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The Expression of MHC Class II Genes in Macrophages Is Cell Cycle Dependent

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Using different drugs, we stopped the cell cycle of bone marrow-derived macrophages at different points. After IFN-γ stimulation, macrophages arrested at the G1 phase of the cell cycle did not increase cell surface expression of the MHC class II IA. This inhibition is specific, because, under the same conditions, IFN-γ induces the expression of Fcγ receptors and the inducible NO synthase mRNA. Treatments that inhibit macrophage proliferation by blocking the cell cycle at the G1 phase, such as adenosine, forskolin, or LPS, blocked the IFN-γ induction of IA. Under IFN-γ treatment, the steady-state levels of IAα and IAβ mRNA did not increase in cells arrested at the G1 phase and the half-life of the MHC mRNA was not modified. These data suggest that the cell cycle modulation of IFN-γ-induced MHC II gene expression occurs at the transcriptional level. The expression of the class II transactivator mRNA induced by IFN-γ was also blocked when macrophages were arrested at the G1 phase of the cell cycle, suggesting that the lack of IFN-γ response occurs at the early steps of MHC class II expression. Finally, macrophages arrested at the G1 phase showed increased basal levels of cell surface IA due to an increase of the translational efficiency. These data show that the expression of MHC class II genes is regulated by the cell cycle. The Journal of Immunology, 2000, 165: 6364–6371.

The expression of several genes correlated with the proliferative state and the cell cycle progression of the cells. This is a broad group of genes known as the cell cycle-dependent (CCD) genes (1), as they are not expressed, or are expressed at a very low level, in quiescent nonproliferating cells, and their expression is markedly increased in growing cells or in cells stimulated to proliferate. These genes could be grouped in several categories, such as histones and DNA-synthesizing machinery (histones, thymidine kinase, thymidylate synthase), growth factors and receptors (IL-2, TGFβ, transferrin receptor), protooncogenes (c-myc, c-fos, p53, N-ras), and other metabolically active and structural proteins (calmodulin, hsp70, IFN-γ) (reviewed in Ref. 1).

To ensure proper progression through the eukaryotic cell cycle, the proteins involved in its regulation must be periodically expressed at appropriate times (2–4). These proteins, in turn, regulate other genes whose products mediate mechanical aspects of the cycle, such as DNA replication. Nevertheless, the regulators of cell cycle progression, such as cyclins and cyclin-dependent kinases, apparently affect both basal and activated transcription of genes not directly involved in cell cycle regulation or cell proliferation (5, 6). Recently, it has been observed that the cell cycle elements could also affect the translational apparatus and regulate in a CCD way the translation of several genes (7, 8).

Therefore, there are genes induced in a CCD fashion that are not involved in cell cycle progression. This is the case of genes that control some cellular activities. For example, it has been described that the sensitivity to apoptotic stimuli observed in some cell types is dependent on the proliferative state of the cells and the progression through the cell cycle (9, 10). Moreover, it has been observed that the phagocytic capacity of macrophages is up-regulated in the S phase of the cell cycle (11) and that, during the G2-M phase, the glucocorticoid receptor is not functionally active in the nucleus (12, 13). None of these functions are directly related to cell cycle regulation, but they are regulated in a cell cycle manner.

MHC II molecules are heterodimers that are expressed on the surface of a limited number of cells and are required to present Ags to T cells. A lack of class II expression leads to severe immunodeficiency (14), whereas an abnormal expression may cause autoimmune diseases (15). Thus, regulation of the expression of MHC II molecules is a critical point in the control and maintenance of the immune response. The expression of MHC class II molecules in macrophages is induced by IFN-γ (16).

Most cells of the immune system are quiescent, and their activation induces a massive proliferation. However, macrophages that are able to proliferate in tissues, thanks to the autocrine production of M-CSF (17, 18), when activated by stimuli such as IFN-γ or LPS, block their proliferation and perform their functional activities (19). Due to the duality between proliferation and activation in macrophages, we were interested in studying how the proliferative state and the cell cycle position of macrophages at the time of activation by IFN-γ could affect their functional activity. Recently, we have found that macrophages may be affected by apoptotic stimuli only in some of the cell cycle positions (19).

