

Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts

(mRNA induction/HLA-DR/HLA-DC/HLA-SB/monoclonal antibody)

TUCKER COLLINS*, ALAN J. KORMAN†, CLAIRE T. WAKE‡, JEREMY M. BOSS†, DIETMAR J. KAPPEST, WALTER FIERS§, KENNETH A. AULT¶, MICHAEL A. GIMBRONE, JR.*, JACK L. STROMINGER†, AND JORDAN S. POBER*

Departments of *Pathology and of ¶Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; ‡Biogen, Inc., Cambridge, MA 02142; †Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138; and the §Laboratory of Molecular Biology, University of Ghent, Ghent, Belgium

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ABSTRACT Immune interferon (IFN- γ) increases the surface expression of HLA-A,B antigens and induces the surface expression of HLA-DR antigens on vascular endothelial cells and dermal fibroblasts. Here we report that IFN- γ induces parallel expression of two other class II major histocompatibility complex (MHC) antigens, SB and DC. Maximal surface expression of all three antigens is reached in 4–6 days, and HLA-DR and -SB are induced to a higher level of expression than HLA-DC. For all three class II antigens, induction is marked by the *de novo* appearance of detectable transcripts of class II heavy and light chains and of the non-MHC-encoded invariant chain, suggestive of the transcription of multiple previously silent genes. Class I message levels and antigen expression are also increased by IFN- γ at similar rates but from initial levels that are 50% of maximal. After removal of IFN- γ , class II antigen expression persists for at least 4 days, while mRNA levels decrease rapidly. The parallel induction and persistence of the several class II MHC antigens may be important in conferring immune accessory function on vascular and stromal cells.

The class II antigens of the major histocompatibility complex (MHC) are products of the immune response genes (1) and serve as restriction elements in antigen presentation to T lymphocytes (2)—i.e., T-helper cells only recognize nominal antigen in combination with the polymorphic determinants of a self-class II MHC antigen. Traditionally, it was thought that only cells of the immune system express cell-surface class II molecules (3, 4). In addition, class II antigens on cells of the monocyte–macrophage line and their tumors could be increased or induced by immune interferon (IFN- γ) (reviewed in ref. 5). On murine macrophages, the expression of class II antigens is a transient event and may regulate the ability of these cells to interact with T cells (5, 6). Unexpectedly, HLA-DR, the best characterized human class II antigen, could also be induced on human umbilical vein endothelial cells (HUVE) and human dermal fibroblasts (HDF) by recombinant IFN- γ (7, 8).

To date, three sets of human class II antigens (HLA-DR, -DC, and -SB) have been identified. All three sets of antigens have been studied at the protein and nucleic acid level; they consist of similar heterodimers with heavy chains of ≈ 34 kDa, and light chains of ≈ 29 kDa (1). Biosynthetic intermediates, but not mature surface-expressed class II antigens, appear associated with a non-MHC non-polymorphic “invariant” chain of ≈ 31 kDa (9). In human, but not in murine

cells, class II MHC loci may be independently expressed (10–12). Furthermore, differential expression (e.g., DR⁺, DC⁻) may be correlated with limitations on the ability of the class II antigen-bearing cell to interact with responding T cells (10–12). To understand more fully the potential of endothelial and stromal cells to interact with T cells, the effects of IFN- γ on these cells have been studied in greater detail, and it has been found that: (i) recombinant human IFN- γ induces the *de novo* appearance of transcripts of multiple class II antigen genes, including the α and β genes of HLA-DR, -DC, and -SB, as well as transcripts of the invariant (γ) chain, with subsequent surface expression of all three class II antigens; and (ii) after removal of IFN, the class II-specific mRNAs disappear relatively rapidly, while the antigens remained expressed on the surface at relatively constant levels for several days.

