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Translational Control of MHC Class II I-A Molecules by IFN-γ

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MHC class II molecules are expressed in a limited number of cell types, including B lymphocytes and macrophages (Mφ). IFN-γ increases the surface expression of class II molecules in a murine B cell line without inducing detectable changes in either I-A or I-A mRNA levels. In bone marrow-derived Mφ, IFN-γ causes an increase in class II expression at both the mRNA and surface levels. In addition to the increase in transcription rates described for Mφ, IFN-γ increases the rate of synthesis of Iα and Iαβ proteins and the ribosome loading for both mRNA molecules in both cell types. Interestingly, there is a significant peak of free I-A mRNA in noninduced cells. Therefore, IFN-γ regulates the expression of MHC class II molecules at the translational level in both B cells and Mφ and, as already reported, at the transcriptional level only in Mφ. The actual mechanism of regulation causes changes in the translation initiation rates in both cell types, as demonstrated by an increase in ribosome loading in polysome gradients. The Journal of Immunology, 1998, 161: 1837–1843.

MHC class II molecules play a key role in the development of specific immune responses against pathogens. Foreign Ags are phagocytosed and processed into small peptides that are subsequently loaded onto MHC class II complexes and exported to the cell surface. Only then are these Ags recognized by CD4 T cells (1). A lack of class II expression leads to severe immunodeficiency (2), whereas abnormal expression may cause autoimmune diseases (3). Consequently, the regulation of the expression of MHC II Ags is a critical point in the control and maintenance of the immune response.

Class II proteins are expressed on a limited set of cell types, which include B lymphocytes, thymic epithelial cells, glial cells, dendritic cells, and macrophages (Mφ). Activated human T cells also express class II molecules but do not express their murine counterparts (4). The expression of class II molecules is regulated by cytokines, mainly IFN-γ (5) and IL-4 (6), primarily through transcriptional activation (7). However, little work has been reported on the posttranscriptional events that take place under these circumstances.

mRNA stability does not play a significant role in IFN-γ-induced MHC II expression (Ref. 8; M. Cullell-Young, E. Goñalons, and A. Celada, manuscript in preparation). Therefore, in this report we have focused on the translational events that take place upon IFN-γ stimulation of both B cells and Mφ. Translational control has been described for a variety of systems (reviewed in Refs. 9 and 10). This control can be a general event consisting of the phosphorylation of the protein factors involved in the translational process (11) or can take place through highly specific mechanisms. The latter include events at the level of elongation (12, 13) or at the termination phase, such as the frame shifting described in the expression of many viral proteins, but also in some mammalian mRNAs (14). However, most translationally controlled mRNAs are regulated at the initiation step by multiple specific mechanisms (15).

In the present study, we demonstrate that IFN-γ is a translational activator of MHC class II Ag expression. In murine B lymphocytes, IFN-γ increases I-A surface expression, but the levels of Iα and Iαβ mRNAs remain unchanged. In Mφ, the observed increase takes place both at the mRNA and the surface expression levels. In both cell types, independently of the transcriptional effects, IFN-γ increases the binding of ribosomes to I-A mRNAs, thus increasing the translation rates of MHC class II molecules.

Materials and Methods

Cells

Bone marrow-derived Mφ (BMDMs) were obtained as described previously (16). Briefly, 6- to 8-wk-old C3H/HeJ mice (Charles River Laboratories, Wilmington, MA) were killed by cervical dislocation, and both femurs were dissected free of adherent tissue. The ends of the bones were cut off, and the marrow tissue was eluted by irrigation with culture media. The cells were desegregated by passing the suspension several times through an 18-gauge needle. The cells were then plated in 150-mm petri dishes in 40 ml of high-glucose DMEM containing 20% FCS (Sigma, St. Louis, MO) and 30% L cell-conditioned media as a source of Mφ CSF. The cells were cultured at 37°C in a humidified 5% CO2 atmosphere for 7 to 8 days until reaching confluence. The murine lymphoma A20 cell line was also used (17). A20 cells were maintained in high-glucose DMEM supplemented with 50 M M 2-ME and 5% FCS.