In these studies, we have used bone marrow-derived macrophages, because they are nontransformed cells that respond to both proliferative and activating stimuli. We have observed that IFN-γ induces the expression of MHC class II molecules in macrophages in a CCD manner. Despite having a higher basal expression of
class II molecules, macrophages arrested at the G1 phase of the cell cycle do not increase class II expression after stimulation with IFN-γ. This effect is not observed in macrophages arrested at other phases of the cell cycle and is specific for MHC class II expression. Moreover, the effect of G1-arrested macrophages on the IFN-γ-induced MHC expression is mainly found at the transcriptional level, whereas the increase of the basal expression is due to an effect at the translational level.

Materials and Methods

Reagents

Adenosine, 5′-N-ethyl-carboxamido-adenosine, mimosine, OH-urea, no-codazole, and LPS were obtained from Sigma (St. Louis, MO). Forskolin was obtained from Fluka Biochemika (Buchs, Switzerland). [3H]Thymidine was obtained from Amersham International (Buckinghamshire, U.K.). 4,6-di-amidino-2-phenylindole (DAPI) was purchased from Calbiochem (La Jolla, CA). All of the other products were of the best grade available and were purchased from Sigma. Deionized water further purified with a Millipore Milli-Q System (Bedford, MA) was used. IFN-γ was a kind gift from Genentech (South San Francisco, CA).

Cell culture

Bone marrow-derived macrophages were isolated from 6-wk-old BALB/c mice (Charles River Breeding Laboratories, Wilmington, MA) as previously described (19, 20). The cells were cultured in plastic dishes (150 mm) in 40 ml DMEM containing 20% FBS and 3% L cell-conditioned media as a source of M-CSF. The cells were incubated at 37°C in a humidified 5% CO2 atmosphere. After 7 days of culture, a homogeneous population of adherent macrophages was obtained.

Antibodies

Surface expression of the MHC class II molecules (IAa) was analyzed by using purified mouse mAb anti-mouse IAa (PharMingen, San Diego, CA) as previously described (21). Fluorescein-conjugated rat anti-mouse IgG Ab from Cappel (Turnhout, Belgium) was used as a secondary Ab. To block Fc receptors, we used an anti-CD16/CD32 Ab (PharMingen). The same Ab was used as primary Ab for the surface analysis of Fcγ receptor, and a fluorescein-conjugated anti-rat IgG (Cappel) as a secondary Ab.

Plasmids and constructs

The cDNA probes for IAα and IAβ used for Northern and slot blotting were a kind gift from P. Cosson (Basel Institute für Immunobiologie, Basel, Switzerland) (22). A rat-inducible NO synthase (iNOS) cDNA fragment (23) was used for the detection of IFN-γ-induced iNOS expression. The class II transactivator (CIITA) probe was made by PCR with the following primers: 5′-GACCTGGATCTGTCCTGCTG and 3′-CTTCAGCTTAGGAGGGACTTG, as indicated by Richard Flavell (Yale University, New Haven, CT). A total of 10^6 cells previously subjected to a specific treatment was resuspended and fixed in ice-cold 70% ethanol (19, 26). The cells were then analyzed with the Multicycle program (Phoenix Flow Systems, San Diego, CA).

Northern blot analysis

Total cellular RNA (20 μg) was extracted and separated as previously described (27) in 1% agarose gel with 5 mM MOPS (pH 7.0) 1 M formamide, and LPS were treated with 300 U/ml during a pulse of 2 h, and class II surface expression was measured after 24 or 48 h. A total of 10^6 cells was harvested and washed in cold PBS. After fixation with 2% paraformaldehyde during 30 min at 4°C, the cells were resuspended in 50 μl PBS containing 5% FBS. In the case of class II MHC expression, they were then incubated at 4°C for 15 min with 1/10 10^6 cells of anti-CD16/CD32 mAb to block Fc receptors. Then the cells were incubated for 1 h at room temperature with mouse mAb anti-mouse IAα and β (1/10 10^6 cells) or directly incubated at 4°C for 60 min with 1/10 10^6 cells of anti-CD16/CD32 mAb to analyze the Fcγ receptor expression. The cells were then washed by centrifugation through a FBS cushion. Finally, they were incubated with FITC-conjugated secondary Ab for 1 h at 4°C. Stained cell suspensions were analyzed using an Epics XL flow cytometer (Coulter). FITC excitation was obtained using a 488-nm argon laser lamp, and its fluorescence was collected with a 525-nm band-pass filter. The parameters used to select cell populations for analysis were forward and side light scatter. A nonrelated Ab was used as a control for nonspecificity.