MATERIALS AND METHODS

HUVE cells were isolated and grown in primary culture as described (13). In some experiments, HUVE cells were passaged (5–10 subcultures) in medium containing 100 μ g of endothelial cell growth factor per ml (a generous gift of Thomas Maciag, Meloy Laboratories, Springfield, VA) and porcine heparin (Sigma) on 0.1% gelatin-coated (Difco) dishes (14). HDF (a generous gift of James Rheinwald, Dana-Farber Cancer Institute, Boston; see ref. 15) strains were cultured as described (8). HUVE and HDF cultures were treated where indicated with IFN- γ by addition of 0.1% (vol/vol) medium conditioned by a transfected Chinese hamster ovary fibroblast line [final IFN activity, 100 units/ml (16)].

The monoclonal antibodies reacting with MHC antigens are listed in Table 1. Monoclonal antibody binding measurements and fluorescence flow cytometry were carried out with HUVE and HDF cells as described (7, 24).

Total cytoplasmic RNA was prepared as described by Maniatis *et al.* (25). Denatured total cytoplasmic RNA and poly(A)⁺ RNA from the B lymphoblastoid cell line LB (26) was electrophoresed in a formaldehyde-containing 1% agarose gel, transferred to nitrocellulose, and probed with nick-translated cDNA inserts, as described (25). RNA blots were routinely washed 3 times with 0.5 \times standard saline citrate (NaCl/Cit; 1 \times NaCl/Cit is 150 mM NaCl/15 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulfate at 65°C. Additional washing with 0.1 \times NaCl/Cit/0.1% sodium dodecyl sulfate at 65°C did not qualitatively alter any of the blots.

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Abbreviations: FACS, fluorescence-activated cell sorter; HDF, human dermal fibroblast; HUVE, human umbilical vein endothelial cell; IFN- γ , immune interferon; MHC, major histocompatibility complex; bp, base pair(s); NaCl/Cit, standard saline citrate.

Table 1. Expression of MHC antigens on dermal fibroblasts

Antigen	Antibody	Without IFN- γ	With IFN- γ
HLA-A,B	W6/32 (17)	25.8 \pm 2.1	65.2 \pm 3.0
HLA-DR	LB3.1 (18)	1.1 \pm 0.4	31.2 \pm 0.7
HLA-DC	Leu-10 (19)	0.3 \pm 0.4	4.4 \pm 0.4
HLA-DC	Genox 3.53 (20)	0.1 \pm 0.3	2.8 \pm 0.6
HLA-SB	ILR1 (21, 22)	0.5 \pm 0.5	19.6 \pm 3.6
HLA-SB	TU39 (23)	0.5 \pm 0.3	26.7 \pm 2.2

Replicate cultures of a human dermal fibroblast strain (HDF-5) were treated with or without IFN- γ for 5 days and monoclonal antibody binding to confluent monolayers was quantified as described (8). Nonspecific binding ($2.8 \pm 0.3 \times 10^{-3}$ cpm) was measured using UPC 10, a mouse myeloma protein, and subtracted. All values are means of triplicate determinations and represent specific binding, cpm $\times 10^{-3}$ per well \pm SD.

Cross-hybridization was observed between DC α and DR α only above $2 \times$ NaCl/Cit (27). Blots were autoradiographed using XAR-5 film (Kodak) and intensifying screens, and the intensity of mRNA bands from autoradiograms was measured with a Quick Scan densitometer (Helena Laboratories, Beaumont, TX) working within the linear range of the film.

The cDNA probes used include DR α [an 1100-base-pair (bp) *Pst* I partial fragment of pDR α -15 (28) subcloned into pUC9]; DR β [a 900-bp *Pst* I partial *Hind*III fragment of pDR β 1 (29) subcloned into pUC9]; DC α [a *Pst*/*Sst* partial fragment of HB20 (30), subcloned into pUC12]; DC β [a 750-bp *Pst*/*Eco*RI fragment of pII β 1 (31)]; SB α [a 500-bp 5' *Pst* I fragment of LB14 (30) subcloned into pUC9]; SB β [an 1100-bp *Hind*III/*Eco*RI fragment of pHAB-10-DD2: pHAB-10 (32) spliced at the *Acc* I site with pDD2, subcloned into pUC13]; HLA-A,B [pHLA2, *Pst* I fragment (33)]; and the

invariant chain [in 5, a 700-bp *Pst* I fragment of a cDNA isolated from a Raji lymphoblastoid cell cDNA library by hybridization using clone 33-10 (34)].