IFN-γ

For IFN-γ stimulation studies, saturating amounts (300 U/ml) of murine rIFN-γ (18) were added to the media for the indicated times. The cytokine was a kind gift from Genentech, Inc. (South San Francisco, CA).
Antibodies

For the surface staining of MHC class II molecules, we used Ab 11-5.2.1.9 (anti-I-\(\alpha\), PharMingen, San Diego, CA) for M\(\delta\) and 34-5-3 (anti-I-\(\beta\), PharMingen) for B cells. In both cases, FITC-labeled sheep anti-mouse IgG (Cappel, Turnhout, Belgium) was used as a secondary Ab. To block FcRs, we used anti-CD16/CD32 Ab (PharMingen). For the immune- 


oprecipitation experiments we used, rabbit antiserum FF282–4 as anti-I-\(\alpha\) (19) and 10-2.16 Ab (20) as anti-I-\(\beta\). The anti-I-\(\alpha\) antiserum was kindly provided by Dr. R. N. Germain (National Institutes of Health, Bethesda, MD), and the anti-I-\(\beta\) Ab was provided by Dr. P. Cosson (Bord Insitute for Immunology, Basel, Switzerland). These Abs were chosen for their ability to recognize I-\(\alpha\) \(\alpha\) and \(\beta\) subunits independently of their association (21). For the immunoprecipitation of \(\beta\)-actin we used mouse anti-mouse \(\beta\)-actin AC-15 Ab (Sigma).

Determination of cell surface expression of I-A molecules

Cell surface staining was conducted using specific Abs and cytofluorometric analysis as described previously (22). Cells were washed in PBS and resuspended in 100 \(\mu\)l of PBS containing 5% FCS. Next, they were incubated at 4°C with 1 \(\mu\)g/\(10^6\) cells of anti-CDI/CD32 mAb to block FcRs. After 15 min, the primary Ab was added, and the cells were further incubated for 45 min at 4°C. The cells were then washed by centrifugation through an FCS cushion. Finally, they were incubated with fluorescein-conjugated secondary Ab for 45 min at 4°C. Cytometry analysis was conducted using an Epics XL (Coulter, Miami, FL) apparatus or a FACSkan apparatus (Becton Dickinson, Bedford, MA).

RNA blot analysis

Northern blotting, slot blotting, and RNAS protection techniques were used for the analysis of polysome gradients. For Northern blotting, one-fifth of each fraction of the gradient or 30 \(\mu\)g of total RNA were electrophoretically separated in a 1.2% agarose/formaldehyde gel. The RNA was then transferred by capillarity onto a nylon membrane (GeneScreen, DuPont, Boston, MA). For slot blotting, one-fifth of each fraction of the gradient was applied to the membrane in 5\(\times\) SSC, 20 \(\mu\)M Tris-HCl (pH 7.5), and 18.5% formaldehyde using a vacuum manifold (Minifold II, Schleicher and Schuell, Dassel, Germany). Hybridization was conducted overnight in 50% formamide at 65°C for riboprobes and 42°C for cDNA probes (23).

RNase protection assay

A fragment of I-\(\beta\)\(\beta\) cDNA (21) ranging from position 1 to 230 of the open reading frame was used for the analysis of I-\(\alpha\)-\(\alpha\)B RNA. For I-\(\alpha\)-\(\alpha\), we used a fragment of I-\(\alpha\)-\(\alpha\) cDNA (21) covering positions 1 to 489 of the open reading frame. Both fragments were subcloned into the pGEM3 vector (Promega, Madison, WI). As a control, we used a PsrI (-1) +BgIII (173) fragment of mouse \(\beta\)-actin cDNA (24).

Labeled RNA that was complementary to the mRNA was generated in all cases from the SP6 promoter using SP6 polymerase. Probe synthesis, hybridization, digestion, and acrylamide gel electrophoresis of the protected probe was performed as described previously (25). Quantification of the bands was conducted using a Molecular Imaging System (Bio-Rad, Hercules, CA) and a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Polysome gradients

The cells were collected and washed in ice-cold PBS. The pellet was resuspended in 1 ml of lysis buffer (10 mM Tris-HCl (pH 8), 150 mM NaCl, 1.5 mM MgCl\(_2\), and 0.5% v/v Nonidet P-40) supplemented with 10 \(\mu\)l of RNase inhibitor (RNAguard, Pharmacia Biotech, Uppsala, Sweden). The cell lysate was centrifuged for 2 min at 3000 \(\times\) g at 4°C. The supernatant was then transferred to a new tube containing heparin to 0.6 \(\mu\)g/ml, cytochrome c to 0.15 \(\mu\)g/ml, DTT to 20 mM, and PMSF to 1 mM. Finally, the lysate was centrifuged again for 5 min at 4°C; loaded onto a 10 ml linear 15 to 40% sucrose gradient that had been prepared as described previously (26) in 10 mM Tris-HCl (pH 7.5), 140 mM NaCl, and 1.5 mM MgCl\(_2\); and centrifuged for 3 h at 28,000 rpm in a Beckman SW28.1 (Fullerton, CA). Fractions of ~550 \(\mu\)l were collected into tubes containing SDS to 1%, EDTA (pH 8) to 10 mM, and protease K to 200 \(\mu\)g/ml. The fractions were incubated for 30 min at 37°C followed by phenol/chloroform extraction and ethanol precipitation. The specific mRNA content of each fraction was analyzed by Northern blotting, slot blotting, or the RNase protection assay. The position of ribosomes was assessed by hybridization with a 28S ribosomal RNA (rRNA) probe. To ensure that the denser fractions contained polysome-bound mRNA, we prepared sucrose gradients in which the 1.5 mM MgCl\(_2\) had been substituted with 10 mM EDTA. In all cases, the mRNA accumulated in the top fractions (data not shown), which indicates that the mRNAs migrating to denser fractions are polysome-bound.

