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Stabilized Expression of mRNA Is Associated with Mycobacterial Resistance Controlled by *Nramp1*

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Control of innate resistance to the growth of mycobacteria is mediated by a gene termed *Nramp1*. Although the role of the protein product of *Nramp1* in mediating resistance to mycobacterial growth is not known, the effect of the gene is pleiotropic and it has been suggested that the gene controls macrophage priming for activation. We have found that the functional capacity of macrophages from *Mycobacterium bovis* BCG-susceptible mice can be suppressed by corticosterone, while the function of macrophages from BCG-resistant mice remains unaffected. In this study, we show that corticosterone differentially affects the stability of mRNAs of several recombinant gamma interferon (rIFN-γ)-induced genes. Treatment of macrophages from BCG-susceptible mice with corticosterone accelerates the decay of *Nramp1* mRNA. The mRNA of IFN-γ-induced genes of macrophages from BCG-resistant mice was more stable than the mRNA of macrophages from BCG-susceptible mice in the presence or absence of corticosterone. The results of this investigation suggest that *Nramp1* acts by stabilizing the mRNA of genes associated with macrophage activation, thus accounting for the functional differences that have been attributed to these macrophage populations.

Resistance to the growth of several mycobacterial species is controlled by a gene (*Bcg*) that maps to mouse chromosome 1 (31). The *Bcg* gene codes for Nramp1, a membrane protein product termed the natural-resistance-associated macrophage protein (34). *Mycobacterium bovis* BCG-resistant mice were found to differ from BCG-susceptible mice in a nonconservative Gly-to-Asp substitution within a predicted transmembrane domain of Nramp1. The structural motif of Nramp1 is that of a transport protein, but the function of Nramp1 is still unknown. 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-resistant mice to control the growth of mycobacteria. Alternatively, it has also been suggested that the protein may play a part in nitric oxide-mediated signal transduction or ion transport resulting in priming-activation of macrophages, thereby accounting for the pleiotropic effects that have been reported to be under the control of *Bcg* (11, 12, 25, 30, 31).

Previous studies in our laboratory have shown that corticosteroids increased the susceptibility of BCG-susceptible mice to in vivo growth of *Mycobacterium avium* (5). In contrast, corticosteroids did not affect the resistance of BCG-resistant mice. In vitro treatment of macrophages with corticosterone paralleled the in vivo effects in that treatment of macrophages from BCG-susceptible mice increased the permissiveness of cells for mycobacterial growth, while treatment of macrophages from BCG-resistant mice did not alter their resistance to the growth of *M. avium* (6). We also found that *Nramp1* mRNA expression by macrophages from both BCG-resistant and BCG-susceptible mice was up-regulated by treatment of these cells with recombinant gamma interferon (rIFN-γ) and recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) (7). However, corticosterone suppressed the expression of *Nramp1* mRNA only by macrophages from BCG-susceptible mice. The purpose of this study was to characterize the differential effects of corticosterone on the expression of *Nramp1* mRNA by macrophages from BCG-resistant and BCG-susceptible mice. Our results show that corticosterone accelerated the decay of *Nramp1* mRNA, as well as the mRNAs of several other rIFN-γ-induced genes, in macrophages from BCG-susceptible mice. Macrophages from BCG-resistant mice were resistant to the suppressive effects of corticosterone. Functional *Nramp1* resulted in the more stable expression of *Nramp1*, *Mag-1*(*GBP-1*) (37), *Mg21*(*GTP-2*) (21), and major histocompatibility complex (MHC) class II mRNAs, as well as tumor necrosis factor alpha (TNF-α) and inducible nitric oxide synthase (iNOS) mRNAs, in the presence or absence of corticosterone. Since differences in mRNA stability can have dramatic effects on protein levels, the increased mRNA stability in macrophages from BCG-resistant mice could result in the sustained production of anti-mycobacterial effector molecules and thus could account for the increased resistance of macrophages from BCG-resistant mice.

MATERIALS AND METHODS

Animals. Male BCG-susceptible BALB/c (BALB/c.*Bcg*^{s}) 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 BCG-resistant C.D2*Idh*^{b}-*Ity*^{r}-*Pep-3*^{b} (BALB/c.*Bcg*^{r}) mice were initially obtained from Michael Potter (National Cancer Institute) (29) and bred in our facilities.