RESULTS

IFN- γ Induces HLA-DR, -DC, and -SB Antigens. By monoclonal antibody binding, HUVE and HDF cells express class I (HLA-A,B) but not class II antigens (7, 8, 24). IFN- γ increases the level of HLA-A,B and induces the appearance of HLA-DR antigens as measured with anti-human HLA-A,B and HLA-DR monomorphic monoclonal antibodies, respectively (8). As shown in Table 1, IFN- γ also induces antigens on some cultures of both HUVE and HDF recognized by polymorphic monoclonal antibodies putatively specific for HLA-DC (Leu-10 and Genox 3.53) and HLA-SB (ILR1 and TU39). In both HDF and HUVE, the amount of putative HLA-DC was significantly less than HLA-DR (10%–20% of HLA-DR, depending on the cell strain).

These results suggest that IFN- γ treatment of HDF and HUVE induces expression of HLA-DR, -DC, and -SB antigens. However, because none of the DC and SB polymorphic anti-class II monoclonal antibodies has been shown to react exclusively with products of a single class II locus for all alleles, it is not possible to conclude rigorously from these antibody-binding data that class II loci other than HLA-DR are expressed.

IFN- γ Induces mRNAs for the α and β Chains of HLA-DR, -DC, and -SB and for Invariant Chain. To determine unequivocally whether IFN- γ increased expression of the various MHC class II genes, cytoplasmic RNA was prepared and mRNA was analyzed by RNA blot analysis with class II cDNA probes. After 4 days of IFN- γ treatment, the DR α and - β cDNA probes detected a single mRNA band in both

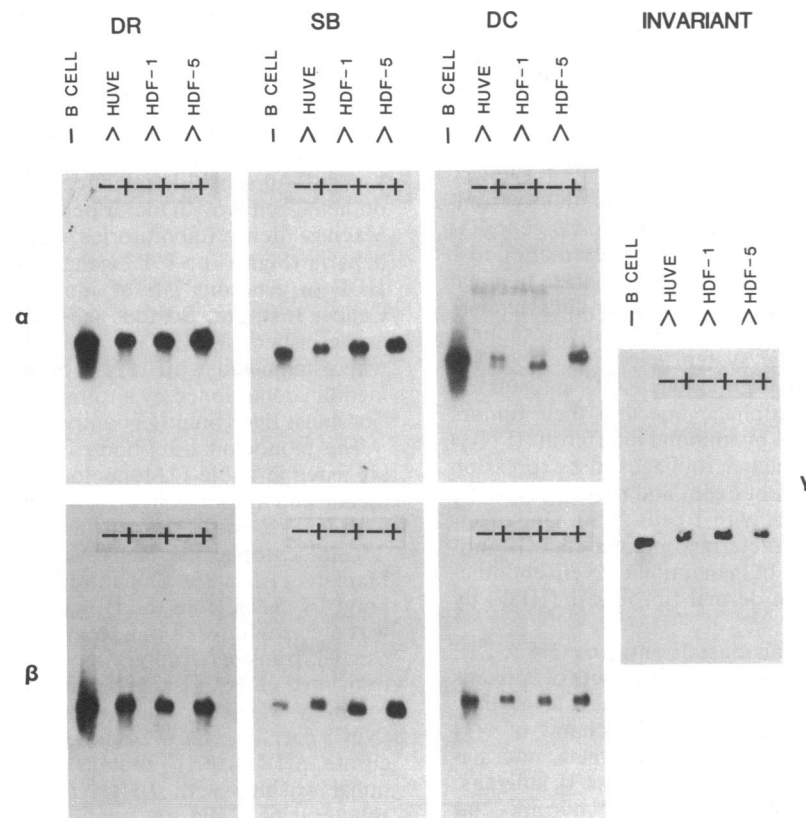


Fig. 1. IFN- γ -dependent induction of transcripts of α and β chain of HLA-DR, -SB, and -DC and invariant chain in HUVE and two strains of HDF. Denatured total cytoplasmic RNA (25 μ g per lane) from passaged HUVE cells or two strains of HDF, either control (-) or IFN- γ treated (+), was analyzed by RNA blotting techniques, using the probes described in *Materials and Methods*. Each RNA blot contained poly(A)⁺ RNA (250 ng per lane) from the B-lymphoblastoid cell line LB.

