assay. The slight differences in the initial CFU counts were also not important since there were never infection ratios above 1:1. BALB/c macrophages allowed bacterial growth resulting in a 2·5-fold increase in the CFU counts in 96 h of the infection (Fig. 9a). Macrophages from C.D2 were more bacteriostatic than those from BALB/c allowing for only a 1·6-fold increase in the CFU counts (Fig. 9b). The daily addition of spleen cell-conditioned medium containing macrophage activation factors led to an increase in the bacteriostatic capacities of the macrophages from both mouse strains (Fig. 9a, b). However, the relative differences between the two strains were still maintained, BALB/c macrophages allowing a 1·7-fold increase in CFU counts compared with a 1·2-fold increase with C.D2 macrophages.

DISCUSSION

Mycobacteria are intracellular parasites residing inside the macrophages of the infected host (Edwards & Kirkpatrick, 1986). The protection against these bacteria appears to depend upon mechanisms of cell-mediated immunity involving the recognition of bacterial antigens by T cells and the activation of the phagocytes by T cell-derived lymphokines (Edwards & Kirkpatrick, 1986; Gaugas & Rees 1968; Mackaness, 1964; Patterson & Youmans, 1970; Rook *et al.*, 1985). In addition to these immune pathways, innate resistance traits are able to affect the initial growth of these bacteria before the development of the acquired phase of immunity (Buschman *et al.*, 1988). The best studied of these traits is under the regulation of a gene located in the mouse chromosome 1, named *Bcg* (Forget *et al.*, 1981; Gros, Skamene & Forget, 1983), that probably is the same as those named *Lsh* (Bradley *et al.*, 1979) and *Ity* (Plant & Glynn 1979). The *Bcg* gene controls some as yet unidentified macrophage function that is manifested by the anti-mycobacterial

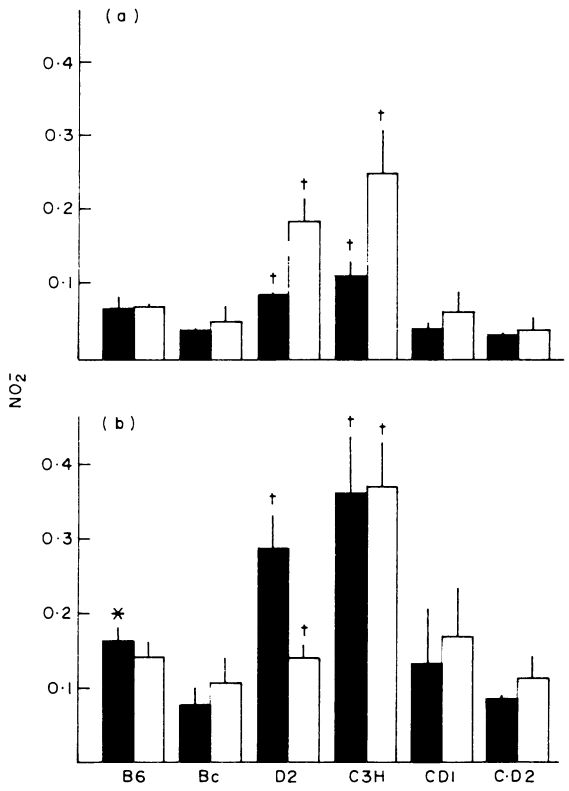

Fig. 8. Production of nitrite by peritoneal macrophages from C57BL 6 (B6), BALB/c (Bc), C3H/He (C3), DBA/2 (D2), CD-1 and C.D2 mice intraperitoneally infected with $2{\cdot}5 \times 10^8$ CFU of *M. avium* for 15 (closed columns) or 30 (open columns) days. Nitrite was detected in the culture supernatants of adherent cells incubated for 24 h in RPMI medium plus 10% FCS in the absence (a) or presence (b) of 10 ng of LPS ml. Each column represents the mean value for three mice. Comparison between infected and non-infected mice: statistically significant $*P<0{\cdot}01$, $\dagger P<0{\cdot}05$; Mann Whitney *U*-test.

