

Cytokine regulation of expression of class I MHC antigens

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Accepted 12 January 1998

Abbreviation: MHC, major histocompatibility; IFN, interferon; TNF, tumor necrosis factor; LT, lymphotoxin; NK, natural killer; MHC-AF, MHC class I augmenting factor; NF-RIF, natural killer-lysis resistance inducing factor; EC, embryonal carcinoma; CRE, class I regulatory element; ISGF-3, IFN stimulated gene factor-3; GAS, IFN- activation site; GBP, guanylate binding protein; IRS, interferon response sequence; ICSBP, interferon consensus sequence binding protein; TAP, transporter associated with antigen presentation

Introduction

The major histocompatibility complex (MHC) is a chromosomal region that has been extensively characterized in several mammals, particularly man and mouse (Flavell *et al.*, 1986 and Ploegh *et al.*, 1981). The region which represents about 0.1% of the total genome in mouse, comprises a large number of genes classified into class I and class II MHC genes encoding a large number of polymorphic class I or class II MHC molecules respectively. Class I genes include HLA-A, B, C genes in man and H-2D, K, L in the mouse. Class II MHC genes include HLA-DR, DP, DQ in human and H-2IA and IE genes in the mouse. Class I MHC molecules are made of a highly polymorphic glycosylated transmembrane α -chain of about 45 kDa, associated with a non-polymorphic, non-glycosylated, 12-kDa chain, called β_2 -microglobulin, not encoded within the MHC locus. Class II molecules encoded entirely within the MHC locus are made of two transmembrane polymorphic chains, α and β , which associate to form heterodimer. Class I heavy and light chains comprise three and one extracellular domains respectively, whereas both α and β chains of class II molecules have two extracellular domains each. Both class I and class II MHC antigens show very high polymorphism and two of the extracellular domains, α_1 and β_1 in class I and α_1 and β_1 in class II heterodimers display much more variability within different alleles than the other so called constant domains (α_2 and α_3 in class I and β_2 and β_3 in class II molecules). Constant domains resemble immunoglobulin domain, which make MHC molecules members of the

immunoglobulin super family (Williams *et al.*, 1988).

Class I molecules are constitutively expressed on most types of nucleated cells. Class II molecules are expressed largely by cells of immune lineage, particularly B cells, macrophages, and dendritic cells. MHC class I molecules present antigenic peptides to CD8⁺ T cells. The majority of class I-binding peptides are derived from nuclear and cytosolic proteins. MHC class II molecules present peptides from antigens degraded by the endosomal/lysosomal pathway to CD4⁺ T cells (Benham *et al.*, 1995). Antigen receptor on T-cells (T-cell receptor or TCR) can recognize antigenic peptides only when the latter are presented associated with class I or class II MHC antigens. In addition, during T-cell differentiation, only those T-cells are permitted to develop which can recognize antigen associated with self MHC molecules, a phenomenon which is referred to as MHC restriction (Zinkernagel *et al.*, 1979). While T-cells can only be activated in a MHC restricted manner, a class of non-T cytotoxic lymphocytes called natural killer (NK) cells, can spontaneously kill target cells in a MHC non-restricted manner. Levels of expression of class I MHC antigens on tumor cells however determine the susceptibility of tumor cells to NK cells (Haridas and Saxena, 1995a, 1995b, Saxena *et al.*, 1996). Expression of class I MHC molecules thus play a crucial role in determining the susceptibility of target cells to both T cells and NK cells.

Keywords: cytokine regulation, MHC class I, expression

Cytokines which modulate expression of MHC class I molecules

Interferons

Interferons (IFN)- α and β were originally described as anti-viral and anti-proliferative agents which were subsequently also shown to have immunoregulatory properties. IFN- γ has successfully been used in the treatment of some leukemias, and to lesser extent renal cells carcinomas. Interferon- α and β can antagonize the effects of IFN- γ on MHC class II expression on murine macrophages and endothelial cells (Inaba *et al.*, 1986).