passed HUVE and two strains of HDF. The identified mRNAs were ≈ 1400 nucleotides and comigrated with the mRNA bands detected in 250 ng of poly(A)⁺ RNA from a B-lymphoblastoid line (LB; DRw6,6). Neither α - nor β -chain HLA-DR mRNAs were detected in untreated HUVE or HDF preparations. Similarly, DC α -, DC β -, SB α -, and SB β -specific probes detected mRNAs present in all the IFN-treated cultures but not in controls (Fig. 1). Transcripts of invariant chain were also detected in IFN-treated but not in untreated HUVE and HDF cells (Fig. 1).

Three points deserve further comment. (i) Two sizes of DC α -specific mRNAs were observed of 1100 and 1300 nucleotides (Fig. 1). The DC α -chain genes are polymorphic [and correlated with the serologically defined MT1 (DC1), MT2 (DC3), and MT3 (DC4) specificities in linkage disequilibrium with DR1,2,6; DR3,5,6,8; and DR4,7, respectively]. Sequences of the DC1 α and DC4 α cDNAs have revealed that the former mRNA is 196 nucleotides larger than the latter as the result of the use of different splice sites for the exons encoding the 3' untranslated regions (30, 34); DC3 α also encodes the larger 1300-bp mRNA (34). Thus, HUVE (derived from multiple pooled donors) contained both sizes of DC α mRNA, while HDF-1 (from a DR7-, thus DC4 donor) had only the 1100-bp mRNA and HDF-5 (from a DR5,6, thus either DC3,3 or DC1,3) had only the 1300-bp mRNA. These results confirm the specificity of the DC α -chain probe used.

(ii) Cross-hybridization between induced mRNA species for HLA-DR α and - β chains and the full length HLA-SB and DC α - and DC β -chain cDNA probes cannot explain the observed induction of HLA-SB and -DC transcripts. Probes specific for the 3' untranslated regions of several genes with low sequence homology to other genes were generated. Such probes for SB α (HB20: *Pst*/*Bgl* II fragment), SB β (pHAB-10-DD2: *Sac* I/*Hind*III fragment), and DC β (p2 β 1: *Pvu* II fragment) detected transcripts of the same size as the full-length cDNA probes under high stringency (data not shown). Small differences in size (≈ 100 nucleotides) between the induced class II α - and β -chain transcripts and invariant chain with the corresponding B-cell transcripts may result from slight electrophoretic mobility differences on these denaturing gels between purified B-cell poly(A)⁺ RNA used as the reference and total cytoplasmic RNA from HUVE and HDF.

(iii) The invariant chain probe identified an abundant 1400-nucleotide transcript from the B-lymphoblastoid line poly(A)⁺ RNA; however, in both HDF strains and passaged HUVE, the cDNA probe identified two mRNAs: a major species of ≈ 1400 nucleotides and a minor larger species of ≈ 1500 nucleotides. Previous work with a similar clone (35) found that in some B-cell lines, in addition to the 1400-nucleotide RNA, the cDNA probe hybridized to a larger (1800 nucleotide) minor species. β_2 microglobulin, another non-polymorphic protein associated with MHC antigens, has also been noted to show multiple-sized transcripts, in this case resulting from use of alternative polyadenylation sites (36).

