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*J Immunol* 1999; 163:2688-2696; 
http://www.jimmunol.org/content/163/5/2688

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Attenuation of MHC Class II Expression in Macrophages Infected with Mycobacterium bovis Bacillus Calmette-Guerin Involves Class II Transactivator and Depends on the Nramp1 Gene

Wojciech Wojciechowski,* Juan DeSanctis, † Emil Skamene,* and Danuta Radzioch**

The natural resistance associated macrophage protein 1 (Nramp1) gene determines the ability of murine macrophages to control infection with a group of intracellular pathogens, including Salmonella typhimurium, Leishmania donovani, and Mycobacterium bovis bacillus Calmette-Guérin (BCG). The expression of the resistant allele of the Nramp1 gene in murine macrophages is associated with a more efficient expression of several macrophage activation-associated genes, including class II MHC loci. In this study, we investigated the molecular mechanisms involved in IFN-γ-induced MHC class II expression in three types of macrophages: those expressing a wild-type allele of the Nramp1 gene (B10R and 129/Mb), those carrying a susceptible form of the Nramp1 gene (B10S), and those derived from 129-Nramp1-knockout mice (129/Nramp1-KO). Previously, we published results showing that Ia protein expression is significantly higher in the IFN-γ-induced B10R macrophages, compared with its susceptible counterpart. In this paper, we also show that the higher expression of Ia protein in B10R cells is associated with higher I-Aβ mRNA expression, which correlates with a higher level of IFN-γ-induced phosphorylation of the STAT1-α protein and subsequently with elevated expression of class II transactivator (CIITA) mRNA, compared with B10S. Furthermore, we demonstrate that the infection of macrophages with M. bovis BCG results in a down-regulation of CIITA mRNA expression and, consequently, in the inhibition of Ia induction. Therefore, our data explain, at least in part, the molecular mechanism involved in the inhibition of I-Aβ gene expression in M. bovis BCG-infected macrophages activated with IFN-γ.

The evidence obtained from the analysis ofNramp1 homologues in yeasts SFM1 and SFM2 (from Saccharomyces cerevisiae) suggests that the Nramp1 protein may be involved in the transport of divalent cations, such as Mn2+, Zn2+, or Fe2+, across the membrane (12). The Nramp2 protein, the second member of the Nramp family in mammals, has been shown to transport iron in many different tissues (13). Overall, the above evidence, together with the experiments showing the intracellular localization of the Nramp1 protein and the late phagosomal/lysosomal membrane (14), and an involvement of Nramp1 protein in phagosomal acidification (15) suggest that Nramp1 controls and/or regulates the intraphagosomal environment.

The Nramp1 gene has been shown to be directly involved in the inhibition of Ia expression in response to infection with several unrelated pathogens, including Leishmania donovani, Salmonella typhimurium, and Mycobacterium bovis BCG. The inhibition of I-Aγ expression in the presence of M. bovis BCG is due to the inability of the host’s macrophages to control the growth of the microorganism in the early phase of infection (7, 17–20). The inefficient bactericidal activity of macrophages derived from mice susceptible to infection with M. bovis BCG seems to be associated with an inadequate magnitude of activation (21–28). It has been demonstrated that Nramp1, through a number of pleiotropic effects, influences the process of macrophage activation. These effects include the differential expression of IL-1β, TNF-α, and (IL-10-sensitive gene) KC, inducible NO synthase.

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Received for publication February 22, 1999. Accepted for publication June 16, 1999.

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1 This work was supported by Grant MT10707 and Grant MT 13059 from Medical Research Council of Canada. W.W. is supported by a Hans and Eugenia Jutting Fellowship, and D.R. is the recipient of a Fonds de la recherche en santé du Québec Senior Scholarship.

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3 Abbreviations used in this paper: Nramp1, natural resistance associated macrophage protein 1; CIITA, class II transactivator; BCG, bacillus Calmette-Guérin; PKC, protein kinase C; iNOS, inducible NO synthase; RNI, reactive nitrogen intermediates.
phages leads to the phosphorylation and activation of two tyrosine
compared with B10S macrophages.

