Cytokine-Mediated Activation of Macrophages from *Mycobacterium bovis* BCG-Resistant and -Susceptible Mice: Differential Effects of Corticosterone on Antimycobacterial Activity and Expression of the *Bcg* Gene (Candidate *Nramp*)

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Previous work in our laboratory has shown that corticosterone increases the susceptibility of macrophages from *Bcg*^s mice to the growth of *Mycobacterium avium*. The innate antimycobacterial activity of macrophages from *Bcg*^r mice was not affected by corticosterone. In contrast to the differential effect of corticosterone on the antimycobacterial activity of the macrophages, corticosterone suppressed the production of tumor necrosis factor alpha and nitric oxide by macrophages from both *Bcg*^r and *Bcg*^s mice. The purpose of this investigation was to compare the effects of corticosterone on the antimycobacterial activity of macrophages from *Bcg*^r and *Bcg*^s mice that have been activated in vitro with recombinant gamma interferon or granulocyte-macrophage colony-stimulating factor. We found that macrophages from both strains of congenic mice responded equally to the activation stimuli. The capacity of the activated macrophages from *Bcg*^s mice to suppress the growth of *M. avium* was inhibited by the addition of corticosterone to the cultures. The addition of *N*^G-monomethyl-L-arginine to the cultures did not affect the capacity of resident splenic macrophages from *Bcg*^r mice to limit the growth of *M. avium*. However, *N*^G-monomethyl-L-arginine reduced the capacity of gamma interferon-activated, but not granulocyte-macrophage colony-stimulating factor-activated, macrophages to limit the growth of *M. avium* by macrophages from both *Bcg*^r and *Bcg*^s mice. The addition of corticosterone suppressed *Nramp* expression by macrophages from *Bcg*^s mice. *Nramp* expression by macrophages from *Bcg*^r mice was not affected by corticosterone.

Resistance to mycobacterial growth is controlled by a gene (*Bcg*) that maps to chromosome 1 in mice (28). The candidate *Bcg* gene, *Nramp*, codes for a product, termed natural resistance associated-macrophage protein, that has a motif of a transporter protein, but the function of *Nramp* is not known (31). It has been suggested that the protein may be involved in the transport of nitrates to the phagolysosome of infected macrophages, which accounts for the increased capacity of macrophages from *Bcg*^r mice to control the growth of mycobacteria. Others have suggested that the protein may play an important role in nitric oxide (NO)-mediated signal transduction resulting from priming and activation of macrophages, thereby accounting for the pleiotropic effects that have been reported to be under *Bcg*-mediated control (12, 13, 26, 28).

Studies in our laboratory have shown that corticosteroids increased the susceptibility of *Bcg*^s mice to the growth of *Mycobacterium avium* (4). In contrast, the corticosteroids did not affect resistance of *Bcg*^r mice to mycobacterial growth. The in vivo effects of corticosterone mimic the effect of the steroid on the capacity of the macrophage to control the growth of *M. avium* in vitro (5). Treatment of macrophages from *Bcg*^s mice with corticosterone increased the permissiveness of the macrophages for mycobacterial growth. Corticosterone treatment of macrophages from *Bcg*^r mice did not alter their resistance to

the growth of *M. avium*. We also showed that corticosteroids suppressed the production of tumor necrosis factor alpha and of reactive nitrogen intermediates by macrophages from both strains of mice without affecting the resistance of macrophages from *Bcg*^r mice.