Results

IFN-\(\gamma\) is the strongest inducer of the expression of MHC class II molecules in cells of the M\(\delta\) lineage (27). Moreover, previous work has suggested that IFN-\(\gamma\) also induces the expression of these Ags in B lymphocytes (28). In our studies, we used the mature B cell line A20, which is devoid of any M\(\delta\) characteristics (17). A20 cells constitutively express MHC class II I-A molecules on the cell surface; after stimulation with IFN-\(\gamma\), the surface expression of MHC class II molecules increased two- to threefold over basal conditions (Fig. 1A). Interestingly, this increase in I-A surface expression was not accompanied by changes at the mRNA steady-state levels of either \(\alpha\) and \(\beta\) I-A chains, as determined by Northern blotting using either total or cytoplasmic RNA (Fig. 1B and data not shown). This finding suggests that the effect of IFN-\(\gamma\) on the surface levels of I-A molecules is not due to a different distribution of the messenger between the nucleus and the cytoplasm.

After the biosynthetic labeling of the cells with \(^{35}\)S) methionine and subsequent immunoprecipitation using anti-I-\(\alpha\)-specific Abs able to recognize I-A\(\alpha\) chains independent of the presence of I-\(\beta\)-\(\beta\) and/or invariant chains (Iis) (p31 and p41), a two- to threefold increase in the translation rate of I-A\(\alpha\) protein was detected (mean of three different experiments) (Fig. 2). The translation rates of I-\(\beta\)-\(\beta\) and Iis were not quantified, since it has not been demonstrated whether the Abs used were able to recognize these proteins independently of their association to I-\(\alpha\). As a control, we used an anti-\(\beta\)-actin Ab (Fig. 2).

The fact that mRNA levels remained unchanged after IFN-\(\gamma\) treatment despite inducing an increase in the rate of I-A\(\alpha\) protein synthesis suggested a translational control for this mRNA; several mechanisms may be responsible for this control. For most of the examples known, initiation is the step during which translation is controlled. To analyze translational control at the initiation step, polysome gradients were performed; the profiles of I-A\(\alpha\) and I-\(\beta\)-\(\beta\) mRNAs were analyzed both before and after IFN-\(\gamma\)-treatment. The fractioning of the cellular mRNAs in a sucrose gradient allows the separation of free mRNA from mRNA that is bound to one or more ribosomes. In noninduced B lymphocytes, I-\(\alpha\)-\(\beta\) mRNA was bound to several ribosomes, but I-A\(\alpha\) mRNA displayed quite a large peak of mRNA (60%) in the fractions devoid of ribosomes, indicating that this quantity of I-A\(\alpha\) mRNA was not being translated (Fig. 3, top). After 48 h of IFN-\(\gamma\)-treatment, there was a shift toward the
polysome-bound fractions for both mRNAs; this shift was particularly important in the case of I-A<sub>a</sub>, which showed a marked reduction (from 60% to 35%) in the unbound mRNA peak. These data indicated that IFN-γ induced an increase in the translation of MHC class II molecules in B lymphocytes, causing the dissociation between the levels of mRNA and the protein synthesis rate. As controls, the distribution of 28S rRNA and β-actin mRNA were used (Fig. 3).

Since B cells are a model in which MHC class II molecules are constitutively expressed, we analyzed translational regulation in Mφ, a cell type in which the expression of class II molecules is inducible. For this purpose we used BMDMs, which represent a homogeneous population of nontransformed, quiescent cells.