Reagents. rIFN-γ was obtained from Gibco Bethesda Research Laboratories (BRL), Grand Island, N.Y. rGM-CSF was purchased from Boehringer Mannheim (Indianapolis, Ind.) and corticosterone was obtained from Sigma (St. Louis, Mo.). Restriction enzymes were obtained from Boehringer Mannheim. [^{32}P] dCTP (specific activity, 3,000 Ci/mmol) was purchased from Amersham (Arlington Heights, Ill.). The *M. avium* used was a recent clinical isolate and was prepared as previously described by us (5). The cDNA probe for Nramp1 was produced by reverse transcription-PCR as previously described by us (7). The cDNA probe for iNOS was produced by reverse transcription-PCR by using primers obtained from Clontech (Palo Alto, Calif.). The cDNA clones of Mag-1 (37), Mg21 (21), and MHC class II I-E$_\beta$ (22) were isolated from a macrophage cDNA subtraction library of rIFN-γ-induced genes. The actin cDNA probe was obtained by screening a macrophage cDNA library with an actin-specific oligonucleotide. The TNF-α probe used was a 1.1-kb cDNA containing the entire coding region (Genosys, The Woodlands, Tex.).

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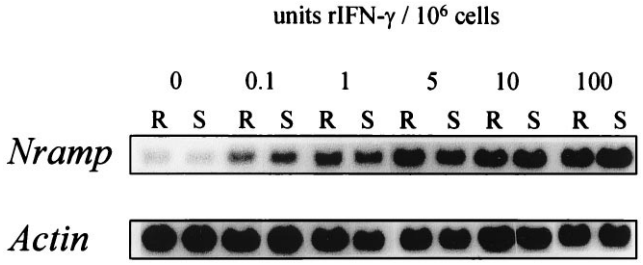

FIG. 1. Induction by IFN-γ of expression of *Nramp1* mRNA by macrophages from both BCG-resistant and BCG-susceptible mice. Purified splenic macrophages from BCG-resistant and BCG-susceptible mice were treated with increasing doses of rIFN-γ for 20 h to determine the dose required for maximal expression of *Nramp1*. Following treatment, total RNA was extracted and IFN-γ-induced expression of *Nramp1* mRNA was determined by Northern analysis. R, BCG resistant; S, BCG susceptible.

Analysis of IFN-γ-induced gene expression in macrophage cultures. Splenic macrophages from both BCG-resistant and BCG-susceptible mice were enriched by overnight adherence to 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 Hanks balanced salt solution (Gibco BRL), and splenic macrophages were removed by scraping with cell scrapers (5). The adherent cells were then added in IMDM containing 20% fetal bovine serum to 35-mm-diameter tissue culture dishes at a concentration of 5×10^6 macrophages per dish. Following a second incubation for 16 h at 37°C, the purified splenic macrophages (>90% pure as determined by differential staining and staining for nonspecific esterase) were washed with Hanks balanced salt solution and the medium was replenished with IMDM containing rIFN-γ and treated as described in Results. Macrophage cultures were incubated at 37°C in 5% CO_2 for the times indicated in Results.

Splenic macrophages were stimulated with rINF-γ (10 U/10^6 cells) or rGM-CSF (250 U/5×10^6 cells) for 20 h to induce *Nramp1* expression. Some of these macrophage cultures were then treated with lipopolysaccharide (LPS) (100 ng) for 8 h to induce TNF-α and iNOS mRNAs. Cultures were subsequently treated with 10^{-6} M corticosterone for an additional 12 h. After treatment, the macrophage monolayers were washed with ice-cold phosphate-buffered saline (Gibco BRL) and incubated on ice for 20 min. The phosphate-buffered saline was removed and replaced with lysing buffer containing 8 M guanidine hydrochloride, 0.3 M sodium acetate, and 10% sarcosyl lauryl sulfate. Total RNA was extracted as described by Evans and Kandar (10). The isolated RNA was size fractionated by 1.5% formaldehyde-agarose gel electrophoresis, and the RNA was transferred to Hybond-N+ membrane (Amersham) by capillary blotting. Following electrophoresis, a separate lane containing a 0.24- to 9.5-kb RNA marker ladder (Gibco BRL) was stained with ethidium bromide and used to determine RNA size. Northern hybridization of the mRNA was carried out by the protocol of Maniatis et al. (24). Gel-purified insert cDNAs were radiolabelled with [^{32}P]dCTP by random priming. Autoradiographs were quantified by using the UVP ImageStore 5000 gel documentation program (San Gabriel, Calif.) and NIH Image, version 1.58. To determine if corticosterone transcriptionally regulates Nramp1, a nuclear runoff assay of newly synthesized mRNA was performed as described by Howard and Ortlepp (16).

RESULTS

IFN-γ-induced the expression of *Nramp1* mRNA by macrophages from both BCG-resistant and BCG-susceptible mice. The results in Fig. 1 show that a low level of *Nramp1* mRNA is constitutively expressed by macrophages from both BCG-resistant and BCG-susceptible mice. The macrophages from both strains responded equally to stimulation with rIFN-γ. Maximal expression of *Nramp1* mRNA was induced by 10 U of rIFN-γ. Treatment of the macrophages for a minimum of 8 h with 10 U of IFN-γ per 10^6 cells was necessary to induce maximal expression of *Nramp1* mRNA in both strains of mice (data not shown).