Thus, when the macrophage populations were activated in vitro with recombinant gamma interferon (rIFN- γ), the function of macrophages from *Bcg*^r and *Bcg*^s mice was suppressed by the addition of corticosterone. This finding raised the possibility that the differential effect of glucocorticoids that we have observed was limited to innate resistance and not resistance induced by rIFN- γ or other macrophage-activating cytokines. The purpose of this investigation, therefore, was to evaluate the antimycobacterial activity of macrophages from *Bcg*^r and *Bcg*^s mice following stimulation of the cells with rIFN- γ or granulocyte-macrophage colony-stimulating factor (GM-CSF) and to determine the effects of glucocorticoids on the capacity of the macrophages to control the growth of *M. avium*. We found that macrophages from the two strains of mice responded equally to rIFN- γ - or GM-CSF-mediated activation. Corticosteroids suppressed the ability of rIFN- γ or GM-CSF-activated macrophages from *Bcg*^s mice but not from *Bcg*^r mice to suppress mycobacterial growth. *Nramp* expression, by macrophages from both *Bcg*^r and *Bcg*^s mice, was up-regulated by treatment of the cells with rIFN- γ and by treatment with GM-CSF. Corticosterone suppressed *Nramp* mRNA expression by macrophages from *Bcg*^s mice but did not affect *Nramp* mRNA expression by macrophages from *Bcg*^r mice.

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MATERIALS AND METHODS

Animals. Male BALB/c, *Bcg*⁺ (BALB/c.*Bcg*⁺) mice were obtained from Charles River when 6 weeks of age. The animals were housed in groups of five in microisolation cages and given food and water ad libitum. Congenic C.D2JdH⁺-*Ity*⁻-*Pep*-3⁺ *Bcg*⁺ (BALB/c.*Bcg*⁺) mice were initially obtained from Michael Potter (National Cancer Institute) (25) and bred by us (4).

Mycobacterium. A recent clinical isolate of *M. avium* was obtained from Ohio State University Hospitals (4) and grown in Middlebrook 7H9 broth supplemented with OADC (Difco Laboratories, Detroit, Mich.) at 37°C in 10% CO₂ in air until mid-log phase. Bacteria were divided into 1-ml aliquots and stored frozen at -70°C at a concentration of 3×10^8 to 5×10^8 CFU/ml. Prior to use, each aliquot was thawed, vortexed, and then sonicated (Heat Systems, Farmingdale, N.Y.), using 25 0.25-s pulses of 75 W each. To confirm the number of bacteria used to infect cultures, serial 10-fold dilutions of the mycobacterial stock were cultured onto 7H11 agar plates (Difco), and colony counts were enumerated after 10 days of culture at 37°C in 10% CO₂.

In vitro antimycobacterial activity. The antimycobacterial activity of splenic macrophages was assessed as initially described by Fleisch and Kaufman (11) and adapted by us (5). Briefly, splenic adherent cells were enriched by overnight adherence onto tissue culture dishes (100 by 20 mm, Falcon), using Iscove's modified Dulbecco's medium (IMDM; GIBCO/BRL, Gaithersburg, Md.) supplemented with 20% defined fetal bovine serum (HyClone, Logan, Utah) containing less than 0.03 ng of endotoxin per ml. Nonadherent cells were removed by gentle washing with Hank's balanced salt solution (GIBCO/BRL), and the splenic macrophages were removed by scraping with cell scrapers. Following differential staining and staining for nonspecific esterase, the adherent cells were then added, in IMDM without serum, to 96-well microtiter plates so that a concentration of 10^5 macrophages per well was attained. The purified macrophages (>90% as determined by nonspecific esterase) were then infected with 4×10^5 CFU of *M. avium* suspended in IMDM without antibiotics and incubated overnight to allow for phagocytosis. The cultures were then washed to remove unphagocytized bacteria and incubated for 5 days to allow the intracellular growth of the bacteria. The macrophages were then lysed, and bacteria were pulsed overnight by incubation in medium containing a 1:1 mixture of 7H9 (Difco) and IMDM with [³H]uracil (5 µCi/ml; specific activity, 40 to 60 Ci/mmol; Amersham, Chicago, Ill.) and 0.2% saponin. The bacteria were harvested onto glass fiber filter strips with a PHD cell harvester (Cambridge Technology, Inc., Watertown, Mass.). Radioactivity incorporated by the bacteria was quantitated by liquid scintillation spectrometry.