Using the RNase protection assay with haplotype-specific probes, we observed that Mφ expressed very low levels of both I-A<sub>a</sub> and I-A<sub>b</sub> mRNAs under basal conditions. Incubating BMDMs with saturating amounts of IFN-γ induced a slow increase in mRNA levels that started as late as 8 to 12 h after the treatment and reached a plateau after 48 h (Fig. 4). From then on, the mRNA levels stayed high and constant for at least 24 h. We observed a marked difference between the levels of expression achieved by I-A<sub>a</sub> and I-A<sub>b</sub> mRNAs. I-A<sub>a</sub> reached a maximum increase in the level of expression of 10 to 12-fold compared with that in untreated cells, whereas I-A<sub>b</sub> reached about twice that much (an increase of 25–30-fold). At the cell surface level, BMDMs showed increased I-A expression upon IFN-γ stimulation. This increase in mean fluorescence intensity reached 9- to 10-fold over a 48-h treatment period (Fig. 5) and slowly decreased to 5-fold after 96 h of IFN-γ treatment (data not shown).

We measured the rates of protein expression of I-A<sub>a</sub> and I-A<sub>b</sub> after 48 h of IFN-γ treatment in three independent experiments. For this purpose, [35S] biosynthetically labeled cells were lysed,
and the cytoplasmic extracts were immunoprecipitated using specific Abs against each of the two I-A chains. We found a 6- to 7-fold increase for I-A\(_{\alpha}\) and a 15- to 17-fold increase for I-A\(_{\beta}\) over basal levels. \(\beta\)-actin was used as a control and did not show any variation in its protein synthesis rate after 48 h of treatment with IFN-\(\gamma\) (Fig. 6).

Since IFN-\(\gamma\) is able to modify the translation of I-A mRNAs in B cells, we analyzed the effect of IFN-\(\gamma\) on the polysome gradient profiles of I-A\(_{\alpha}\) and I-A\(_{\beta}\) mRNAs in M\(_{\Phi}\). As seen in Figure 7,

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**FIGURE 3.** Shifts in the polysome gradient distribution profile of I-A\(_{\alpha}\) and I-A\(_{\beta}\) mRNAs in B lymphocytes after IFN-\(\gamma\) stimulation. Cells were treated with 300 U/ml of IFN-\(\gamma\) for 48 h or left untreated. They were subsequently harvested, and RNA was separated into fractions in a 15 to 40% sucrose gradient. The specific mRNA content of each fraction was assessed by Northern blotting. \(\beta\)-actin mRNA was used as a control. Hybridization with 28S rRNA was used to assess the position of one ribosome.

**FIGURE 4.** Kinetics of I-A\(_{\alpha}\) and I-A\(_{\beta}\) mRNA expression in BMDMs after IFN-\(\gamma\) stimulation. Cells were treated with 300 U/ml of IFN-\(\gamma\) for the indicated times. Due to the low expression of class II genes in M\(_{\Phi}\) under basal conditions, mRNA levels were determined by the RNase protection assay. The graph is representative of three independent experiments; both mRNAs showed the same level of basal expression in all experiments. The results are shown as the fold-increase of mRNA over basal levels. \(\beta\)-actin was used as an internal control of RNA load.

**FIGURE 5.** Expression of MHC class II molecules in M\(_{\Phi}\). BMDMs were incubated with 300 U/ml of IFN-\(\gamma\) for the indicated times. Next, the surface expression of MHC class II molecules was assessed as indicated in Materials and Methods.
both mRNAs showed a profile in noninduced cells in which ~30% of the messenger was in free form and the rest was evenly distributed along the ribosome-bound fraction range. However, after IFN-γ-stimulation, both mRNAs underwent a marked shift toward polysome-bound mRNA, indicating that the treatment induced an increase in the average number of ribosomes bound to these specific mRNAs and, consequently, an increase in the efficiency of protein synthesis. As a control, we used β-actin, which did not show any change in its distribution profile or in the mean number of ribosomes attached per molecule of β-actin mRNA. Finally, a general decrease in total protein synthesis per cell was detected after 72 h of IFN-γ stimulation of the cells; this decrease is concomitant with a shift of β-actin mRNA toward the free mRNA fractions. However, this general decrease of protein synthesis did not affect I-α mRNA, which remained accumulated in the denser fractions, confirming the presence of a specific mechanism responsible for the effect of IFN-γ (Fig. 8).