It has been well documented that the expression of MHC class II
genes is essential for the development of an immune response
(34). The MHC class II molecule is encoded by two genes in the
mouse, denominated as I-A and I-E, which are located in two
subregions on murine chromosome 17. The glycoproteins encoded
by the I-A and I-E genes (called Ia surface proteins in mouse) are
able to bind and present foreign peptides to competent T cells.
Both I-A and I-E are expressed as heterodimers formed by the
noncovalent association of α- and β-chains on the surface of APC,
including macrophages, B cells, thymic epithelial cells, glial cells,
and dendritic cells (35). The expression of MHC II molecules can
be either constitutive or inducible, depending on the cellular type
(36). IFN-γ is a potent inducer of MHC class II expression in
macrophages. Although MHC II gene expression is under strict
and highly complex transcription regulation involving interaction
of several regulatory elements with specific transcription factors
(33, 36, 37), it seems that the entire process is controlled by a
single master regulator called class II transactivator (CIITA) (38–
41). CIITA is necessary for both constitutive and inducible ex-
pression of MHC II genes. It does not bind directly to DNA, but
rather interacts with transcription factors bound to the promoter
of the MHC genes perhaps via the N-terminal activation domain
(42–
44). CIITA also contains a GTP-binding domain, which is abso-
lutely essential for its function (45), a proline/serine/treonine-rich
region of unknown function, as well as a leucine-rich region and
two leucine charged domains most likely responsible for direct
interaction with nuclear factors (41, 42).

The induction of CIITA mRNA expression by IFN-γ requires the
presence of the functional STAT1α protein (46, 47). STAT1α
is part of a well-described IFN-γ signal transduction pathway.
The binding of IFN-γ to IFN-γ receptors on the surface of macro-
phages leads to the phosphorylation and activation of two tyrosine
kinases termed Jak1 and Jak2, which associate with the IFN-γRα
(IFNGR-1) and IFN-γRβ (IFNGR-2) subunits, respectively (48).
The two Jak kinases rapidly induce the tyrosine phosphorylation of
the α subunit of IFN-γ receptor providing docking site for
STAT1α (49). The cooperation of the two Jak kinases results in
the phosphorylation of STAT1α protein, which is required for STAT1α
release from the receptor, STAT1α/STAT1α homodimer forma-
tion, and translocation to the nucleus, where it can bind to DNA
and modulate gene expression (50).

The role of the Nramp1 protein in the regulation of the response
of macrophages to IFN-γ has not been elucidated yet. In this re-
port, we present evidence indicating that the higher level of the Ia
Ag, expressed by macrophages carrying the resistant allele of the
Nramp1 gene (B10R), compared with susceptible macrophages
(B10S), correlates with the higher level of CIITA expressed by
those macrophages. The difference in CIITA expression also cor-
relates with the superior capability of B10R macrophages to phos-
phorylate the STAT1α protein in response to IFN-γ stimulation,
compared with B10S macrophages.

Furthermore, this study also shows the effect of M. bovis BCG
infection on I-Aβ and CIITA mRNA expression and, consequently,
on Ia protein production in IFN-γ-stimulated macrophages.

Materials and Methods

Reagents

DMEM, penicillin/streptomycin, Dulbecco’s PBS (DPBS), and Trizol re-
agent were purchased from Life Technologies (Grand Island, NY). FBS, char-
acterized for low level of endotoxin, was obtained from HyClone (Lo-
gan, UT). Recombinant murine IFN-γ was purchased from Arogen (Thou-
sand Oaks, CA). Nonidet P-40 and was purchased from United States Biochemicals (Cleveland, OH). Sodium deoxycholate, sodium or-
thovanadate, sodium fluoride, Tween 80, BSA, aprotinin, leupeptin, p-ni-
 trophenyl guanidinobenzoate, and rabbit anti-mouse IgG polyclonal HRP-
 conjugated Ab were purchased from Sigma (St. Louis, MO). Anti-murine
p91 mouse mAb (C-111), anti-murine p84/p91 rabbit polyclonal Ab (M-
22), anti-phosphotyrosine mouse mAb (PY-20), protein A/G PLUS-Aga-
rose, and goat anti-human/murine CD64 (Fc receptor type I) Ab (N-19)
were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Ab
against murine Fc receptor type II and III (FcBlock), FITC anti mouse I-Aβ
(used to analyze the Ia protein expression of cells derived from 129/J
mice), and FITC anti mouse I-Aβ Ab (used to analyze the surface Ia ex-
pression of cells derived from B10A mice) were obtained from PharMin-
gen (Mississauga, ON, Canada). rp2pCDTP and ECL chemiluminescent
reagent were purchased from Amersham (Amersham, U.K.). Middelebrook
7H9 broth was purchased from Difico Laboratories (Detroit, MI). BBL Mid-
debrook OADC Enrichment was purchased from Becton Dickinson (Mis-
sissauga, ON, Canada).