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 MgCl2, and 0.5% (v/v) Nonidet P-40) supplemented with 10 μl of RNase inhibitor (RNAguard; Pharmacia Biotech). The cell lysate was analyzed the expression of 18S ribosomal RNA. All probes were labeled with [32P]dCTP (ICN Pharmaceuticals, Costa Mesa, CA) using the oligolabeling kit method (Pharmacia Biotech, Uppsala, Sweden). After incubating the membranes for 18 h at 65°C in hybridization solution (20% formamide, 5× Denhardt’s, 5× SSC, 10 mM EDTA, 1% SDS, 25 mM Na2HPO4, 25 mM NaH2PO4, 0.2 mg/ml salmon sperm DNA, and 105 cpm/ml of 32P-labeled probe), they were exposed to Kodak X-AR films (Kodak, Rochester, NY). The bands of interest were quantified with a Molecular Imaging System (Bio-Rad, Richmond, CA).

Surface expression of the MHC class II molecules (IAa) was analyzed by using purified mouse mAb anti-mouse IAa (PharMingen, San Diego, CA) as previously described (21). Fluorescein-conjugated rat anti-mouse IgG Ab from Cappel (Turnhout, Belgium) was used as a secondary Ab. To block Fc receptors, we used an anti-CD16/CD32 Ab (PharMingen). The same Ab was used as primary Ab for the surface analysis of Fcγ receptor, and a fluorescein-conjugated anti-rat IgG (Cappel) as a secondary Ab.

Proliferation assay

Cell proliferation was measured by using a previously described procedure (18, 26) with minor modifications. To analyze the effect of LPS and adenosine on macrophage proliferation, the cells were deprived of M-CSF for 18 h and then 10^5 cells were incubated for 24 h in 24-well plates (Costar, Cambridge, MA) in 1 ml of complete medium in the presence or absence of 100 ng/ml LPS, 10^-3 M forskolin, or 5 × 10^-3 M adenosine. Then the medium was removed and replaced by 0.5 ml of medium containing 1H]Thymidine (1 μCi/ml). After 2 additional h of incubation at 37°C, the medium was removed and the cells were fixed in ice-cold 70% methanol. After three washes in ice-cold 10% TCA, the cells were solubilized in 1% SDS and 0.3% NaOH. Radioactivity was counted by liquid scintillation using a 1500 Tri-Carb Packard scintillation counter (Packard, Meriden, CT). Each point was performed in triplicate, and the results were expressed as the means ± SD.

Analysis of DNA content with DAPI

A total of 10^6 cells previously subjected to a specific treatment was resuspended and fixed in ice-cold 70% ethanol (19, 26). The cells were then washed in PBS, resuspended in 0.2 ml of a solution containing 150 mM NaCl, 80 mM HCl, and 0.1% Triton X-100, and incubated at 4°C for 10 min. Afterward, 1 ml of a solution containing 180 mM Na2HPO4, 90 mM citric acid, and 2 μg/ml DAPI (pH 7.4) was added to each sample. After incubating the cells at 4°C for 24 h, their fluorescence was measured with an Epics Elite flow cytometer (Coulter, Hialeah, FL). For this analysis, we used an UV laser with an excitation beam of 25 mW at 333–364 nm, and fluorescence was collected with a 525-nm band-pass filter. Cell doubles were gated out by comparing the pulse area vs the pulse width. For each histogram, 12,000 cells were counted, and cell cycle distributions were analyzed with the Multicycle program (Phoenix Flow Systems, San Diego, CA).

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Results
In these studies, we have used bone marrow-derived macrophages because they are primary nontransformed cultures with an unmodified cell cycle machinery that respond to macrophage activators (IFN-γ, LPS) or proliferate with several growth factors (M-CSF, GM-CSF, IL-3) (16, 18). Macrophages express low levels of MHC class II molecules under basal conditions, and they must be activated to induce the expression of MHC class II Ags (16). IFN-γ is the major macrophage activator that induces the expression of MHC class II molecules with a maximal induction 24–48 h after stimulation. Moreover, IFN-γ also inhibits macrophage proliferation, blocking the cell cycle at the G1-S boundary (19, 26).