IFN- γ is produced by activated T cells and NK cells. Although IFN- γ displays most of the biologic activities that have been ascribed to the other IFNs, it has 10-100 fold lower specific antiviral activity than either IFN- α or IFN- β . On the other hand IFN- γ is 100-10,000 times more active as an immunomodulator than are other classes of IFNs (Pace *et al.*, 1985). Hence IFN- α or β are primarily antiviral agents displaying some immunomodulatory activity, whereas IFN- γ is primarily an immunomodulator

but exerts some antiviral activity also (Trinchieri *et al.*, 1985). Further more IFN- synergizes with other mediators including lipopolysaccharide, tumor necrosis factor (TNF), granulocyte/macrophage-colony stimulating factor (GM-CSF), and immune complexes to augment the production of cytokines such as IL-1, which are themselves important in growth and differentiation of T and B cells.

A single human IFN- gene codes for a 166 amino acid polypeptide (Derynck *et al.* 1982), which after proteolytic cleavage gives rise to a mature positively charged polypeptide with a predicted molecular mass of 17 kDa. Two polypeptides self-associate to form a homodimer with an apparent molecular weight of 34 kDa (Chang *et al.*, 1984; Nagata *et al.*, 1987). At physiologic concentrations little if any monomer is detectable. Only the dimer can display IFN- biological activity, possibly because only it can effect IFN- receptor dimerization (Fountoulakis *et al.*, 1992). The IFN- is highly sensitive to extremes of heat and pH and is denatured at temperatures above 56°C and pH values below 4.0 and above 9.0 (Mulkerrin *et al.*, 1989).

The murine IFN- gene encodes a mature 134 amino acid polypeptide with a predicted molecular mass of 15.4 kDa (Gray *et al.*, 1983). Like its human counterpart, murine IFN- exists exclusively as a non covalently linked homodimer. Due to a low level of sequence homology, human and murine IFN- display a strict species specificity in their ability to bind to and activate human and murine cells respectively. The individual human and murine polypeptides are differentially glycosylated, thereby giving rise to subunits of differing molecular weights, accounting for the observed molecular weight heterogeneity (range from 30-50 kDa) in fully mature homodimeric human and murine IFN- molecules (Kelker *et al.*, 1983, 1984). Glycosylation seems to be unimportant for biological activity of the molecule but might influence the dynamics of tissue distribution and circulatory half life of the molecule (Kelker *et al.*, 1983).

IFN- is produced by NK cells and certain subpopulation of T lymphocytes, namely TH1 subclass of CD4⁺ lymphocytes and certain CD8⁺ lymphocytes on activation by antigens or mitogens (Fong *et al.*, 1990). In the human system, T cells that express the activation-dependent CD30 membrane antigen are the principal subset producing IFN- (Alzona *et al.*, 1994). Recently production of IFN- by mononuclear phagocytes has also been described (Fultz *et al.*, 1995).

All three types of INF enhance the expression of class I MHC antigens on a number of immunologically important cell types including mononuclear phagocytes, epidermal cells, endothelial cells, B lymphoid cells and macrophages (King *et al.*, 1983; Trinchieri *et al.*, 1985; Chang *et al.*, 1994) IFN- is somewhat more efficient in bringing about this effect. Cells with enhanced MHC I expression may become better targets for CTLs

recognizing viral, tumor or autoantigens present in such cells. Following IFN treatment, the increase of HLA class I antigens on human cells is rapid (detectable after one hour), stable for at least 24 h and ranges from 2 to 10 times the initial levels (Rosa *et al.*, 1986). Northern blot analysis of mRNA encoding HLA class I heavy chain has shown that IFN treatment increases the steady state level of mRNA for HLA-A, -B, -C in every cell type studied (Fellous *et al.*, 1992). IFN- could induce *de novo* expression of class I MHC antigen in K562 human erythroleukemia cells which have no basal expression of these antigens (Rosa *et al.*, 1984). IFN thus appears to be both a modulator, as well as an inducer of MHC genes.

Tumor necrosis factor and lymphotoxin

Tumor necrosis factor- (TNF-) is a 20-25 kDa protein which is produced by activated monocytes, T cells and NK cells. TNF- has a single copy gene which encodes a precursor product of 233 amino acids and a mature product of 157 amino acids after leader sequence is removed. The molecular weight of human recombinant TNF- is 45kDa by gel filtration and 17 kDa by SDS-PAGE. Human and mouse TNF show 80% homology at amino acid level (Old 1985; Pennica *et al.*, 1985). As a result, TNF- lacks species specificity in its action (Batten *et al.*, 1996).