To induce the antimycobacterial activity of the macrophages, the cells were treated with either rIFN-γ (GIBCO) or GM-CSF (Boehringer Mannheim, Indianapolis, Ind.) in IMDM (without antibiotics) for 24 h prior to infection with *M. avium*. Following removal of unphagocytized bacteria, IMDM containing either rIFN-γ or GM-CSF was added back to the macrophage cultures. To assess the role of NO synthase on in vitro antimycobacterial activity, N^G-monomethyl-L-arginine (NMMA) (Sigma Chemical Company, St. Louis, Mo.) was added in concert (at a final concentration of 250 µM) with the cytokines prior to infection with *M. avium* and added back to the cultures after washing to remove any unphagocytized bacteria. To determine the effect of glucocorticoids on macrophage antimycobacterial activity, corticosterone (Sigma), at a concentration of 10^{-6} M, was added to the infected macrophage cultures after phagocytosis of the bacteria.

Production of reactive nitrogen intermediates. To determine the amount of NO, macrophages were stimulated with 5×10^5 *M. avium* together with rIFN-γ or GM-CSF in the presence or absence of NMMA (250 µM) for 72 h at 37°C. Cell-free supernatants were then used to determine the amount of NO. The amount of NO (via nitrite) was determined by using the Griess reagent (15). The amount of NO was calculated from a standard curve and expressed as micromolar.

Nramp expression. To determine the effect of corticosterone on *Nramp* expression, splenic macrophage monolayers, at a concentration of 5×10^6 cells per 35-mm-diameter culture well, were incubated in complete IMDM medium supplemented with 20% fetal bovine serum (no antibiotics) in the presence of rIFN-γ (2,500 U) or GM-CSF (250 U) for 24 h at 37°C in 5% CO₂. The macrophage monolayers were washed with complete IMDM, and the cultures were then replenished with complete IMDM with or without 10^{-6} M corticosterone. After incubation for 24 h at 37°C in an atmosphere containing 5% CO₂, the monolayers were washed and total RNA was extracted with 8 M guanidine hydrochloride as modified by Evans and Kamdar (8, 10, 19). The isolated RNA (7.5 µg per lane) was size fractionated by gel electrophoresis (1.5% formaldehyde agarose) and transferred by capillary blotting onto Hybond-N+ membranes (Amersham). A separate lane containing a 0.24- to 9.5-kb RNA ladder (GIBCO/BRL) was stained with ethidium bromide and used to determine RNA size. Northern (RNA) hybridization of the mRNA was carried out as described by Maniatis et al. (20). Gel-purified insert *Nramp* and actin cDNAs were radiolabeled with [³²P]dCTP by the random primer method (Amersham). Autoradiographs were quantified by scanning with a model 620 CCD densitometer (Bio-Rad, Richmond, Calif.).

The *Nramp* cDNA probe was produced by reverse transcriptase-mediated PCR (18), using primers 5'TCATTGGTTTGCTGGTAGAAGGCTGACC3' and 5'TGAGCATCGCTTTCCTTGACCCGGGA3', originally provided by

FIG. 1. IFN-γ induced activation of macrophages from *Bcg*⁺ and *Bcg*⁺ mice. Splenic macrophages were treated with rIFN-γ for 24 h prior to infection with 4×10^5 CFU of *M. avium*. After 24 h to allow for phagocytosis and following removal of the unphagocytized bacteria, the cultures were incubated in the continued presence of rIFN-γ for 5 days prior to lysis of the macrophages and pulsing of the bacteria with [³H]uracil. The data are expressed as the percentage of the control value, which represents the amount of radioactivity incorporated by the bacteria after infection of cultures of macrophages treated with rIFN-γ divided by that of cultures not treated with rIFN-γ. The effect of rIFN-γ was significant at $P \leq 0.003$ as determined by analysis of variance. There was no statistical difference between macrophages from *Bcg*⁺ and *Bcg*⁺ mice.