Bacteria

M. bovis BCG strain Montreal was cultivated using constant rotation at
37°C for 2 wk in Middelebrook 7H9 broth supplemented with 10% Mid-
debloek OADC Enrichment and containing 0.05% Tween 80. After cul-
ture reached concentration of 0.6–1.0 OD600, the cells were collected and
briefly sonicated to break down bacterial clumps and filtered through a
5-μm syringe filter to eliminate remaining clumps. After estimation of cell
concentration, the culture was aliquoted and frozen in 15% glycerol
solution.

Cell lines

Macrophage cell lines were derived from the bone marrow of B10A.Bcg
and 129/J mice expressing the wild type of the Nramp1 gene (B10R cell
line and 129/M4 cell line, respectively), from B10A mice carrying a mu-
tated Nramp1 gene (point mutation at D169; B10S macrophage cell line),
and from 129/Nramp1 gene knockout mice with genetically disrupted
Nramp-1 (51) gene in the 129/J embryonic stem cells (129/Nramp1-KO
line), as previously described (52). Cell lines were cultured in DMEM
supplemented with 10% heat inactivated FBS and penicillin/streptomycin
antibiotic mixture. The subconfluent cell cultures were used for all of the experiments.

FACS analysis of cell surface Ia expression

Macrophage cell lines were plated at a concentration of 0.5–1 × 10^6/ml
and treated with IFN-γ and/or M. bovis BCG for 48 h. Cells were removed
from the flasks, washed in DPBS, and resuspended in DPBS containing 5%
BSA and 0.1% sodium azide. FcR were blocked for 15 min at 4°C using
Fc receptor type II/III (FcBlock; PharMingen) and anti-murine/human
CD64 Abs against FcR type I (N-19, Santa Cruz Biotechnology). Cells
were washed three times and incubated for 15 min at 4°C with strain-
specific anti I-Aβ (B10R and B10S) and I-Aβ* (129 M4b) Abs directly
labeled with FITC (PharMingen). After washing, cells were fixed in 1%
solution of paraformaldehyde in PBS for 30 min at room temperature.
Stained cells were analyzed using a dual laser FACStarplus flow cytomter
(Becton Dickinson). A green fluorescence histogram of 1000 channel res-
olution was collected from 10,000 cells counted for each sample analyzed.

DNA probes

The pGEM-A4 containing a 500-bp PstI fragment of the I-Aβ* gene was
kindly provided by Dr R. Germain (National Institute of Allergy and In-
fecous Diseases, National Institutes of Health, Bethesda, MD). The
GAPDH probe was generated by PCR amplification of a 456-bp cDNA
fragment using the following oligonucleotide primers: sense primer, 5'-
CCC TCT ATT GAC CTC AAC TAC ATG G-3'; antisense primer, 5'-
AGT CTT CGT GGT GGC AGT GAT GGG-3'. The PCR product was
subcloned in pBluescript KS+ and sequenced. The CIITA probe was ob-
tained by PCR amplification of the 341-bp fragment of macrophage cDNA
using the following oligonucleotide primers: sense primer, 5'-CTT CTT
GCT TCA CCT TCA GGA CTA GAA TCC CTG-3' and anti-sense primer, 5'-
ATT AAG GAC TCA GGG TCC CTG-3'. The products of PCR amplification were sub-
cloned into pGEM-TEasy vector (Promega, Madison, WI).