Differential suppression of *Nramp1* mRNA expression and expression of other macrophage activation genes by corticosterone. Addition of corticosterone to macrophages from BCG-susceptible mice previously stimulated with rIFN-γ resulted in the suppression of *Nramp1* mRNA (Fig. 2). *Nramp1* mRNA expression by macrophages from BCG-resistant mice was not affected by corticosterone. Treatment of the rIFN-γ-stimulated macrophages from BCG-susceptible mice with corticosterone for a minimum of 12 h resulted in maximal suppression of *Nramp1* mRNA (data not shown). Additionally, mRNA expression of other rIFN-γ-inducible genes, i.e., guanine nucleotide binding proteins Mag-1 and 21-1 and MHC class II I-E_β, was differentially suppressed by corticosterone in rIFN-γ-stimulated macrophages from BCG-susceptible mice but not in macrophages from BCG-resistant mice (Fig. 3). rIFN-γ-mediated induction of *Nramp1* mRNA expression by macrophages from both BCG-resistant and BCG-susceptible mice was inhibited when corticosterone was added at the same time as rIFN-γ. The results of these experiments indicate that the differential effect of corticosterone occurs following the induction of mRNA expression and appears to be regulated post-transcriptionally.

Lack of corticosterone effect on *Nramp1* transcription. To confirm that corticosterone does not affect the transcription of *Nramp1* mRNA, macrophages were treated with rIFN-γ for 24 h prior to the addition of corticosterone. Cells were lysed, and nuclei were isolated at the times indicated in Fig. 4 after the addition of corticosterone. The results of the nuclear runoff assay (Fig. 4) showed that corticosterone treatment did not affect the transcription of *Nramp1* mRNA by macrophages from BCG-susceptible mice. In other experiments, we added actinomycin D 8 h after the addition of corticosterone to cultures of macrophages from BCG-susceptible mice. Northern analysis showed that *Nramp1* mRNA levels decreased following the addition of the transcription inhibitor (Fig. 5). The calculated half-life of the Nramp1 mRNA from corticosterone-treated macrophages from BCG-susceptible mice was 7 ± 1.5 h. During this time, the expression of *Nramp1* mRNA by macrophages from BCG-resistant mice remained relatively stable and the mRNA half-life was 27 ± 4 h.

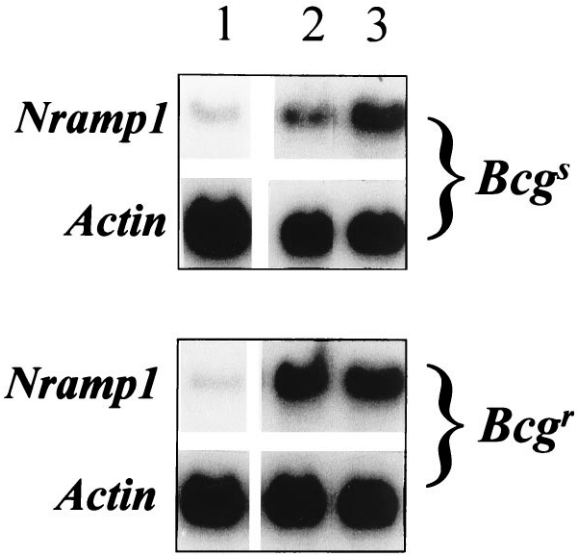

FIG. 2. Differential suppression of *Nramp1* mRNA expression by corticosterone. Purified splenic macrophages from BCG-resistant (*Bcg*^{r}) and BCG-susceptible (*Bcg*^{s}) mice were treated with corticosterone (10^{-6} M) following 24 h of exposure to 10 U of rIFN-γ per 10^6 cells (lane 2). Control cultures (lane 3) were incubated in medium only. Additional macrophages were simultaneously treated with rIFN-γ and corticosterone for 24 h (lane 1). After 24 h, total RNA was extracted and the effect of corticosterone on the induction and expression of *Nramp1* mRNA was determined by Northern analysis.