Phillip Morrissey (Immunex), that amplify nucleotides 1191 to 1214 and 470 to 496 of the published *Nramp* sequence (31). Two micrograms of total RNA from splenic macrophages stimulated with rIFN-γ was reverse transcribed in the presence of oligo(dT) primer, using avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wis.). PCR amplification mixtures contained 10% of the reverse-transcribed RNA, 2.5 U of *Taq* polymerase, 20 µM deoxynucleoside triphosphates, 0.40 µM each primer, and PCR buffer containing 3 mM MgCl₂. Amplification was performed for 35 cycles with 45 s of denaturation at 95°C, 45 s of annealing at 60°C, and 2 min of extension at 72°C. The PCR product was cloned into the pGEM-T vector (Promega) and transformed into *Escherichia coli* DH5α competent cells (GIBCO/BRL). A colony containing an insert of the appropriate size was isolated, and the presence of *Nramp* cDNA was confirmed by dideoxy DNA sequencing using Sequenase (U.S. Biochemical Co., Cleveland, Ohio). The *Nramp* cDNA insert was excised from the pGEM-T plasmid by digestion with *Sst*I and *Sst*II and agarose gel purified for Northern blot analysis.

Statistical analysis. Analysis of the data was carried out by using the SYSTAT statistical program (version 5.2; SYSTAT Inc., Evanston, Ill.). Each observation of antimycobacterial activity or NO production was analyzed by using a two-factor analysis of variance done on both log and absolute scales for comparison. In most cases, the data are expressed as the means of replicate trials. In some cases, representative data are shown. Pairwise comparisons between dosage treatment groups were determined by using the Bonferroni adjusted correlation. All declarations of significance are presented as worst-case values.

RESULTS

Cytokine-induced activation of macrophages from *Bcg*⁺ and *Bcg*⁺ mice. Macrophages from *Bcg*⁺ and *Bcg*⁺ mice responded equally to stimulation with rIFN-γ and GM-CSF. The results in Fig. 1 show that 500 U of rIFN-γ per well resulted in 52% of the growth of *M. avium* within macrophages from *Bcg*⁺ mice not activated with cytokine. Similarly, *M. avium*, within macrophages from *Bcg*⁺ mice, grew to only 44% of control levels. Increasing amounts of rIFN-γ resulted in increased growth inhibition. The ability of GM-CSF to activate macrophages from *Bcg*⁺ and *Bcg*⁺ mice was similar to that observed with rIFN-γ. Thus, 100 U of GM-CSF per well resulted in 48 and 52% of control growth of *M. avium* by macrophages from *Bcg*⁺ and *Bcg*⁺ mice, respectively (Fig. 2).

Differential effect of corticosterone on antimycobacterial activity of macrophages. The results in Fig. 3 and 4 show that corticosterone treatment of rIFN-γ- or GM-CSF-activated macrophages increased the permissiveness of cells from *Bcg*⁺ mice for *M. avium* growth. While treatment with rIFN-γ or

FIG. 2. GM-CSF-induced activation of macrophages from *Bcg^f* and *Bcg^s* mice. Macrophages were treated as described for Fig. 1 except that the cells were treated with GM-CSF for 24 h prior to infection. The effect of GM-CSF was significant at $P \leq 0.02$ by analysis of variance. There was no statistical difference between macrophages from *Bcg^f* and *Bcg^s* mice.

with GM-CSF resulted in 50 to 60% of the growth of control cultures not activated with cytokine, the addition of corticosterone increased mycobacterial growth to 80% of control levels. In contrast, corticosterone did not affect the capacity of the activated macrophages from *Bcg^f* mice to inhibit the growth of *M. avium*.