Northern blot analysis

To isolate total cellular RNA, ~10 million cells, treated with IFN-γ and/or
M. bovis BCG, were lysed using either guanidinium isothiocyanate solu-
tion or Trizol reagent, and RNA was isolated as previously described (53).
A total of 15–20 μg of total cellular RNA extracted from B10R and B10S

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mmunoprecipitation of STAT1α protein

Analysis of STAT1α phosphorylation was performed according to the immunoprecipitation protocol provided by Santa Cruz Biotechnology technical services. Briefly, IFN-γ-stimulated cells were lysed in RIPA buffer (PBS (pH 7.5) containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 25 mM sodium fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 100 μg/ml PMSF, and 0.025 mM p-nitrophenyl guanidino benzoxaate), mixed with rabbit polyclonal anti-p84/ p91 Abs and incubated overnight at 4°C. The immunocomplexes were precipitated for 2 h at 4°C using protein A/G agarose beads. After washing the agarose beads with RIPA buffer, STAT1 proteins were eluted from immunocomplexes by heating at 95°C for 5 min in SDS-PAGE sample buffer. Proteins were subjected to SDS-PAGE using 8 or 10% running gel, according to standard protocols, and transferred to the polyvinylidene difluoride membranes. Non-specific binding was blocked overnight at 4°C using 10% FBS in PBS containing 0.05% Tween 20. To assess the level of STAT1α phosphoprotein, the membranes were incubated in a solution of mouse mAbs against phosphorylase (a a 1:1000 dilution in blocking buffer) at room temperature for 1 h, followed by incubation with a solution of anti-mouse IgG HRP-conjugated Abs (1:10,000 dilution in blocking buffer) at 4°C. The signal was visualized using ECL reagent. Subsequently, the anti-phosphorylase Abs were removed from membranes by a 30-min incubation at 55°C in buffer containing 62.5 mM Tris-HCl (pH 6.7), 2% SDS, and 70 mM 2-ME, and the total amount of the immunoprecipitated STAT1α protein was assessed using mouse mAbs against STAT1α protein, following the same protocol as used for anti-phosphotyrosine Abs. The densities of the bands corresponding to phosphorylated STAT1α protein levels were determined and normalized against the total amount of immunoprecipitated STAT1 protein. The exposure times for loading controls used in normalization were always within the linear range of the film.

Results

Expression of I-A ₅ mRNA in macrophages infected with M. bovis BCG

We have previously shown that the induction of the I-A ₅ gene occurs 12–18 h post IFN-γ stimulation and then continues to rise for at least another 36 h (33). Therefore, in the experiments described below, we tested I-A ₅ mRNA expression 24 h after IFN-γ stimulation of macrophage cell lines derived from the bone marrow of B10.A.Bcg ᵦ (B10R) and from B10.A mice carrying the susceptibility associated mutation of Nramp1 gene (point mutation at D169; B10S). As shown in Fig. 1, following 24 h of treatment with 10 U/ml of IFN-γ, B10R macrophages expressed a high level of I-A ₅ mRNA, whereas B10S macrophages expressed I-A ₅ mRNA at a very low level. The results of the densitometric analysis of four independent experiments are presented in Fig. 1B. Infection with M. bovis BCG decreased the ability of macrophages to express I-A ₅ mRNA in response to IFN-γ by ~75% in B10R cells when a 10:1 bacteria to macrophage ratio was used (Fig. 1). I-A ₅ mRNA expression in IFN-γ-activated B10S macrophages infected with M. bovis BCG was also reduced, compared with uninfected IFN-γ-stimulated B10S macrophages; however, it is difficult to estimate precisely the level of reduction because the expression level was already very low.
Analysis of Ia protein expression

To establish whether the observed difference in the level of I-Aβ mRNA expression between macrophages expressing either the Nramp1* or the Nramp1* allele of the Nramp1 gene was similarly reflected at the level of MHC class II surface protein (Ia) expression, we performed FACS analysis using specific Ab against Ia molecules. As shown in Fig. 2, A and B, the stimulation of B10R macrophages with 10 U/ml IFN-γ resulted in much higher surface Ia protein (99.8%) expression, compared with B10S macrophages (15.6%).

Consistent with the results of Northern blot analysis showed in Fig. 1, the infection of macrophages with M. bovis BCG decreased the ability of both B10R and B10S macrophages to express surface Ia, as determined by FACS analysis. As shown in Fig. 2, A and B, infection of macrophages with M. bovis BCG suppressed the ability of IFN-γ-stimulated macrophages to express surface Ia protein. The expression of Ia in BCG-infected macrophages decreased from 99.8% to 75.8% in B10R and from 15.6% to 9.3% in B10S cells.