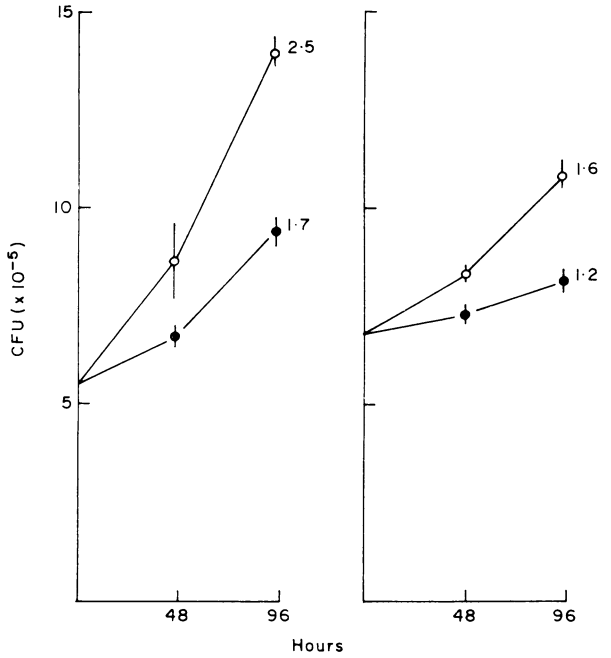

Fig. 9. CFU counts of *M. avium* in infected macrophage monolayers from BALB c (a) or C.D2 (b) mice, cultured up to 96 h in RPMI medium plus 10% FCS in the absence (○) or the presence (●) of 25% of conditioned medium. Each value represents the mean ± 1 s.d. of three determinations. The experiment was repeated three times giving reproducible results. Differences in the numbers of CFU at $t=0$ in the two mouse strains did not account for the differences in the growth rate since they were also observed when CFU numbers were the same at $t=0$ (other experiments).

(Stach *et al.*, 1984) and oxidative activity (Denis *et al.*, 1988c) of resident macrophages as well as by effects on the immune response (Buschman & Skamene, 1988; Denis *et al.*, 1988a, b).

We show here that two groups of mouse strains can be distinguished based on the resistance to *M. avium* infection. The susceptible animals allowed a progressive bacterial growth leading to the death of the animals whereas the resistant mice controlled the infection by inhibiting bacterial growth and showed no signs of disease. Of the seven inbred strains of mice that we studied, the four *M. avium*-susceptible strains bore the *Bcg*^{s} allele whereas the three resistant strains were *Bcg*^{r}. These results extend the data obtained by other authors with *M. avium* (Orme *et al.*, 1986, Stokes *et al.*, 1986) and *M. intracellulare* (Goto *et al.*, 1984), namely by showing that the *Bcg* gene is the major determinant of resistance against *M. avium* in the control of the infection using the pair of congenic mouse strains, BALB/c and BALB/c.*Bcg*^{r} (C.D2) mice (Potter *et al.*, 1983). The presence of the chromosome 1 segment bearing the *Bcg*^{r} gene rendered BALB/c mice as resistan to the infection as the donor DBA/2 strain. However, other genes may still play a role in resistance to these bacteria (Orme *et al.*, 1986). Unlike in the BCG model (Forget *et al.*, 1981; Gros *et al.*, 1983), where the *Bcg*^{s} allele is associated with bacterial growth only in the initial phase of the infection, we found that the effects of the *Bcg*^{s} gene allowing *M. avium* proliferation were observed both in the intial phase of infection where the innate resistance mechanisms are important and at later periods of infection after the acquisition of CMI.