Recombinant human TNF- increases mRNA levels and surface expression of HLA-A, B antigens in normal (untransformed) human vascular endothelial cells and fibroblasts *in vitro*. This effect plateaus in several days and is sustained in presence of TNF. TNF- has also been shown to upregulate constitutively expressed HLA genes in different tumor cell lines (Pfizenmaier *et al.*, 1987) and several neoplastic cell lines of carcinoma and leukemia origin (Scheurich *et al.*, 1986). TNF- by itself is unable to induce *de novo* expression of HLA-A, B, C genes but acts as an enhancer of constitutive or IFN-induced HLA gene expression. The expression of a critical density of class I MHC antigens on tumor cells is a prerequisite for tumor-specific immune responses and tumor rejection in immunocompetent hosts (Hui *et al.*, 1984; Wallich *et al.*, 1985). Enhancement of HLA expression upon combined treatment with IFN- and TNF- may result in a potentiation of the immunogenicity of a tumor. This appears to be of particular importance for those tumor cells expressing little or no HLA antigens and thus are resistant to direct cytotoxic actions of cytotoxic T lymphocytes.

Lymphotoxin (LT) or TNF- was described as a cytotoxic factor produced by activated T lymphocytes (Paul *et al.*, 1988). Both murine and human LT genes are tightly linked to genes for TNF- in the MHC locus. Murine LT is a mature protein of 169 amino acids and human LT a 171 amino acid long mature protein. Both have glycosylation

sites. LT has a molecular weight of 60-70 kDa by gel filtration and 20-25 kDa by SDS-PAGE and a pI of 5.8. The 25 kDa form is the monomeric glycosylated form of LT which aggregates to produce the higher molecular weight forms. *In vitro* transcribed and translated murine lymphotoxin cDNA produces two sizes of LT on SDS-PAGE gel of 18 and 34 kDa (Li *et al.*, 1987), representing monomeric and dimeric non-glycosylated LT. There is a remarkable homology (74%) between amino acid sequences of murine (Li *et al.*, 1987) and human (Nedwin *et al.*, 1985) LT, explaining the lack of species specificity in their biological effects.

Both TNF- and LT cause necrosis of a transplantable methyl cholanthrene-induced sarcoma in mice. The mechanism of this tumor necrosis does not appear to be due solely to direct killing because the Meth A sarcoma is not particularly LT-susceptible *in vitro*. Other effects of LT including increased expression of MHC antigens and subsequent cytotoxic T lymphocyte attack may be responsible for this sarcoma necrosis (Paul *et al.*, 1988). Human endothelial cells (HEC) are not killed by LT but their class I MHC expression increases on treatment with LT (Poerber *et al.*, 1987).

MHC class I augmenting factor (MHC-AF)

Previous work from our laboratory described a potential novel cytokine termed natural killer-lysis resistance-inducing factor (NK-RIF) which is detected in the culture supernatants of mitogen activated rat spleen cells (Saxena 1987, 1992). The factor was so named because it induced resistance to NK cell-mediated lysis of YAC tumor cells, which are otherwise highly susceptible to NK cells. NK-RIF treated YAC tumor cells expressed markedly enhanced levels of class I MHC antigens as compared to control YAC

cells (Saxena *et al.*, 1988). These results are consistent with the idea of an inverse correlation between NK susceptibility and class I MHC antigen expression on target tumor cells as discussed above. This NK-lysis resistance inducing factor (LRIF) has a molecular weight of 12.6 kDa and an isoelectric pH of 4.9, is heat labile, and is deactivated by proteolytic enzymes (Saxena *et al.*, 1988). Because NK-LRIF preparations purified by isoelectric focusing were devoid of any IL-2 or IFN activity, the factor is distinct from these cytokines.