Role of reactive nitrogen intermediates. Previously, we have shown that corticosterone suppresses the production of tumor necrosis factor alpha and NO by splenic macrophages from *Bcg^f* and *Bcg^s* mice stimulated in vitro with rIFN- γ and *M. avium* without affecting the capacity of macrophages from the resistant mice to control the growth of the mycobacteria (5). This finding suggested that the antimycobacterial activity of the macrophages from *Bcg^f* mice was independent of the production of NO. To test this, we determined the effect of NMMA on innate resistance and on the antimycobacterial activity and NO production by rIFN- γ - or GM-CSF-activated macrophages. The results in Table 1 shows that the addition of NMMA to cultures of macrophages did not affect the natural resistance of the macrophages from the *Bcg^f* mice.

Figure 5A shows that the addition of NMMA ameliorated

FIG. 3. Differential effect of corticosterone on rIFN- γ -induced antimycobacterial activity of *Bcg^f* and *Bcg^s* mice. Macrophages were treated and infected as described in the legend to Fig. 1. After infection, the cultures were treated with 10^{-6} M corticosterone (Cort) for 5 days prior to lysis of the macrophages and addition of [3 H]uracil. The effect of corticosterone on the antimycobacterial activity of rIFN- γ -activated macrophages from *Bcg^s* mice was significant ($P \leq 0.03$).

FIG. 4. Differential effect of corticosterone on GM-CSF-induced antimycobacterial activity of *Bcg^f* and *Bcg^s* mice. The cultures were treated as described for Fig. 3. The effect of corticosterone (Cort) on the antimycobacterial activity of the GM-CSF-activated macrophages was significant ($P \leq 0.03$).

the antimycobacterial activity of macrophages from both *Bcg^f* and *Bcg^s* mice activated with rIFN- γ . In contrast, the addition of NMMA did not affect the antimycobacterial activity of macrophages activated by treatment with GM-CSF (Fig. 6A). Similarly, the results in Fig. 5B show that rIFN- γ -activated macrophages produced as much as 40 μ M NO, an amount that was reduced in the presence of NMMA. However, the addition of GM-CSF, which activated the macrophages to suppress mycobacterial growth, induced less than 10 μ M NO (Fig. 6B). Furthermore, the addition of NMMA to these cultures failed to alter the level of NO and did not affect the antimycobacterial activity of the cells.

rIFN- γ and GM-CSF induce the expression of *Nramp*. It has been suggested that *Nramp* may be a nitrate transporter or involved in NO-mediated signal transduction. The results in Fig. 7 and 8 show that both rIFN- γ and GM-CSF induced the expression of *Nramp* by macrophages from both *Bcg^f* and *Bcg^s* mice. However, we found that corticosterone suppressed *Nramp* expression by macrophages from *Bcg^s* mice but did not suppress *Nramp* expression by macrophages from *Bcg^f* mice.

TABLE 1. Effect of NMMA on innate resistance mediated by *Bcg* (candidate *Nramp*)^a

Time of incubation in macrophages	cpm incorporated (mean $\pm$ SD) by <i>M. avium</i> after incubation within macrophages from:	
	<i>Bcg^s</i> mice	<i>Bcg^f</i> mice
Day 0	2,130 $\pm$ 185 ^b	1,982 $\pm$ 223
Day 5	13,280 $\pm$ 721 ^c	8,213 $\pm$ 198
No treatment	13,413 $\pm$ 939 ^d	8,541 $\pm$ 256
+NMMA		

^a Macrophages were infected with *M. avium* by overnight incubation. After being washed to remove unphagocytized bacteria, the macrophages were lysed immediately, and either the bacteria were pulsed with [3 H]uracil (day 0) for 24 h, or the cultures were incubated for an additional 5 days in the absence or presence of 250 μ M NMMA. The macrophages were lysed, and the bacteria were pulsed with [3 H]uracil.

^b The differences in the uptake of *M. avium* between macrophages from *Bcg^f* and *Bcg^s* mice were not significant.

^c The growth of *M. avium* in macrophages from *Bcg^f* and *Bcg^s* mice was significant ($P < 0.001$).

^d The effect of NMMA on mycobacterial growth was not significant.