To study the effects of the cell cycle on the induction by IFN-γ of the expression of MHC class II molecules, we first synchronized macrophages in a reversible way in each of the cell cycle phases. To this purpose, we used cell cycle inhibitors described elsewhere (19). The treatment of macrophages growing in the presence of M-CSF for 16 h with 100 ng/ml mimosine, 100 ng/ml OH-ura, or 1 µg/ml nocodazole-arrested macrophages at the G1, S, or G2-M phases, respectively (19). The concentrations of inhibitors used did not affect cell viability, as measured by flow cytometry and trypan blue exclusion. Mimosine is a plant amino acid that inhibits the initiation of replication and blocks cell cycle in the middle-late G1 phase (29, 30), whereas OH-ura inhibits DNA replication by interfering with DNA polymerases (31) and stops cell cycle at the beginning of the S phase after crossing the restriction point (32). Nocodazole inhibits the cell cycle of macrophages at the G2-M phase by blocking the polymerization of tubulin needed for the microspindle formation (33, 34).

Macrophages were arrested at each phase of the cell cycle by treatment with the specific inhibitors for 16 h. Then the cells were activated with 300 U/ml IFN-γ for 2 h. After that, the cells were washed to remove any remaining inhibitors and IFN-γ and cultured again in fresh complete media for another 24 or 48 h, after which the expression of MHC class II molecules was determined. The incubation with saturating amounts of IFN-γ for 2 h resulted, 24 h later, in a maximal expression of MHC class II molecules on cell surface that was similar to the level of expression obtained with a continuous stimulation with IFN-γ (35). According to this protocol, the macrophages were first incubated in the presence of the cell cycle inhibitors alone to arrest all of the cell populations at the same cell cycle stage. Once the blockage had been established, the pulse of IFN-γ was administered. Finally, both the inhibitor and IFN-γ were removed and the incubation was resumed in fresh medium, allowing the cells to fully express the effect of the IFN-γ pulse on the expression of MHC class II molecules. The surface expression of MHC Ags was analyzed by flow cytometry using purified mAbs against IA<sub>a</sub> molecules. In these conditions, macrophages arrested in the G1 phase did not respond to IFN-γ and the expression of MHC molecules on the cell surface was not induced (Fig. 1, A and B). This correlated with an inhibition of the MHC mRNA expression induced by IFN-γ (Fig. 1C). No significant differences in MHC class II-induced expression were observed in cells arrested at other phases of the cell cycle.

To determine whether the effects of cell cycle on MHC class II gene expression were a general feature affecting many different genes, we measured the IFN-γ-induced expression of Fcγ receptor under the same conditions. Macrophages arrested at different points of the cell cycle did not show differences in the IFN-γ-induced expression of Fcγ receptor (Fig. 2).

Besides, we did not detect differences between the IFN-γ-induced expression of iNOS mRNA in macrophages arrested at the G1 phase with mimosine and in control macrophages (Fig. 3).

Therefore, the lack of IFN-γ induction of the MHC class II Ags in the G1 phase of the cell cycle was specific and not a general inhibition of IFN-γ-induced macrophage activation. These results also suggested that the effect observed on MHC class II genes was not due to a reduction in the expression of IFN-γ receptors on the surface of macrophages during the G1 phase of the cell cycle or to a blockage of the signal transduction mechanism. Moreover, the effect observed in G1-arrested cells could not be related to any toxic effects of mimosine, because it did not affect the surface cell cycle.

![FIGURE 1](http://www.jimmunol.org/)
expression of Fcγ receptors or iNOS mRNA expression. Therefore, we concluded that the cell cycle position could specifically modulate MHC class II expression on macrophages and its induction by IFN-γ.

Our results so far indicated that cell cycle arrest at the G1 phase inhibited IFN-γ-induced expression of MHC class II genes. Therefore, those treatments that inhibit macrophage proliferation by blocking the cell cycle at the G1 phase might be inhibiting MHC class II expression induced by IFN-γ in these cells. In fact, it has been suggested that the agents that increase cAMP levels could be inhibitors of macrophage activities (21, 36). It has also been reported that LPS inhibits, in some cases, certain aspects of IFN-γ-induced macrophage activation, whereas, in other cases, it increases activation (16, 37–39). Both cAMP-increasing agents and LPS inhibit M-CSF-dependent proliferation of macrophages. The treatment of macrophages with adenosine, forskolin (Fig. 4A), or LPS (Fig. 4B) inhibited the [3H]thymidine incorporation induced by M-CSF completely.