NK-LRIF activity has also been isolated from culture supernatants of activated human peripheral blood mononuclear cells (Saxena *et al.*, 1996; Raval *et al.*, 1997) or mouse spleen cells (Sarin *et al.*, 1995). While looking for the human or mouse equivalent of the rat NK-RIF, MHC class I augmentation was used as a primary bioassay and therefore, it is now referred to as MHC-augmenting factor (MHC-AF). In these studies an approach similar to that used for the purification of rat NK-RIF was utilized, only difference being the standardization of use of serum-free medium for the generation of human MHC-AF, to simplify the purification of this activity (Raval *et al.*, 1997). The molecular weight of human MHC-AF was around 35 kDa with a pI of 6.0 (Saxena *et al.*, 1996). The activity was found to be susceptible to heat and proteolytic enzyme treatments (Raval *et al.*, 1997). Mouse NK-RIF has a molecular weight of about 10 kDa and a pI around 6.0 (Saxena *et al.*, 1992).

Several lines of experimental evidence indicate that MHC-AF is distinct from IFN. Table 1 shows a comparison of MHC-AF and IFN- : (a) IFN activity is a molecular entity of about 50 kDa (Saxena *et al.*, 1992), rat NK-RIF, on the other hand, has an apparent molecular weight of 12.6 kDa (Saxena *et al.*, 1988), (b) MHC-AF preparations lack detectable antiviral activity associated with IFN. The

Table 1. Comparison of properties of MHC-AF, IFN- and TNF- .

Property	MHC-AF	IFN-	TNF-
Molecular weight on Sephadex G100	10-35 kDa	30-55 kDa	45 kDa
Susceptibility to pH-2.0 treatment	No	Yes	n.d.
Antiviral activity in VSV/L929 assay	Absent	Present	Absent
Potency in terms of augmentation of MHC I on tumor cell lines	High	Relatively lower	Very low
Ability to induce de novo synthesis of MHC I (e.g. in K562)	Yes	Yes	No
Blocking of activity by anti- IFN- antibodies	No	Yes	No
Blocking of activity by anti-TNF- antibodies	No	No	Yes
Retention of activity on anti- IFN- affinity column	No	Yes	No
Augmentation of class II MHC antigens	No	Yes	No
Augmentation of adhesion molecules (ICAM-1)	No	Yes	n.d.
Susceptibility to Trifluoroacetic acid (Reverse phase HPLC)	No	Yes	n.d.
Species specificity	No	Yes	No
Requirement of glycosylation for activity	Yes	No	No

n.d.: not determined.

purification protocol for all MHC-AFs involves a step of pH-2.0 dialysis for 2 days. By being acid stable, MHC-AF is quite distinct from IFN- γ , (c) the extent of enhancement in MHC I antigen expression of MHC-AF preparations cannot be explained by the presence of small amounts of contaminating IFN- γ in these preparations, (d) the anti-IFN antisera could not block the effects of MHC-AF on YAC cells, and (e) IFN- γ induces the expression of both class I and class II MHC antigens whereas MHC-AF enhances the expression of class I MHC antigens only (Raval, 1997). MHC-AF does not act through IFN because it does not induce the production of IFN (Puri and Saxena,

1997). Neutralizing antibodies to TNF- α and IFN- γ had no effect on class I MHC augmenting activity of human MHC-AF preparations. In addition, an affinity column with immobilized monoclonal antibodies to IFN- γ with proven efficacy to absorb IFN- γ , did not show any significant absorption of MHC-AF activity (Saxena *et al.*, 1996).

Besides YAC cells, rat MHC-AF was found to induce increased levels of class I MHC antigen expression in a variety of other tumor cell lines, including EL4 lymphoma, L929 fibroblast cell line, methylcholanthrene-induced adenocarcinomas (MCA102 and MCA105), and melanoma (BL6) cell lines (Saxena *et al.*, 1992). Human MHC-AF

Table 2. Transcription factors involved in Class I MHC gene in mammalian cells.