FIG. 5. NMMA suppresses the antimycobacterial activity and the production of reactive nitrogen intermediates (NO) by rIFN- γ -activated macrophages. (A) Antimycobacterial activity. Macrophages were treated with rIFN- γ and infected with *M. avium* as described in the legend to Fig. 1 except that the cultures were treated with 250 μ M NMMA concurrently with the addition of rIFN- γ and throughout the 5-day incubation period prior to the addition of [3 H]uracil. The effect of NMMA on the antimycobacterial activity of macrophages from *Bcg*^r and *Bcg*^s mice was significant ($P \leq 0.03$). (B) Production of NO. Splenic macrophages were cultured in the presence of 250 U of rIFN- γ and 4×10^5 CFU of *M. avium* with or without 250 μ M NMMA for 72 h. The amount of NO (nitrate) in the cell-free supernatant fluids was determined by the Griess reaction. The effect of NMMA on NO production by macrophages from *Bcg*^r and *Bcg*^s mice was significant ($P \leq 0.02$).

DISCUSSION

The results of this investigation show that the effects of corticosterone on macrophages from congenic *Bcg*^r and *Bcg*^s mice differ and that the difference appears to be related to the different effects of the hormone on the *Bcg* gene, (candidate *Nramp*). The results also show that rIFN- γ and GM-CSF induce different antimycobacterial pathways. The rIFN- γ -induced pathway involves the production of NO and is inhibited by NMMA, while the GM-CSF pathway does not appear to be dependent on NO production and is not inhibited by NMMA. Nevertheless, both the rIFN- γ - and GM-CSF-induced pathways appear to be regulated by *Bcg* because they are differentially affected by corticosterone.

Our results also appear to rule out a role for NO as an effector molecule in *Bcg*-mediated resistance for two reasons. First, NMMA does not interfere with innate resistance mechanisms. Second, corticosterone suppresses the production of NO by macrophages from *Bcg*^r mice but does not affect their capacity to control the growth of *M. avium* (4, 5).

Our results are similar to those reported by others who have

FIG. 6. Lack of NO production by GM-CSF-activated macrophages from *Bcg*^r and *Bcg*^s mice. (A) Effect of NMMA on antimycobacterial activity. Macrophages were stimulated and infected as described for Fig. 2 and treated with 250 μ M NMMA. The effect of NMMA was not significant. (B) NO production. Macrophages were treated with 250 U of GM-CSF and 4×10^5 *M. avium* as well as 250 μ M NMMA. NO production by GM-CSF-treated macrophages was not significantly different from NO production by macrophages not treated with GM-CSF. The effect of NMMA was not significant.

also shown that the capacity of macrophages to limit the growth of mycobacteria and other intracellular pathogens is induced by IFN- γ and is dependent on the production of reactive nitrogen intermediates (7, 13, 16, 29). The antimycobac-

FIG. 7. Differential effect of corticosterone on *Nramp* expression by rIFN- γ -activated macrophages from *Bcg*^r and *Bcg*^s mice. Splenic macrophages (2×10^6) were treated with 2,500 U of rIFN- γ for 24 h prior to the addition of 10^{-6} corticosterone (Cort). After 24 h, the mRNA was extracted with 8 M guanidine hydrochloride, and the expression of *Nramp* mRNA was determined by Northern analysis.

FIG. 8. Differential effect of corticosterone (Cort) on *Nramp* expression by GM-CSF-activated macrophages from *Bcg^s* and *Bcg^r* mice. Splenic macrophages were treated as described for Fig. 7 except with 500 U of GM-CSF. The expression of *Nramp* mRNA was determined by Northern analysis.

terial activity of macrophages from both *Bcg^r* and *Bcg^s* mice was inhibited by the addition of NMMA to the culture media. Our observation also support those of Barrera et al. (2) and Roach et al. (27), who showed that macrophages from *Bcg^r* and *Bcg^s* mice that are activated in vitro with rIFN- γ are more bacteriostatic or leishmanicidal than are macrophages not treated with rIFN- γ . The antimicrobial activities in both of those studies were ameliorated by the addition of NMMA. Similarly, we also found that NMMA reduced the inhibition of *M. avium* growth by rIFN- γ -activated macrophages. However, NMMA did not affect the innate capacity of macrophages from *Bcg^r* mice to control the growth of *M. avium*.