The analysis of DNA content using flow cytometry of DAPI-stained cells showed that bone marrow macrophage cultures growing in the presence of M-CSF were not cell cycle-synchronized and showed a random distribution, with 51% of cells in G0-G1, 30% in S, and 17% in G2-M (Fig. 4C). In response to adenosine, macrophages appeared to be distributed homogeneously (88% of total cells) in a peak corresponding to the G1 phase of the cell cycle. The treatment of macrophages with 100 ng/ml LPS also induced a G1 arrest of >75% of viable cells, but, in contrast with adenosine, LPS induced the appearance of a subdiploid peak that accounts for 35% of the culture and corresponds to cell death by apoptosis.

As shown by Northern blotting (Fig. 4, D and E), the treatment of macrophages with cAMP-increasing agents or LPS also inhibited the expression of MHC class II genes induced by IFN-γ. Therefore, both cAMP-increasing agents and LPS inhibited macrophage proliferation by blocking the cell cycle at the G1 phase, and all three inhibited the IFN-γ-induced expression of MHC class II molecules.

It has been described that the cell cycle modulates the expression of several genes not only at the transcriptional level but also through posttranscriptional mechanisms (6–8). To determine whether the modifications observed in the CCD regulation of MHC expression are due to transcriptional changes or to the rate of mRNA degradation, we analyzed the half-life of MHC II transcripts. The rate of degradation was measured after 24 h of IFN-γ treatment in cells arrested with mimosine or in control cells. Actinomycin D was added to a concentration (5 μg/ml) sufficient to block all further RNA synthesis, as determined by [3H]UTP incorporation (16, 40), and the RNA was isolated at intervals of 2 h thereafter. IAb mRNA was very stable both under the treatment with mimosine and in control macrophages (Fig. 5), thus suggesting that the lack of IFN-γ induction of mRNA levels in G1-arrested cells was due to a lack of transcriptional activation.

Recently, we have shown that IFN-γ regulates the expression of MHC class II molecules in bone marrow macrophages not only at the transcriptional level (16) but also at the translational level (28). Therefore, we also analyzed whether the blockage effect in IFN-γ-induced MHC class II expression observed in G1-arrested cells could be caused by the translational machinery. As we have reported elsewhere, initiation is the step where translation of IAα...
and Iαβ is controlled (28). Thus, polysome gradients were obtained and the profiles of Iαα and Iαβ mRNAs were analyzed both before and after IFN-γ treatment in cells arrested or not at G1 with mimosine. Fractionation of the cellular mRNAs in a sucrose gradient allows the separation of free mRNA from mRNA that is bound to one or more ribosomes. As seen in Fig. 6, in noninduced cells, 30 and 24%, respectively, of the Iαα and Iαβ mRNAs were free and the rest was evenly distributed along the ribosome-bound fractions. However, after IFN-γ-stimulation, both Iαα and Iαβ mRNAs underwent a marked shift toward polysome-bound mRNAs, and only 8 and 7% were free. A similar shift was observed in G1-arrested cells that had not been stimulated with IFN-γ (8 and 1%), and this shift was not modified after IFN-γ activation (10 and 5%). This indicated that the G1 arrest induced an increase in the average number of ribosomes bound to these specific mRNAs, and consequently increased protein synthesis efficiency. As a control we used β-actin (28, 40), which did not show any changes in its distribution profile or in the mean number of ribosomes attached per molecule of β-actin mRNA after treatment with IFN-γ or with mimosine. The distribution of 18S mRNA was used to determine the position of one ribosome (28, 40).

Thus, G1 arrest induced an increase in the efficiency of MHC class II protein synthesis that may account for the increase of basal MHC surface expression observed in the G1 phase of the cell cycle. As shown in Fig. 7, when the cells are arrested at the G1 phase of the cell cycle, there is an increase in the levels of MHC class II Iαα molecules on the cell surface. However, translational control was not involved in the inhibition of IFN-γ-induced MHC class II expression observed in cells arrested at the G1 phase of the cell cycle.