Differential tyrosine phosphorylation of STAT1α protein in resistant and susceptible macrophages

The activation of STAT1α protein requires its phosphorylation at a specific tyrosine residue. Only phosphorylated STAT1α is capable of forming homodimers that are subsequently translocated to the nucleus. Therefore, macrophages carrying the resistant allele of the Nramp1 gene (B10R and 129.Mf) and macrophages that do not express intact Nramp1 protein (B10S), as well as macrophages derived from Nramp1 knockout mice (129/Nramp1-KO), were examined for their ability to phosphorylate STAT1 protein in response to IFN-γ stimulation. Macrophages were stimulated with 10 U/ml of IFN-γ for 5, 10, and 30 min, lysed, and STAT1 protein was immunoprecipitated with anti-STAT1 Abs and subjected to Western blot analysis using anti-phosphotyrosine Ab.

As shown in Fig. 3A, significantly higher levels of the phosphorylated form of STAT1 were present in B10R, compared with B10S, macrophages. The densitometric analysis of the level of phosphorylated STAT1 compensated for total immunoprecipitated STAT1 protein were plotted. In Fig. 3B, the results show that the
level of the phosphorylated STAT1α protein induced by IFN-γ in B10R macrophages was at least 4-fold higher, compared with the B10S macrophages. Similar results were obtained using 129/Mφ macrophages (carrying Nramp1 r allele) and macrophages derived from Nramp1 gene knockout mice on the same genetic background. As shown in Fig. 4, A and B, 129/Mφ macrophages expressed 2.5–3 times more phosphorylated STAT1α, compared with the 129/Nramp1-KO macrophages.

Since our immunoprecipitation analyses shown in Figs. 3 and 4 seem to be more efficient for B10R macrophages than for 129/Mφ (both carrying the resistant allele of the Nramp1 gene), we decided to compare the level of I-Aβ mRNA and Ia surface protein expression in the two cell lines following activation with 10 U/ml of IFN-γ. We have found no significant differences between IFN-γ-activated B10R and 129/Mφ, either at the level of I-Aβ mRNA expression or at the level of surface Ia protein (Fig. 5). Therefore, we can conclude that the apparent difference at the level of STAT1α expression was most likely due to different efficiency of immunoprecipitation between the two sets of experiments illustrated in Figs. 3 and 4.

Effect of infection with M. bovis BCG on IFN-γ-induced CIITA mRNA expression

Recently, it has been shown that phosphorylated STAT1α is acting as a transactivating factor that is able to induce transcription of the CIITA gene (54). Since we have observed a significant difference in STAT1α phosphorylation between B10R and B10S macrophages, it was important to test whether the higher level of STAT1α phosphorylation observed in B10R cells correlated with higher level of CIITA mRNA. We analyzed the IFN-γ-induced CIITA mRNA expression in B10R and B10S macrophages. We found that the expression of CIITA mRNA was induced as early as 3 h post IFN-γ stimulation, reaching highest level at 12–18 h of stimulation both in B10R and B10S macrophages (data not shown). At each of the time points analyzed, the expression of CIITA was a few-fold higher in B10R than in B10S macrophages. However, the level of induction was 10-fold higher in B10R, compared with B10S, macrophages. Interestingly, infection of macrophages with M. bovis BCG suppressed the ability of macrophages to augment CIITA mRNA in response to IFN-γ stimulation. In the B10R macrophages infected with M. bovis BCG, the induction of CIITA mRNA with IFN-γ was diminished by 70%.

FIGURE 3. Tyrosine phosphorylation of STAT1 protein in B10R and B10S macrophages. A, B10R and B10S macrophages were stimulated with 10 U/ml of IFN-γ for 5, 15, or 30 min. STAT1 protein was then immunoprecipitated using anti-STAT1 Ab and subjected to the Western blot analysis using anti-phosphotyrosine Ab. The data shown are representative of three replicative experiments. B, The densities of the bands corresponding to phosphorylated STAT1α protein levels were determined and normalized against for the total amount of immunoprecipitated STAT1 protein.