The Rate of MHC Class II mRNA Induction Is Faster Than Antigen Expression. The kinetics of class II antigen induction was monitored by examining both surface expression of HLA-DR and cytoplasmic mRNA content. After a 1-day lag period, surface expression of HLA-DR antigen, measured by the binding of a monomorphic monoclonal antibody (LB3.1), increased from an undetectable level to plateau values at 5–6 days (Fig. 2). The percentage of HLA-DR positive cells in the population was determined by fluorescence-activated cell sorter (FACS). Binding of LB3.1 to HDF-5 in untreated cultures (Fig. 3a, large dots) is indistinguishable from nonspecific binding with a mouse myeloma protein (Fig. 3a, small dots). After 2 days of IFN- γ treatment, the entire population has become HLA-DR positive (Fig. 3b), reaching a

plateau value at 4–6 days (Fig. 3c). Uniform induction of HLA-DC was also observed, although at a slower rate and to a lower final level.

mRNA levels for HLA-DR α chain, following IFN- γ treatment, increased from undetectable levels to plateau values by 24–48 hr (Fig. 2). Since the amount of total mRNA present in the cytoplasmic RNA sample and the lymphoblastoid poly(A)⁺ RNA are approximately equivalent (250 ng), relative abundance can be assessed by direct comparison. Blots were reprobbed with a tubulin cDNA, confirming that equivalent amounts of mRNA were transferred to each lane (data not shown). HLA-DR is estimated to comprise 0.01%–0.05% of total mRNA in B cells (33, 37). In the IFN- γ -treated samples, HLA-DR mRNA becomes as abundant as found in the B-cell line and remains at that level during the course of IFN- γ treatment. The plateau value of HLA-DR mRNA content preceded maximal surface expression of the antigen by 1–2 days. HLA-DC α -chain mRNA appeared to be expressed at lower levels during IFN- γ treatment. The rate of appearance of invariant chain mRNA (Fig. 2) exactly paralleled that of HLA-DR α -chain mRNA.

In contrast to the class II induction, surface expression of a class I antigen measured with a monomorphic monoclonal antibody (W6/32) increased linearly to plateau values 1.5- to 2.5-fold greater than controls at 3–4 days after treatment (Fig. 2). By FACS analysis, each cell expressed more HLA-A,B, accounting for the increase. The class I α -chain probe detected a single 1700-nucleotide transcript in cytoplasmic RNA from HDF and HUVE, which comigrated with the mRNA identified in B-cell poly(A)⁺ RNA. Class I mRNA increased 3.5-fold during 1 day of treatment, and it increased 6-fold over untreated samples by day 3. This increase in class I mRNA level exceeds the 2-fold increase of class I antigen expressed on the cell surface.

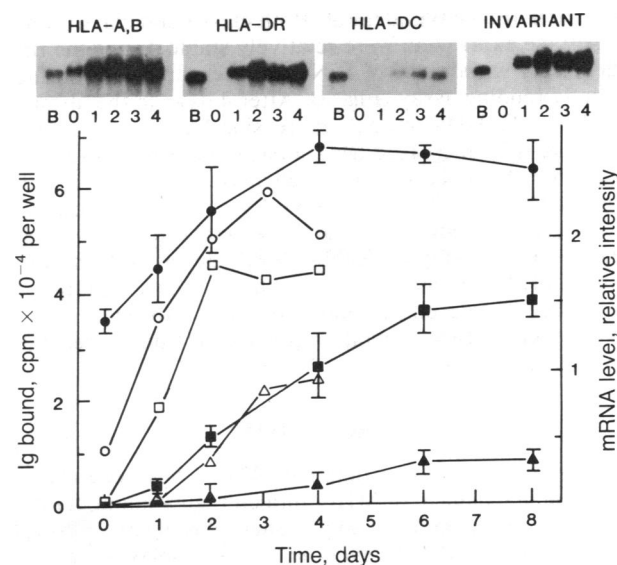


FIG. 2. Kinetics of the IFN- γ -dependent class I and class II mRNA induction and surface antigen expression in HDF. Surface expression of class I and class II antigens was measured by monoclonal antibody binding using antibody reactive with HLA-A,B (W6/32), HLA-DR (LB3.1), and HLA-DC (Leu-10). Nonspecific binding was measured using a mouse myeloma protein (UPC 10) and subtracted. Values are $\text{cpm} \times 10^{-4}$ and are means of triplicate determinations \pm SD. Levels of mRNA were measured by scanning autoradiograms of RNA blots (*Upper*) of cytoplasmic RNA (25 μg per lane) or of LB poly(A)⁺ RNA (250 ng in lane B) with a class I, class II α -chain insert, or an invariant chain cDNA probe. Numbers below the blots refer to the time (days) of IFN- γ treatment. (*Lower*) The symbols used are as follows: class I antigen (\bullet) and mRNA (\circ) levels; class II HLA-DR antigen (\blacksquare) and α -chain mRNA (\square) levels; class II HLA-DC antigen (\blacktriangle) and α -chain mRNA (\triangle) levels.