Trans-acting factor	Presence	Function	Reference
H-2IIBP	Induced by retinoic acid in undifferentiated embryonic carcinoma cells.	Developmental regulation of class I MHC gene.	Nagala <i>et al.</i> , 1992
CREB	Constitutive in cyclic AMP and Calcium responsive cells and tissues.	Developmental regulation of class I MHC gene.	Israel <i>et al.</i> , 1989 Roesler <i>et al.</i> , 1988
KBF1	Constitutively present in cells and tissues expressing MHC I.	Developmental and tissue specific regulation of class I MHC gene.	Israel <i>et al.</i> , 1987 Kimura <i>et al.</i> , 1986
H2TF1	Constitutively present in cells and tissues expressing MHC I.	Developmental and tissue specific regulation of class I MHC gene.	Baldwin <i>et al.</i> , 1988 Kimura <i>et al.</i> , 1986
NF- κ B	Preexisting in inactive form in association with I κ B in many cells and needs to be activated by certain agents through phosphorylation of I κ B.	Developmental regulation of class I MHC gene. It also participates in the induction of class I MHC gene by TNF- α .	Baldwin <i>et al.</i> , 1988 Israel <i>et al.</i> , 1989
ISGF-3	Preexisting in IFN responsive cells. Forms ISGF-3 complex with ISGF-3 γ in response to type I IFN and acts as DNA binding factor.	Involved in JAK-STAT pathway.	Kessler <i>et al.</i> , 1990
IRF-1	Present in basal levels and further inducible in many cell types by certain agents.	Acts as a positive regulatory factor in response to both type I and type II IFN.	Miyamoto <i>et al.</i> , 1988
IRF-2	Present in basal levels and further inducible in many cell types by certain agents.	Repressor for IRF-1 mediated activation of gene expression.	Harada <i>et al.</i> , 1989
IRF-3	Constitutively present in a variety of tissues but steady state mRNA levels not increased by either IFN or virus treatment of cells.	Binds to ISRE, may associate with other transcription factor(s) to form complex.	Au <i>et al.</i> , 1995
ICSBP	Constitutively present in macrophage and B cells and can be induced in T cells by IFN- γ .	Act as a repressor of class I MHC antigen. This repression can be attenuated by IFN- γ .	Shirayoshi <i>et al.</i> , 1988

could enhance the class I MHC expression on Molt-4 (human T cell leukemia) and HR-7 (human gastric carcinoma) tumor cells (Saxena *et al.*, 1992) and could induce a marked dose dependent expression of MHC I antigens on human erythromyeloid leukemia cell line K562, which otherwise does not express HLA antigens (Sarin *et al.*, 1995). This is a property which TNF lacks (Pfizenmaier *et al.*, 1987). Table 2 further highlights the differences between MHC-AF and TNF. Mouse MHC-AF, like the rat counterpart, enhances the expression of class I MHC antigens on YAC tumor cells and renders them resistant to NK lysis (Sarin *et al.*, 1995; Puri and Saxena, 1997). Mouse MHC-AF also enhances the class I MHC expression on EL4 and BW5147 (both murine T cell lymphomas) tumor cell lines but had no effect on class I MHC expression of K562 or HR-7 (both human tumor cell lines) cells (Puri *et al.*, 1997). Human MHC-AF on the other hand, could enhance the class I MHC expression on murine EL4 and BW5147 tumor cell lines also. In this respect MHC-AF is quite different from IFN- γ which is highly species specific. The class I MHC antigen expression on tumor cells *in vivo* is an important factor in determining the susceptibility of these cells to immunosurveillance mechanisms. In this context, MHC-AF which is a potent inducer of class I MHC antigen expression *in vitro*, has a potential for tumor class I MHC expression augmentation *in vivo*.

Regulation of MHC gene expression

The regulation of MHC gene expression plays a fundamental role in the homeostasis of the immune response. Tissue specific expression of class I and class II genes is developmentally regulated and is principally controlled at the level of transcription. In cells of the central nervous system that do not express class I genes, the crucial promoter/regulatory sequences of class I and β_2 -microglobulin genes are largely unoccupied *in vivo* although factors that bind to the regulatory regions are present in the nuclear extracts of the cells (Burke *et al.*, 1989). This suggests the existence of mechanisms that determines accessibility of regulatory nuclear binding factors to relevant *cis*-acting elements during development.

The expression of MHC gene products can also be regulated at the posttranscriptional level. It is reported that the 3' untranslated region of HLA-DR mRNA interacts with at least two compartmentalized proteins, which seem to participate in mRNA partitioning in the nucleus and in the cytoplasm, respectively. Transfection of cDNA from the crucial 3' untranslated region results in an mRNA that is preferentially released in the cytoplasm, where it mainly associates with ribosomes (Accolla *et al.*, 1995). Thus, recognition of retention signals by distinct proteins may play a role in the distribution of MHC mRNA and, therefore, in the amount of mRNA translated

within a cell.