Discussion

The data presented here demonstrate that IFN-γ regulates the expression of MHC class II molecules at the level of translation in both B cell lines and primary Mφ. An increase in the surface expression of MHC class II molecules after IFN-γ stimulation has been shown for Mφ (27) as well as for B lymphocytes (28). The mechanism underlying this effect has been described exclusively as an increased mRNA expression. According to our results, this is not the case for either Mφ or B lymphocytes. Under our experimental conditions, we have found increased I-A surface expression in B cells treated with IFN-γ but no changes in the levels of I-α and I-β mRNAs. Furthermore, we have found that IFN-γ does not alter the t1/2 of I-A mRNAs; since the amount of mRNA is the same, we conclude that this cytokine does not increase the level of transcription of I-A mRNA. Therefore, there must be some post-transcriptional event that can account for the increased MHC class II surface expression. Another possibility is that IFN-γ increases the transit of mRNA from the nucleus to the cytoplasm, thus increasing the amount of mRNA available for translation; however, we have found that IFN-γ does not significantly modify the amount of cytoplasmic mRNA either. Finally, our data also showed that the protein synthesis rate in B cells was increased by the IFN-γ treatment. Consistently, the polysome gradient analyses that were performed showed that IFN-γ induces both a shift of free I-α mRNA toward polysome-bound fractions and an increase in the density of I-β mRNA in B lymphocytes, indicating that there is an increase in translational efficiency. Our study is not a unique case in which translation is the only regulatory step, as the expression of other genes such as ferritin (29) or insulin-like growth factor-II (30) is regulated only at this level. The analysis of the polysome distribution profile of I-α and I-β mRNA in B cells shows that I-α is mainly accumulated in the polysome-bound fractions, whereas I-β shows a large peak of free mRNA. Therefore, I-β is being actively translated, while the translation of I-α is repressed. These data might seem to be in disagreement with the fact that B cells show a constitutive expression of MHC class II molecules. However, despite a peak of free mRNA, 40% of I-α mRNA is still in the polysome-bound fractions which, when added to the long t1/2 of MHC class II molecules on the cell surface (8), would account for the maintenance of surface protein turnover.

It has been well-established that MHC class II gene expression is induced in Mφ (27). Before IFN-γ treatment, we found low levels of I-A mRNAs as well as I-A protein synthesis in these cells. This observation is in agreement with previous models in which residual amounts of class II expression were detected despite a lack of response to IFN-γ, such as the targeted gene inactivation of class II transactivator (31) or STAT-1 genes (32). In Mφ, IFN-γ not only increases the levels of class II mRNAs but also increases their translational rates; this finding is in contrast to that seen with B cells, in which this cytokine only acts at the translational step. Also, the polysome gradients showed a marked shift of both I-α and I-β mRNA from free toward polysome-bound mRNA in IFN-γ-treated Mφ, indicating that IFN-γ also has effect on the translational process in this case. In addition, IFNs have been shown to induce a general decrease in protein synthesis through direct or indirect mechanisms (33). Accordingly, β-actin shows a partial shift toward free mRNA at the 72-h timepoint, indicating a generalized inhibition of protein synthesis. However, I-α mRNA stays bound to ribosomes, thus confirming the specificity of translational induction by IFN-γ. Reviewing our model, we must point out that existing data (34) indicate the presence of a translational regulatory mechanism in Mφ. Also, Sicher et al. (35) have shown that LPS, acting as a second signal after IFN-γ pretreatment, induces an increase in the surface cell expression of MHC class II molecules without changing the mRNA levels. Finally, our data with regard to B cells demonstrate that the binding of mRNAs to ribosomes is not a process that is controlled by the amount of mRNA present in the cell; rather, this process is completely independent and is regulated by different mechanisms. In accordance with this hypothesis, the results presented show that I-α and I-β mRNAs have different polysome distribution profiles in B lymphocytes, despite showing similar levels of expression.
The data in this work is in agreement with other studies that have shown the relevance of translational regulation in the control of gene expression in a variety of systems. For the past few years, growing evidence has arisen regarding the role of translational processes as a key step in the regulation of gene expression, and every day new genes appear to be regulated at this level by mechanisms that are just as complex as those involved in transcriptional regulation (36). Translational machinery is controlled by several mechanisms that regulate the various steps of the process. However, most mRNAs have their expression modulated at the initiation phase by increasing the binding of new ribosomes to the 5′ end of the mRNA (15). Since treating the cells with IFN-γ induces a change in the population of free mRNA, inducing it to bind to ribosomes, we can conclude that initiation is the step during which this cytokine exerts its effects in this case.

In conclusion, IFN-γ has a regulatory effect on the translation of I-Aα molecules in both Mφ and B cells; in the latter case, IFN-γ is the only regulatory mechanism. These data provide evidence of a new step in the regulation of the expression of MHC class II molecules by IFN-γ.

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