FIGURE 4. Tyrosine phosphorylation of STAT1 protein in 129/Mφ and 129/Nramp1-KO macrophages. A, 129/Mφ and 129/Nramp1-KO macrophages were stimulated with 10 U/ml of IFN-γ for 5, 15, or 30 min. STAT1α protein was then immunoprecipitated using anti-STAT1α Ab and subjected to the Western blot analysis using anti-phosphotyrosine Ab. The data shown are representative of three replicative experiments. B, The densities of the bands corresponding to phosphorylated STAT1α protein levels were determined and normalized against for the total amount of immunoprecipitated STAT1α protein.
compared with IFN-γ-stimulated noninfected macrophages. The similar effect of *M. bovis* BCG infection on the induction of CIITA by IFN-γ was observed in B10S macrophages; however, since the level of CIITA mRNA induction by IFN-γ was a few-fold lower in B10S macrophages, compared with B10R macrophages, the level of CIITA in the BCG-infected B10S macrophages was barely detectable.

**Discussion**

In mice, resistance to the early growth of *M. bovis* BCG is controlled by the *Bcg* gene. The *Bcg* gene, now termed natural resistance associated macrophage protein 1 (*Nramp1*), was cloned in 1993 and shown to encode a phagocyte-specific membrane protein, which bears significant structural similarity to transporter proteins (51). Recently, it has been shown that the Nramp1 protein is localized in the late phagosomal/lysosomal membrane, and that it is involved in regulation of iron balance (14, 55, 56). It has been suggested that Nramp1 may also be involved in the transport of other metal ions, such as Mn²⁺, Zn²⁺, and Mg²⁺ (55, 57). Alternative alleles of the *Nramp1* gene (*Nramp1r* and *Nramp1s*) confer susceptibility to infection of mice with *M. bovis* BCG, *L. donovani*, *S. typhimurium*) are expressed in mice by mature tissue macrophages.

The *Bcg/Lsh/Ity* gene was shown to be associated with regulation of macrophage activation (9, 30, 58). This has been demonstrated by a wide range of pleiotropic effects, including regulation of KC chemokine, iNOS and RNI production, respiratory burst, and IL-1β and TNF-α levels (27, 29–32). RNI were shown to play a decisive role in the control of the replication of intracellular pathogens (21–28).

A proper analysis of the molecular basis of macrophage activation for effective bactericidal function requires access to homogeneous cell populations of defined genetic background. We have generated macrophage cell lines from mouse strains that carry either the *Nramp1r* or *Nramp1s* allele (52), and we have made a detailed comparison of these cell lines (27, 33, 59, 60). In addition, we have generated macrophage cell lines from the *Nramp1* gene knockout mice on the 129/J genetic background and from their 129/J littermate controls that carry the *Nramp1r* allele.

Recently, using our macrophage cell lines, we have found that PKC-specific activity was significantly more increased in the cytosolic fractions derived from *Nramp1r*, compared with *Nramp1s*. 
macrophages. Furthermore, during the course of macrophage activation, particulate fractions from Nramp1− macrophages contained significantly greater PKC activity, compared with Nramp1+. The differences in PKC activity between Nramp1− and Nramp1+ macrophages contributed to altered responsiveness to IFN-γ that resulted in more efficient production of RNI by Nramp1+ macrophages. Nramp1+ macrophages also had a superior ability to phosphorylate endogenous substrate compared with Nramp1− macrophages (60).

Previously, we found that macrophages carrying the susceptible allele of Nramp1 expressed much lower levels of MHC class II surface proteins and I-Aα mRNA when compared with macrophages with resistant allele (33). We also demonstrated a significantly reduced amount of produced nitrates, a decreased production of TNF-α, and a decrease in the level of MHC class II in response to IFN-γ stimulation in macrophages transfected with Nramp1 antisense ribozyme (Nramp1-Rb), compared with the controls transfected with mock vector (52). Overall, these studies supported the hypothesis that the Nramp1 gene is involved in the regulation of the early signaling that occurs in macrophages activated with IFN-γ.

In this paper, we have focused on the mechanism of IFN-γ-induced expression of MHC class II Ags by macrophages carrying either the resistant or susceptible allele of Nramp1 and macrophage cell lines derived from Nramp1-knockout mice. We also analyzed the effect of M. bovis BCG infection on the expression of MHC class II using these cell lines. We found that infection with M. bovis BCG leads to a very significant inhibition of IFN-γ-induced I-Aα mRNA and Ia protein expression in tested macrophage cell lines.