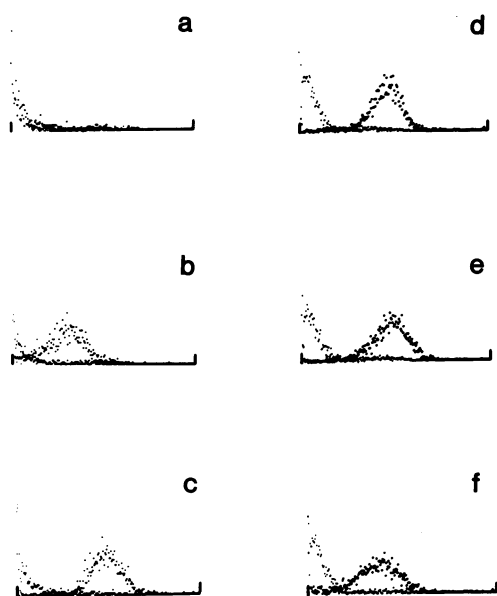


FIG. 3. Time course of the induction of HLA-DR in the presence of IFN- γ and the relative stability of the induced class II antigen after withdrawal of IFN, as measured by fluorescence flow cytometry. The data are presented as histograms, plotting cell number (y axis) vs. log fluorescence intensity (x axis). The binding of a monomeric anti-HLA-DR (LB3.1; large dots) is compared with a non-specific mouse immunoglobulin (UPC 10; small dots). (A-C) Cultures with 0, 2, and 4 days of IFN- γ treatment, respectively; (D-F) cultures with 4 days of IFN- γ treatment followed by 0, 2, and 4 days in the absence of IFN.

Class II mRNA but Not Antigen Expression Rapidly Declines After IFN- γ Withdrawal. Both class I and class II antigen surface expression were relatively stable during the first 2 days after withdrawal of IFN- γ , as measured by a surface antibody-binding assay (Fig. 4). After 4 days in the absence of IFN- γ , HLA-DR surface expression had decreased 32% and HLA-A,B decreased 64%. FACS analysis showed that the entire cell population lost small amounts of antigen at the same rate (Fig. 3; data not shown for class I). By contrast, HLA-DR, -DC, -SB α -chain, and invariant mRNA levels decreased to minimally detectable levels during 4 days in the absence of IFN- γ . Similarly, expression of class I antigen at the surface decreased by a factor of about 2 during 4 days in the absence of IFN- γ , while mRNA levels decreased by a factor of at least 5.

DISCUSSION

In the present study, recombinant IFN- γ has been used to study the mechanism of MHC antigen regulation in cultured vascular endothelial cells and stromal fibroblasts. IFN- γ increases class I mRNA levels in B-lymphoblastoid cells at concentrations far lower than those needed to induce the antiviral state (38). In this study, we confirm that IFN- γ increases class I mRNA expression, but the rates of surface expression on vascular endothelial cells and dermal fibroblasts are slower than that previously reported for lymphoblastoid cells and foreskin fibroblasts (39) or in murine L cells transfected with a human class I gene (40). As in previous work, we find that the percentage increase in class I surface-antigen expression is smaller than the increase in the amount of mRNA.