The transcriptional regulation of the expression of MHC class II genes involves at least three cis-acting elements located 5′ upstream of the transcription initiation site (reviewed in Refs. 41–43). These elements have been referred to as W, X, and Y, and NFs have been shown to bind to each element. The expression of these transcription factors is ubiquitous and is not regulated by IFN-γ. However, the correct expression of MHC class II genes requires the presence of CIITA, a trans-activator that does not bind directly to the DNA, is tissue specific, and whose expression is regulated by IFN-γ in macrophages (44). To determine whether this transcription factor is involved in the CCD expression of MHC class II molecules, we analyzed the IFN-γ-induced expression of this gene in macrophages arrested at the different phases of the cell cycle.
The Journal of Immunology

The steady-state levels of CIITA mRNA in macrophages were monitored during the treatment of the cells with IFN-γ (Fig. 8). The expression of CIITA was detected after 6 h of treatment with IFN-γ. Macrophages arrested at the G1 phase of the cell cycle after treatment with mimosine showed a higher basal expression, but no induction was observed after treatment with IFN-γ. These results could explain the specific effect of the cell cycle on the expression of CIITA and MHC class II molecules. The detectable levels of IA mRNAs as well as IA proteins on the cell surface are in accordance with previous models, where residual amounts of class II expression were detected despite a lack of response to IFN-γ. This is the case of the targeted gene inactivation of CIITA (24) or STAT1 genes (52).

The basal increase found in MHC class II expression in macrophages is in accordance with early studies performed with B cells (53) or macrophages arrested by M-CSF starvation (54). The effect on IFN-γ-induced expression observed in G1-arrested cells is a new observation. This effect is not due to mimosine toxicity for several reasons: first, mimosine arrest is reversible and does not modify macrophage viability at the concentrations used; second, mimosine arrest does not inhibit basal expression but up-regulates it; and third, IFN-γ-induced surface expression of Fcγ receptors or iNOS mRNA are not abolished in the same experimental conditions.

We have observed that IFN-γ induces the expression of MHC class II molecules in macrophages in a CCD manner, because macrophages arrested at the G1 phase of cell cycle do not show an increase in the expression of class II molecules after stimulation with IFN-γ; however, these cells do have a higher basal expression of class II molecules. The detectable levels of IA mRNAs as well as IA proteins on the cell surface are in accordance with previous models, where residual amounts of class II expression were detected despite a lack of response to IFN-γ. This is the case of the targeted gene inactivation of CIITA (24) or STAT1 genes (52).

The effect of G1-arrested macrophages on the IFN-γ-induced MHC expression takes place mainly at the transcriptional level, because we have found an inhibition of the CIITA and MHC class II mRNAs and a decrease of MHC class II expression in macrophages treated with each cell cycle inhibitor. The Fc receptors or iNOS mRNA are not abolished in the same experimental conditions.

The effect of G1-arrested macrophages on the IFN-γ-induced MHC expression takes place mainly at the transcriptional level, because we have found an inhibition of the CIITA and MHC class II mRNAs after IFN-γ stimulation in control and G1-arrested cells. The cells were treated with 300 U/ml IFN-γ for 2 h or left untreated after G1 synchronization with 100 ng/ml mimosine. After IFN-γ treatment, the cells were washed and cultured for 48 h to assess class II expression. Then they were harvested and the RNA was separated into fractions in a sucrose gradient. The specific mRNA content of each fraction was assessed by slot blotting. Hybridization with 18S rRNA was performed to assess the position of one ribosome (indicated with an arrow). β-actin was used as control and no modifications in the polysome distribution were observed after treatment with IFN-γ and/or mimosine. These results are representative of two independent experiments.

The term CCD genes designates a very broad group of genes that are expressed in a CCD manner. This means that they are not expressed, or are expressed at very low level, in quiescent nonproliferating cells, and their expression is markedly increased in growing cells or in cells stimulated to proliferate. This includes genes that are directly involved in cell cycle regulation, together with other genes not involved in cell proliferation and that participate in several signaling cascades (reviewed in Ref. 8). Moreover, the activity of some elements of the cell cycle machinery may modulate the expression or the activity of several genes acting on the transcriptional and/or translational machinery (45–51). Therefore, it is not surprising that the cell cycle position or the proliferative state of a cell could modulate its activity.

FIGURE 6. Shifts in the polysome gradient distribution profile of IAα and IAβ mRNA in macrophages after IFN-γ stimulation in control and G1-arrested cells. The cells were treated with 300 U/ml IFN-γ for 2 h or left untreated after G1 synchronization with 100 ng/ml mimosine. After IFN-γ treatment, the cells were washed and cultured for 48 h to assess class II expression. Then they were harvested and the RNA was separated into fractions in a sucrose gradient. The specific mRNA content of each fraction was assessed by slot blotting. Hybridization with 18S rRNA was performed to assess the position of one ribosome (indicated with an arrow). β-actin was used as control and no modifications in the polysome distribution were observed after treatment with IFN-γ and/or mimosine. These results are representative of two independent experiments.