We looked at the level of macrophage activation during the *M. avium* infection since this parameter is thought to be the effector mechanism in resistance to mycobacterial infection (Patterson & Youmans, 1970; Rook *et al.*, 1985; Edwards & Kirkpatrick, 1986) and because it reflects the degree of immune response that takes place in the infected host (North 1974). We studied three parameters of macrophage activation in infected mice of six strains including the congenic pair, BALB/c and CD-2. Our results showed that the levels of macrophage activation for non-specific antibacterial activity and H_2O_2 production were as high or even higher among susceptible strains as they were in strains resistant to *M. avium* thus showing that susceptibility to these mycobacteria was not due to a lack of macrophage activation. The secretion of reactive nitrogen intermediates, which has been reported to be enhanced following immunological activation of macrophages (Ding *et al.*, 1988; Stuehr & Marletta, 1985), was not associated with any pattern of resistance/susceptibility to infection by *M. avium*. It is thus apparent that this bacterium is quite resistant to the antibacterial activity of the activated macrophage as was also found by others (Orme & Collins 1983, Stokes & Collins 1988). It must be stressed, however, that any of the three parameters of macrophage activation studied represent markers of such functional macrophage alterations but may not represent the molecular mechanisms of antimycobacterial activity. Indeed, it has been

suggested that reactive oxygen intermediates are not involved in the macrophage control of mycobacterial growth (Flesch & Kaufmann 1988). As for the NO_2^- ions, there are no data concerning their action on mycobacteria even though they were shown to be active against fungi (Granger *et al.*, 1988). Moreover, there are numerous reports on the dissociation of the parameters of macrophage activation. Thus, it has been shown that tumoricidal and listericidal activities are independently regulated in exudate peritoneal macrophages of mice (Campbell *et al.*, 1988) and that antibacterial activities against different species of bacteria are dissociated (van Dissel *et al.*, 1987). It must also been taken into account that we measured macrophage activation of a population of cells that had a minority of infected macrophages. Thus, it cannot be excluded that the infected cells might not exhibit the same general pattern of macrophage activation.

We showed that congenitally athymic C57BL/6 nude mice and T cell-depleted BALB/c mice developed a smaller degree of NSR to listeria compared with normal littermates, suggesting that at least partially, macrophage activation involves T cell-dependent mechanisms in agreement with previous reports (North, 1974). This would argue in favour of the hypothesis that susceptibility to *M. avium* is not due to the lack of an efficient immune response leading to macrophage activation. The susceptibility to infection could be due to either a defective macrophage antimicrobial function or to the resistance of *M. avium* to the antibacterial activity of the macrophages as suggested before. Likewise, the lack of growth of *M. avium* in resistant mice could be explained by the presence of an efficient antibacterial function in Bcg^r macrophages or by the lack of a suitable environment for bacterial growth.

Our observation that the macrophage activation is directly proportional to the bacterial load in the infected mice prompts us to speculate that in the presence of higher amounts of antigen or when innate susceptibility allows bacterial proliferation, the T cells are more active in the regulation of the macrophage functions, or mycobacterial products by themselves directly activate macrophages.

The differences in resistance to *M. avium* between mice were, at least partially, mimicked *in vitro* using cultures of resident peritoneal macrophages. Unlike Stokes & Collins (1988), we found that Con A-induced lymphokines could activate mycobacteriostatic activity in resident peritoneal macrophages of distinct innate resistance to mycobacteria. Interestingly, the differences between resistant and susceptible animals were maintained after *in vitro* activation by spleen cell-derived lymphokines. Since the activating stimulus in the macrophage culture was the same for the two cell populations, this result supports the notion that the susceptibility is indeed due to some defective antibacterial function of Bcg^s macrophages that cannot be completely overcome by immune-mediated activation. Such function could be related either directly to an effector molecule or to the triggering of the antimicrobial mechanisms.

In conclusion we have shown that the *Bcg* gene plays an important role in the control of the resistance to *M. avium* infection in mice and we hypothesize that this resistance depends on macrophage functions rather than on the immune response and that the activation of these phagocytes cannot completely overcome, as happens with the BCG bacilli, the inability of Bcg^s macrophages to control bacterial proliferation.

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