Class II MHC antigens show a limited tissue distribution *in vivo* and are involved in the presentation of antigen to T-helper cells. IFN- γ has been shown to increase the expression of class II genes in many human and murine cell types

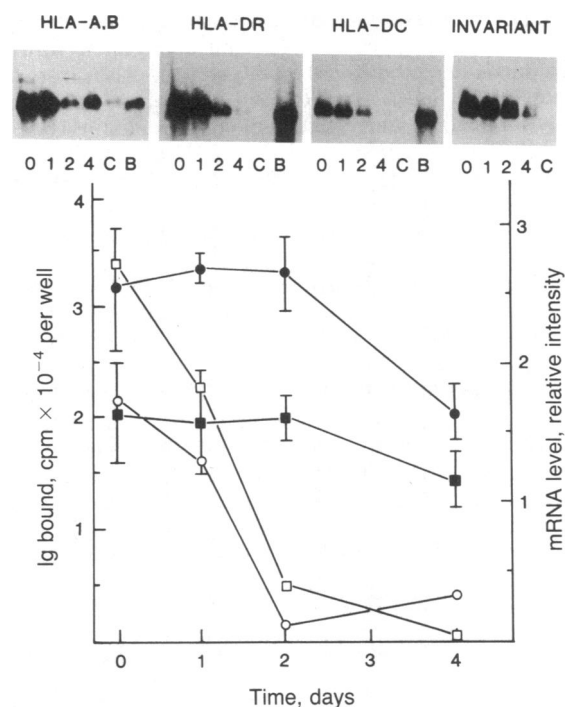


FIG. 4. Class I and class II mRNA content and surface expression after withdrawal of IFN- γ . HDF were treated for 4 days with IFN- γ , rinsed 3 times with Hanks' balanced salt solution, and returned to standard culture medium for various lengths of time. Surface antigen and mRNA levels were determined as in Fig. 2. (Upper) RNA blots. Numbers beneath the lanes refer to the length of time (days) after 4 days of treatment that the cultures were held in standard culture medium. Lane C is a control (untreated) culture; B identifies the lanes containing 250 ng of LB poly(A)⁺ RNA. Symbols are as in Fig. 2.

(7, 8, 41-47, 11). In some murine mast cell progenitors, IFN- γ may also induce the class II-associated invariant chain (48). In the results reported here, IFN- γ appears to induce HLA-DR, -DC, and -SB α - and - β chains and invariant (γ) chain in HUVE and HDF by transcription of apparently silent genes, although the stabilization of previously undetectable transcripts cannot be rigorously excluded.

Finally, we have examined the stability of the changes in MHC antigen expression induced by IFN- γ in fibroblasts and have found a striking difference in results from those previously reported for other IFN effects. For example, 3'-5' oligo(A) synthetase decreases within a few hours after removal of IFN (49). Murine macrophages express class II antigens for <24 hr after IFN- γ induction, and synthesis stops rapidly after IFN is removed (6). In contrast, we find little decrease in class II antigen expression by fibroblasts for several days after removal of IFN- γ . Interestingly, mRNA levels decrease significantly over this time period.

In summary, we have shown that IFN- γ increases the level of mRNA for class I MHC antigens and induces the appearance of at least six previously undetectable transcripts for class II MHC polypeptides as well as of the non-MHC-encoded invariant chain transcripts in fibroblasts and endothelial cells. The increase in mRNA results in expression of surface antigens, but our studies on the relative rate of change of mRNA and surface protein levels suggest additional levels of control. The persistence of the protein products on human vascular and stromal cells, in contrast to

¹¹Sztein, M. B., Steeg, P. S., Mann, D., Stiehm, R., Blaese, R. M. & Oppenheim, J. J., Third International Lymphokine Workshop, Haverford, PA, Aug. 1-5, 1982, p. 16 (abstr.).

murine macrophages, may have biological implications. For example, the widespread anatomic distributions of endothelium and fibroblasts may permit these cells to participate in the development and sustenance of an immune response in virtually every peripheral tissue. The fact that all known class II MHC products are induced in parallel further supports the idea of "immunocompetence" of these cell types, consistent with reports of their ability to present antigen (50–52). Finally, the uniformity of the cell population in responding to IFN- γ and the appearance of new transcripts in previously negative cells provide a powerful system for further analyzing the molecular mechanism of regulation of the MHC.

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