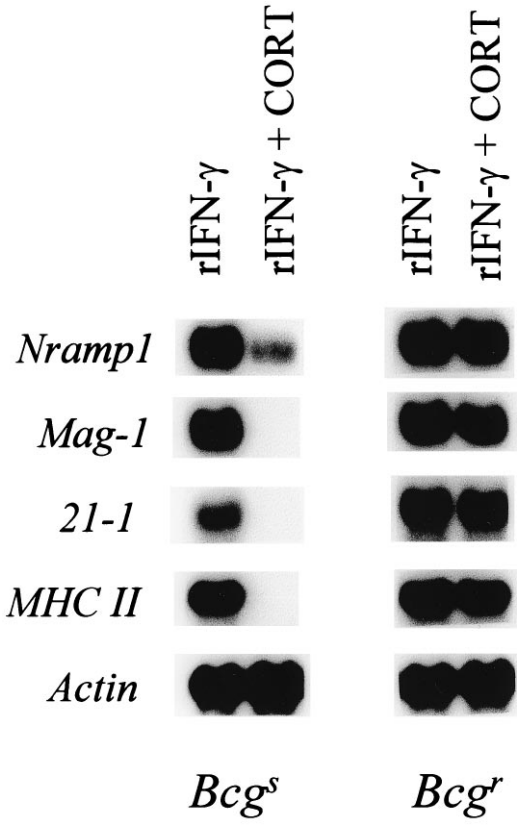

FIG. 3. Differential effect of corticosterone on rIFN-γ induced genes by macrophages from BCG-resistant (*Bcg*^{r}) and BCG-susceptible (*Bcg*^{s}) mice. Splenic macrophages from BCG-resistant and BCG-susceptible mice were stimulated with rIFN-γ for 24 h and then treated with corticosterone (CORT). After 20 h, total RNA was extracted and the effect of corticosterone on the expression of Mag-1, 21-1, MHC class II, and *Nramp1* mRNAs was determined by Northern analysis.

If differences in functional capacity that have been attributed to macrophages from BCG-resistant and BCG-susceptible mice can be accounted for by the differences in mRNA stability between these macrophages, then differences in mRNA stability should be observed in the absence of corticosterone. The results in Fig. 6 show that the half-life of *Nramp1* mRNA and those of Mag-1 and MHC class II I-E_β mRNAs were also different in macrophages from BCG-resistant and BCG-susceptible mice in the absence of corticosterone. The half-life of *Nramp1* mRNA in macrophages from BCG-susceptible mice was 17 ± 2 h, compared to 24 ± 3 h for mRNA in macrophages from BCG-resistant mice. Similarly, the half-life of Mag-1 mRNA was 10 ± 3 versus 15 ± 1 h for mRNA in macrophages from BCG-susceptible and BCG-resistant mice, respectively, and those of MHC class II I-E_β were 13 and 20 h, respectively.

Requirement of prior induction of *Nramp1* for resistance to corticosterone-mediated suppression. We have previously shown that rIFN-γ and rGM-CSF induce *Nramp1* mRNA (7). However, GM-CSF alone does not induce iNOS mRNA and induces only low levels of TNF-α mRNA (13). To determine if prior induction of *Nramp1* is required for iNOS and TNF-α mRNA stability, we treated macrophages from BCG-resistant and BCG-susceptible mice with GM-CSF to induce *Nramp1*. The macrophages were then treated with LPS for 8 h. This resulted in the induction of both TNF-α and iNOS mRNAs. The cultures were then treated with corticosterone for 12 h, and the presence of TNF-α and iNOS mRNAs was determined by Northern analysis. The results in Fig. 7 showed, as expected, that rGM-CSF did not induce TNF-α or iNOS mRNA (lane 2); treatment of primed or unprimed macrophages with LPS resulted in the induction of both TNF-α and iNOS mRNAs (lanes 3 and 5). The addition of corticosterone resulted in a decrease in the level of both TNF-α and iNOS mRNAs in macrophages from both BCG-resistant and BCG-susceptible mice not previously treated with rGM-CSF in which *Nramp1* expression was not induced prior to the addition of LPS (lane 6). Thus, in the presence of a functional *Nramp1* product in BCG-resistant mice, the TNF-α and iNOS mRNAs were stable (lane 4). In contrast, when *Nramp1* is not functional, as in the macrophages from BCG-susceptible mice, addition of corticosterone reduced the expression of TNF-α and iNOS mRNAs.

When we added actinomycin D to cultures stimulated with rGM-CSF and LPS in the absence of corticosterone, we found that the half-life of TNF-α and iNOS mRNAs was 2 ± 0.4 h in macrophages from BCG-susceptible mice and 4 ± 1 h in macrophages from BCG-resistant mice. Additionally, the half-life of *Nramp1* mRNA was 15 ± 2.5 h in macrophages from BCG-susceptible mice and 25 ± 3 h in macrophages from BCG-resistant mice (Fig. 8). The half-life of *Nramp1* mRNA in macrophages stimulated with GM-CSF and LPS was similar to that following stimulation of the cells with rIFN-γ.