FIGURE 7. Macrophages arrested at the G1 phase have an increased basal expression of MHC class II molecules on the cell surface. A. Mimosine arrest increases basal IA surface expression in bone marrow-derived macrophages. A total of 10^6 macrophages was treated with 100 ng/ml mimosine, 100 ng/ml OH-urea, or 1 μg/ml nocodazole for 16 h, and MHC class II surface expression was analyzed by flow cytometry. The continuous line histogram shows basal IA surface expression in nonsynchronized cells, whereas the noncontinuous line histogram corresponds to IA expression in cells synchronized with each cell cycle inhibitor. The Fc receptors were blocked using a specific Ab (anti-CD16/CD32). B. Quantification of IA surface expression using the Immuno4 software. Similar results were obtained in three independent experiments.
general transcription factors (55–57), a local change in the structure of chromatin near the promoter (58–60), and inhibition of DNA binding by competition or steric hindrance (61, 62).

In some promoters, in vivo footprinting techniques have identified two contiguous regulatory elements, known as the CCD element (CDE) and the cell cycle genes homology region (CHR) (63–65). These elements are located near the transcription initiation sites and play a key role in the periodic transcription of the cdc25C, cdc2, and cyclin A genes (63, 66). CDE and CHR are bound by a transcriptional repressor during the G2/M phase that is released at the S and G2/M phases (63, 64). Apparently, CDE does not interfere with basal transcription from the core promoter (64).

This CDE-interacting factor, termed CDI-1 (66), represses the activity of transcription factors with glutamine-rich DNA-binding domains, such as NF-Y or Sp-1. There are several putative CDE boxes, but no CHR boxes, in the promoter of the transcription factor CIITA (67). Using oligonucleotides covering several CDE boxes from the CIITA promoter, we found no differences in the gel retardation assay when nuclear extracts from macrophages treated with mimosine, nocodazole, or OH-urea were used (data not shown).

In the active cell cycle, CIITA mRNA expression is controlled by the engagement of the CDE-enhancer element (CDE), which acts as a block to basal transcription from the core promoter (64). This effect is mediated by CDE-interacting factors, which are generated during cell proliferation (64). CIITA mRNA expression is downregulated by CDE-binding factors in G1, which is consistent with the observed cell cycle-dependent regulation of CIITA mRNA expression. The CDE-binding factor, termed CDI-1 (66), represses the activity of transcription factors with glutamine-rich DNA-binding domains, such as NF-Y or Sp-1. There are several putative CDE boxes, but no CHR boxes, in the promoter of the transcription factor CIITA (67).

The cell cycle dependency of the IFN-γ-induced MHC class II expression may have its physiologic consequence and could act as a mechanism of repression of an excessive macrophage activation during inflammation and a mechanism of resolution of the immune response. Both LPS and IFN-γ, the major macrophage activators, as well as other macrophage activators, inhibit macrophage proliferation at different points in the G1 phase of the cell cycle, and macrophages arrested at G1 phase do not express MHC class II molecules in response to IFN-γ-activation. This also may help to explain the differences in the immune response observed against small vs large overwhelming numbers of Gram-negative bacteria. Early during infections by small numbers of Gram-negative bacteria, IFN-γ is produced and should diffuse into adjacent tissues. On the other hand, LPS and bacterial products should be contained at the site of infection by neutrophils and resident macrophages. Macrophages would then encounter first IFN-γ as they approach the site of infection, and endotoxin only after they enter yet activated at the site of infection, and the MHC class II expression could be optimal, permitting Ag presentation and an effective host defense. However, with overwhelming infections, as occurs during septic shock (68), the quantities of LPS and other bacterial components are too great and they cannot be contained by local phagocytes. Macrophages migrating toward sites of infection might then encounter LPS before IFN-γ. In these situations, LPS might block macrophage cell cycle, and the late IFN-γ activation will be inefficient and the MHC class II expression insufficient to develop a correct host defense. This would explain the decreased MHC class II expression and Ag presentation by macrophages observed in sepsis on both animal models and clinically.

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