The observed inhibition of I-Aα gene expression by M. bovis BCG infection may represent a protective mechanism that allows the pathogen to persist longer in the host. This phenomenon does not seem to be unique for the infection of macrophages with M. bovis BCG. It was previously reported that the protozoan Toxoplasma gondii is able to inhibit MHC class II expression by infected macrophages. The mechanism of that inhibition is still unknown, but does not seem to be related to the increased production of prostaglandin E2, IL-10, TGF-β, or NO (61). The infection of macrophages with L. donovani also leads to the inhibition of IFN-γ-induced MHC class II gene expression (62–64). Similarly, it was shown that murine CMV (MCMV) was able to inhibit MHC class II transcription (65). Macrophages infected with MCMV were not able to express Ia in response to IFN-γ and, consequently, failed to present Ags and activate CD4+ T lymphocytes. The CMV virus most likely interferes with the expression of transcription factors involved in I-Aα gene expression. In addition, Wadee et al. (66, 67) showed that a 25-kDa glycoprotein encoded by M. tuberculosis was able to inhibit MHC class II expression in monocytes. IFN-γ has been shown to be a potent inducer of MHC II molecules in a variety of cell types. The analysis of IFN-γ-knockout mice showed that they were able to develop normally in the absence of pathogens, but their macrophages were unable to produce antimicrobial products and had reduced expression of MHC class II genes. Thus, IFN-γ-deficient animals were extremely sensitive to infection and died shortly after administration of the pathogen, i.e., M. bovis (68). Similarly, the lack of IFN-γ receptor expression in knockout mice led to an inability of the animals to control Mycobacterial as well as other types of infection. Macrophages derived from IFN-γ receptor-deficient mice produced much lower...
levels of TNF-α and NO, making them inefficient in killing bacteria (69). IFN-γ-induced signal transduction requires activation of specific receptor coupled to the JAK/STAT signal transduction system. This primary effect leads to induction of expression of a number of genes, including many transcription factors and regulatory proteins. One of them, CIITA, was characterized as a master regulator of MHC genes, and it was shown to be essential for both constitutive and inducible expression of MHC class II genes (43). Interestingly, it was shown that STAT1 phosphorylation is required for the expression of CIITA gene. The aberrations in constitutive or infection-induced levels of CIITA expression may lead to severe immunological disorders or chronic infections.

Our studies demonstrate that, after IFN-γ stimulation, CIITA expression is elevated in macrophages carrying either resistant or susceptible allele of Nramp1, but the level of expression of CIITA was higher in macrophages carrying the “r” allele of Nramp1. Since IFN-γ-inducible expression of CIITA is STAT1α-dependent, we decided to evaluate its phosphorylation in response to IFN-γ stimulation. Our results demonstrate that, also at this level, Nramp1 macrophages are superior to the Nramp1* macrophages and were able to express much higher levels of the tyrosine-phosphorylated form of STAT1α in response to IFN-γ stimulation. A higher level of phosphorylated STAT1α protein would lead to a higher level of STAT1α homodimer being translocated to the nucleus and bound to the GAS element of the CIITA promoter in Nramp1*, compared with Nramp1*, macrophages. Consequently, the lower level of CIITA gene expression in susceptible macrophages results in less efficient transcriptional activation of MHC class II gene expression. Our data clearly support this hypothesis and explain our previously published findings, showing a difference between Nramp1* and Nramp1* macrophages at the level of transcription activation of I-Aβ (33).

Overall, the presented results indicate that the differential MHC class II expression observed between Nramp1* and Nramp1* macrophages results from differences found at the level of transcription activation of the I-Aβ gene controlled by CIITA. Alternative explanations, such as translational control and 1a Ag stability, have also been proposed (73–76). Recent evidence suggests that one possible function of the Nramp1 protein is the transport of iron out of the bacteria-parasite-containing phagosome (14, 77). Therefore, it is possible that transport of iron by Nramp1 also has an impact on an iron homeostasis in macrophages. Iron and other divalent ions (e.g., Zn2+) were shown to regulate the activity of various transcription factors and other regulatory proteins involved in posttranscriptional regulation of gene expression. The exact link between the regulation of iron metabolism by the Nramp1 gene and transcriptional and posttranscriptional regulation of MHC class II expression remains to be established, and more studies are required to further characterize the molecular mechanism that regulates this important function of macrophages in immunity against bacterial infection.

Acknowledgments

We thank Dr. Ellen Buschman, Sergio DiMarco, and Jim Garnon for useful discussions and for critical reading of this manuscript.

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