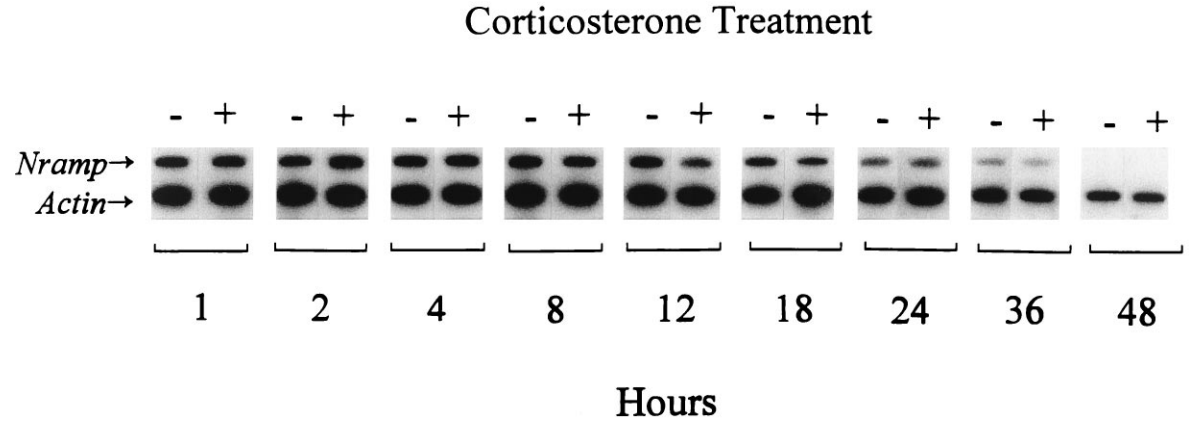

FIG. 4. Absence of corticosterone effect on *Nramp1* transcription. Splenic macrophages from BCG-susceptible mice were treated with rIFN-γ for 24 h prior to the addition of corticosterone and incubation for the times given. The cells were lysed, and mRNA was extracted from isolated nuclei. To determine if corticosterone affected *Nramp1* mRNA transcription, the nuclei were radiolabelled and hybridized to membrane-bound *Nramp1* cDNA and control β-Actin cDNA.

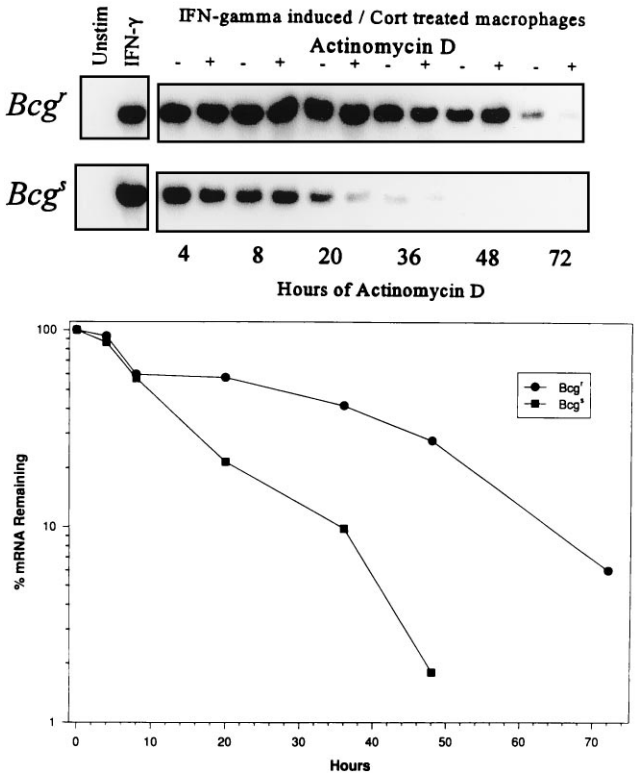

FIG. 5. Corticosterone (Cort)-induced accelerated decay of *Nramp1* mRNA expression by macrophages from BCG-susceptible (*Bcg*^{s}) mice. Splenic macrophages from BCG-resistant (*Bcg*^{r}) and BCG-susceptible mice were stimulated for 12 h with rIFN-γ and then treated with corticosterone. Actinomycin D (10 μg/10^6 cells) was added 8 h after corticosterone. Total RNA was extracted, and the effect of corticosterone on the *Nramp1* mRNA half-life was determined by Northern and densitometric analyses. Unstim, unstimulated.

DISCUSSION

The results of this investigation indicate that the differences in resistance to mycobacterial growth mediated by *Nramp1* correlate with increased mRNA stability. Thus, addition of corticosterone results in the destabilization of mRNA in macrophages from BCG-susceptible mice. Differences in the decay of mRNA in macrophages was also observed without the addition of corticosterone. The glucocorticoid hormone, however, resulted in an accelerated decay of mRNA in macrophages from BCG-susceptible mice. For example, the calculated half-life of *Nramp1* mRNA in macrophages from BCG-susceptible mice was 17 h in the absence of corticosterone and 7 h in the presence of the hormone.