Our observation that corticosterone suppressed the production of NO by macrophages from both *Bcg^r* and *Bcg^s* mice while suppressing the capacity of macrophages from only *Bcg^s* mice to control *M. avium* growth confounds the interpretation of these results. These observations suggest that macrophages from *Bcg^r* mice can control mycobacterial growth by a mechanism that is, in part, independent of the production of NO but controlled by the *Bcg* gene. This possibility is supported by our observation that NMMA did not affect the innate resistance mediated by resident splenic macrophage from *Bcg^r* mice. Indeed, several recent reports have suggested that IFN- γ -activated macrophages control the growth of intracellular parasites, including mycobacteria, by a mechanism that is independent of the production of NO as well as the production of reactive oxygen intermediates, depletion of tryptophan, or sequestration of iron (14, 23, 24).

The differential effect of corticosterone on the capacity of rIFN- γ - or GM-CSF-activated macrophages from *Bcg^r* and *Bcg^s* mice is similar to that which we have previously reported for macrophages not activated by cytokines (4, 5). Thus, corticosterone increased the permissiveness of resident splenic macrophages from *Bcg^s* mice to the growth of *M. avium* but did not result in an alteration of the growth pattern of the microorganism from *Bcg^r* mice.

GM-CSF has also been reported to increase the resistance of mice to the in vivo growth of *Salmonella typhimurium* and to activate macrophages in vitro to control the growth of intracellular pathogens, including *M. avium* (1, 3, 17, 21, 22, 30, 32). The in vivo effects of GM-CSF appear to be limited to an effect on macrophages from *Bcg^r* mice (23). We have found, however, that the abilities of GM-CSF to activate macrophages from both *Bcg^r* and *Bcg^s* mice in vitro were comparable. The capacity of GM-CSF-activated macrophages to control the growth of *M. avium* was independent of the production of NO.

This observation is similar to that reported by Buisman et al. (6), who found that GM-CSF did not induce the production of NO. Our observations are different, however, from those of Denis (9), who found that the inhibition of growth of an avirulent strain of *M. avium* by GM-CSF-activated human monocytes was suppressed by treating the cells with NMMA.

The results of this investigation show, for the first time, that the expression of *Bcg* (candidate *Nramp*) is up-regulated by the addition of either rIFN- γ or GM-CSF. There does not appear to be a difference in the level of expression of *Nramp* mRNA by macrophages from *Bcg^r* and *Bcg^s* mice. Rather, the differences appear to be related to the effect of corticosterone on *Nramp* mRNA. The differences in the effect of corticosterone could be either on the stability of the mRNA or on its transcription. In preliminary experiments, we have found that other rIFN- γ -inducible genes are also differentially regulated by corticosterone (unpublished observations). These genes do not map to chromosome 1, which suggests that differential regulation by corticosterone is a general phenomenon of IFN- γ induction in macrophages from *Bcg^r* and *Bcg^s* mice.

Glucocorticoids have been shown to have inhibitory and stimulatory effects on macrophage function (4). The effects of glucocorticoids are produced following the binding of the steroid to its receptor, which translocates to the nucleus and binds to glucocorticoid response elements (GREs), resulting in regulation of gene expression. Several studies concerning glucocorticoid-induced modulation of gene expression have shown that positive or negative effects of glucocorticoids are altered by a single nucleotide difference in the GRE. It is possible, therefore, that the differential regulation of *Nramp* expression by corticosterone that we have observed is due to differences in the GRE within the promoter region *Nramp* in *Bcg^r* and *Bcg^s* mice.

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