The differential effect of corticosterone appears to require the expression of a functional *Nramp1* gene. Thus, following treatment of macrophages from BCG-resistant mice with rIFN-γ, expression of Mag-1 and MHC class II I-E_β mRNAs, as well as *Nramp1* mRNA, was stable. When TNF-α and iNOS mRNAs were induced prior to the induction of *Nramp1*, they remained unstable and their expression decreased following the addition of corticosterone. In contrast, if TNF-α or iNOS mRNA was induced after the induction of *Nramp1* in macrophages from BCG-resistant mice, the iNOS and TNF-α mRNAs were stably expressed. Since *Nramp1* produced by macrophages from BCG-susceptible mice is likely to be nonfunctional, addition of corticosterone results in the destabilization of the mRNA.

Macrophages from BCG-resistant and BCG-susceptible mice have been reported to be functionally different. However, different results have been reported when measuring the functional capacity of macrophages to control the growth of mycobacteria, *Salmonella typhimurium*, and *Leishmania donovani* (15). *Nramp1* mRNA expression was up-regulated by rIFN-γ in macrophages from both BCG-resistant and BCG-susceptible mice. There were no apparent differences in the level of expression of *Nramp1* mRNA. The amount of rIFN-γ required to induce maximal *Nramp1* mRNA expression by macrophages from both strains of mice was the same. Similarly, we have previously reported that the level of expression of MHC class II glycoproteins by macrophages from both strains of mice was the same immediately following treatment with rIFN-γ. We have also reported that the level of MHC class II glycoprotein

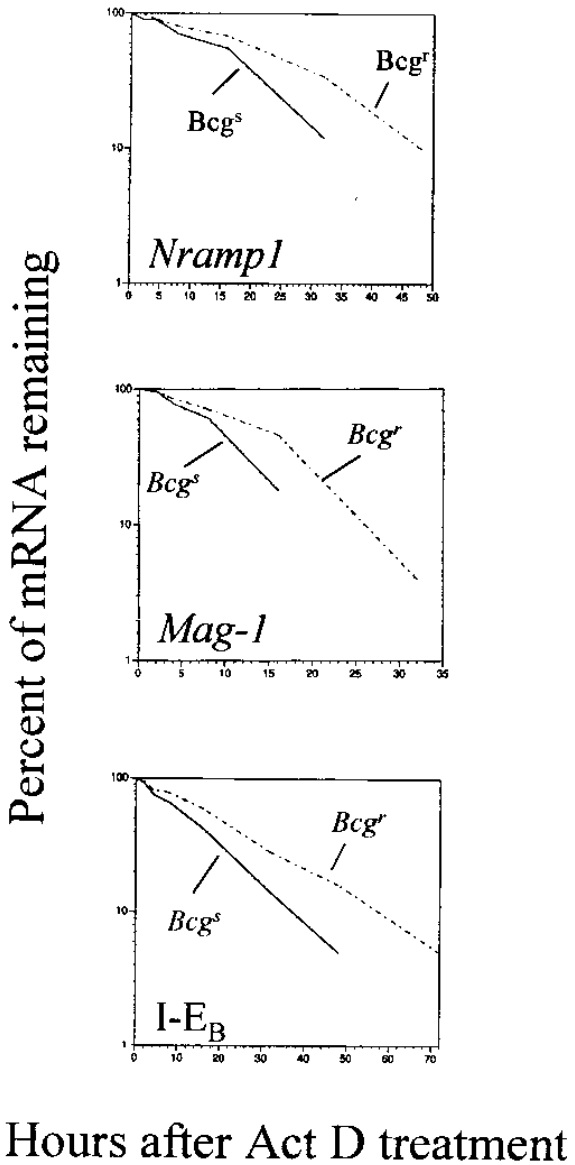

FIG. 6. Difference in *Nramp1*, Mag-1, and I-E_β mRNA half-lives between macrophages from BCG-resistant (*Bcg*^{r}) and BCG-susceptible (*Bcg*^{s}) mice. Splenic macrophages from BCG-resistant and BCG-susceptible mice were treated with rIFN-γ as described for Fig. 1. Actinomycin D (Act D; 10 μg/10^6 cells) was added to the macrophage cultures 12 h after the addition of rIFN-γ, and the resultant half-lives of *Nramp1*, Mag-1, and I-E_β mRNAs were determined by Northern and densitometric analyses.

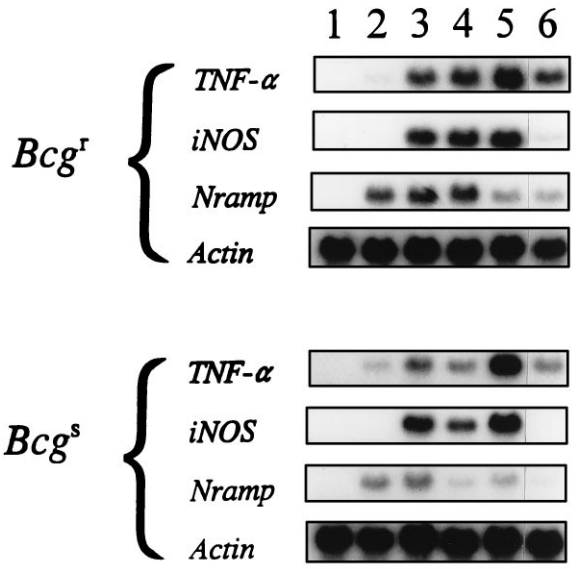

FIG. 7. Dependence of resistance to corticosterone-mediated suppression on prior induction of *Nramp1*. Splenic macrophages were stimulated with GM-CSF (250 U/5 × 10^6 cells) for 20 h to induce *Nramp1* expression (lanes 2, 3, and 4). These macrophage cultures were then treated with LPS (100 ng) for 8 h to induce TNF-α and iNOS mRNAs (lanes 3 and 4). Some cultures were then treated with corticosterone for 12 h (lane 4). Other macrophage cultures were stimulated with LPS only (lane 5) and then treated with corticosterone (lane 6). Unstimulated control cultures (lane 1) were maintained in medium throughout the treatment protocols. Total RNA was concurrently extracted from the various macrophage cultures, and the effect of prior induction of *Nramp1* expression on the stability of induced TNF-α and iNOS mRNAs was determined by Northern analysis with the corresponding cDNA probes. *Bcg*^{r}, BCG resistant; *Bcg*^{s}, BCG susceptible.

expressed by macrophages from BCG-susceptible mice decreased following the removal of rIFN-γ. However, macrophages from BCG-resistant mice continued to express MHC class II glycoproteins (39). This difference can be accounted for by the differences we have observed in the stability of the mRNA. Similar differences in the functional capacity of macrophages from BCG-resistant and BCG-susceptible mice may also be accounted for by differences in mRNA stability. Thus, differences in the levels of TNF-α or nitric oxide produced by the cells may be the result of differences in the stability of the respective mRNAs.

RNA stability has been the subject of considerable investigation. Stability of mRNA is determined by different elements along the message (14, 27). These mRNA sequence elements include the cap structure, 5′ untranslated secondary structures, premature termination codons, open reading frame sequences, 3′ untranslated region sequences, AU-rich regions, and the poly(A) tail. Degradation of mRNA requires translation, as well as deadenylation, of the poly(A) tail. Protection from degradation is the result of the binding to mRNA by proteins binding to mRNA sequence elements. This results in masking or activation of these elements that lead to deadenylation. Once deadenylation has occurred, the sequence elements of the mRNA may provide binding sites for nucleases that result in rapid degradation of the mRNA.

Glucocorticoids can stimulate or inhibit macrophage gene expression (2, 18, 38). Treatment of macrophages with glucocorticoids can result in the stimulation of migration inhibition factor production (8) or Fc receptor expression (32). In contrast, glucorticoids inhibit the production of other cytokines, such as interleukin 1 or TNF-α, as well as MHC class II glycoprotein expression (17, 19, 20, 23, 26, 40). The mechanism of glucocorticoid-mediated effects on gene expression has received much attention recently. Glucocorticoids can affect cell function following binding of the receptor to glucocorticoid response elements 5′ to the transcription start site and stimulate or suppress the initiation of transcription, depending on whether the activated receptor binds to positive or negative glucocorticoid response elements. Alternatively, glucocorticoid receptors can interfere with the binding of other transcriptional activators to their response elements. Both mechanisms result in transcriptional regulation of gene expression.

The results of our investigation indicate that glucocorticoid-mediated alteration of mRNA is not transcriptionally regulated. However, corticosterone did prevent the initial activa-

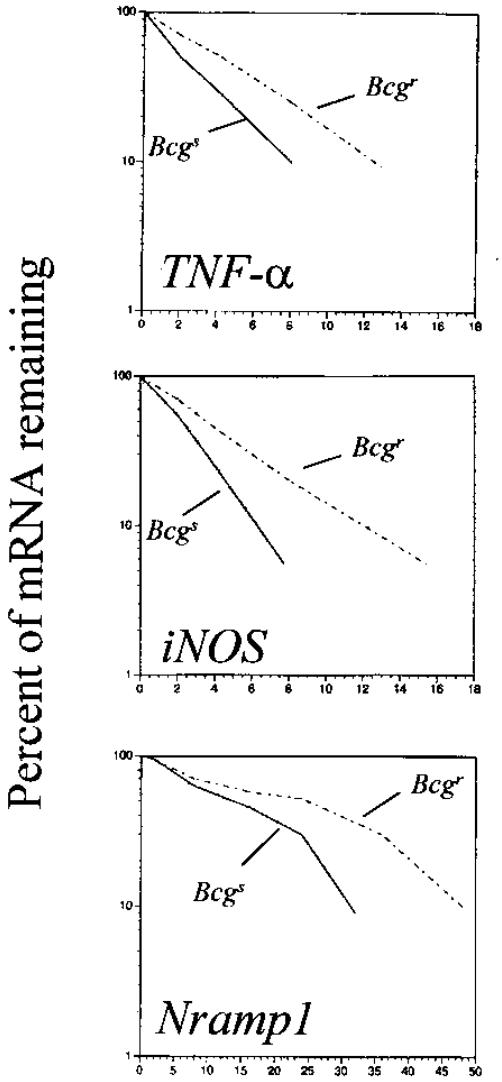

FIG. 8. Difference in TNF-α, iNOS, and *Nramp1* mRNA half-lives between macrophages from BCG-resistant (*Bcg*^{r}) and BCG-susceptible (*Bcg*^{s}) mice. Splenic macrophages from BCG-resistant and BCG-susceptible mice were treated with GM-CSF and LPS as described in the legend to Fig. 7. Actinomycin D (Act D; 10 μg/10^6 cells) was added to the macrophage cultures 8 h after the addition of LPS, and the resultant half-lives of TNF-α, iNOS, and *Nramp1* mRNAs were determined by Northern and densitometric analyses.

tion of transcription. Presumably, this occurred when the activated type II glucocorticoid receptor interfered with the binding of transcriptional initiating factors to their consensus sequences within the promoter region of *Nramp1* (2, 18, 38). The observation that the corticosterone-mediated effect does not result in inhibition of *Nramp1* mRNA transcription indicates that the loss of *Nramp1* mRNA is the result of its degradation. The exact mechanism of mRNA destabilization is not known. A recent report by Peppel et al. (28) has suggested that glucocorticoids may directly activate RNases that degrade mRNA-containing AU-rich sequences in the 3′ untranslated region. The increase in mRNA turnover was not prevented by cycloheximide, indicating that the mechanism of the glucocorticoid-mediated effect did not require new protein synthesis. In contrast, the effect mediated by corticosterone in our experiments was inhibited by the addition of cycloheximide (unpublished observations). Thus, it appears that glucocorticoids can mediate mRNA instability by at least two different mechanisms.

What is the relationship of *Nramp1* expression, mRNA stability, and disease resistance? We believe that glucocorticoid-mediated suppression serves to define a possible role of *Nramp1*. Corticosterone induced mRNA destabilization when *Nramp1* was not functional. Similarly, when functional *Nramp1* was not induced, TNF-α and iNOS mRNAs were not stable. The stability of mRNAs of several rIFN-γ-induced genes, as well as TNF-α and iNOS mRNAs, in macrophages from BCG-resistant mice was longer than that of the same mRNAs in macrophages from BCG-susceptible mice, even in the absence of corticosterone. Corticosterone resulted in an accelerated decay of the mRNA and exaggerated the difference between the macrophage populations.

Our results indicate that a functional *Nramp1* gene correlates with stability of the mRNAs of several rIFN-γ-induced genes. These experiments were done with macrophages from congenic BCG-resistant and BCG-susceptible mice. Since the two strains of mice differ by more than just the expression of *Nramp1* mRNA, similar experiments need to be done with macrophages from transgenic mice or transfected macrophage cell lines that carry the *Nramp1*$^{Gly\text{-}169}$ allele on a BCG-susceptible background. By using *Vil*-congenic mice, we have mapped these differences in mRNA stability to a 4.6-cM region on mouse chromosome 1 containing *Nramp1* (unpublished data). When the mRNA is stable, activated macrophages can produce antimycobacterial effector molecules for prolonged periods, accounting for increased resistance. A recent report by Barton et al. (1) has suggested that Nramp1 may mediate its effects via mitochondrial generation of oxygen radicals for intracellular signalling. Iron serves as a key element in these reactions and is an important cofactor in the stabilization of some mRNAs (9, 33, 35, 36). Does *Nramp1* control iron transport in a way that maintains mitochondrial iron but causes low levels of intracellular iron and stabilize mRNA? This possibility is reinforced by the observation that both the murine and human Nramp proteins show 53 to 58% sequence similarity to two yeast proteins, SMF1 (3) and SMF2 (4), which regulate protein transport across mitochondrial membranes and by the recent observation that the amino acid sequence of a yeast manganese transport protein is 30% identical to the corresponding sequence of the *Bcg* gene product Nramp1 (33).

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