Down regulation of MHC genes may be a mechanism by which pathogens escape from immune attack. The Tat gene of human immunodeficiency virus (HIV) can generate two forms of the Tat protein, one-exon Tat and two-exon Tat. One exon tat protein suppresses MHC class I expression by up to 50% by acting at the level of the class I gene promoter (Accolla *et al.*, 1995). The rapid progression towards oncogenic transformation of cells infected with adenovirus 12 is associated with highly reduced class I expression. This is not due to a reduction in the amount of class I mRNA but, is a consequence of the very low level of TAP-1 (transporters associated with antigen presentation-1, tenfold less) and TAP-2 (100-fold less) mRNA, which results in a lower transport of MHC class I heterodimers to the cell surface (Proffitt *et al.*, 1994). Suppression of peptide transporter genes might be an important mechanism by which virus-transformed cells escape immune recognition *in vivo*.

Assembly of MHC class I molecule

MHC class I molecules present antigenic peptides to cytotoxic T cells. The expression of peptide loaded class I MHC molecule at the cell surface takes place in three steps. First, there is degradation of cellular proteins (or viral proteins in case of virus infected cells) to peptides in the cytosol, followed by active translocation of these peptides across the endoplasmic reticulum membrane and then the assembly of MHC class I heterodimers and their transport to the cell surface (Lehner *et al.*, 1996). Peptide pool present in endoplasmic reticulum is crucial for the assembly of class I molecule since in absence of peptides occupying the groove on α_1 - α_2 domains of the alpha chain of class I MHC antigen, proper assembly of class I MHC molecule does not take place.

Proteasome, a 700 kDa complex present in the cytoplasm and nucleus of all cell types, is involved in nonlysosomal protein degradation of cytosolic proteins (Fehling *et al.*, 1994; Rock *et al.*, 1994; Van *et al.*, 1994). The proteasome efficiently cleaves proteins at basic and hydrophobic residues. This facilitates the production of peptides with carboxy termini suitable for binding to class I MHC groove. Transportation of peptides generated in cytoplasm, to endoplasmic reticulum is carried by TAP-1 and TAP-2 proteins present on endoplasmic reticulum membrane (Monaco *et al.*, 1990; Spies *et al.*, 1990; Neefjes *et al.*, 1993; Wang *et al.*, 1996). Both TAP-1 and TAP-2 are multimembrane spanning proteins with an ATP-binding cassette. They can transport peptides across endoplasmic reticulum membrane in an energy dependent manner.

The peptide transporters are selective for peptide length and for the carboxy-terminal amino acid (Momburg *et al.*, 1994; Schumacher *et al.*, 1994). TAP has the highest

affinity for peptides of 8-13 amino acids in length, which is the approximate size of MHC class I peptides. Within the endoplasmic reticulum lumen, assembly of the three components of class I MHC molecule i.e. the α -chain, the β_2 -microglobulin and the peptide that occupy the groove on the alpha chain, takes place. This process requires the participation of several chaperone proteins of which calnexin, calreticulin and tapasin are the most well studied ones. Calnexin transiently associates with the nascent heavy chain and plays an important role in the assembly process (Hochstenbach *et al.*, 1992). It mediates disulfide bond formation and promote dimerization of α chain of class I MHC molecules with β_2 -microglobulin. Calreticulin plays a key role in the association of class I molecule with TAP (Sadasivan *et al.*, 1996). Tapasin, on the other hand, functions as a bridge connecting class I molecule and TAP (Kovacsovics *et al.*, 1994; Sadasivan *et al.*, 1996).

Basal expression of class I MHC antigen

The MHC class I molecule is broadly expressed on a variety of different cell types of mammals but its constitutive level varies markedly between cell types and tissues (David-Watine *et al.*, 1990). The highest level of cell surface expression of class I MHC antigens occurs in lymphoid tissues. Whereas lower levels are found in other somatic tissues, such as kidney and liver. There is no detectable cell surface class I MHC expression on brain cells, germline cells and undifferentiated embryonal carcinoma (EC) cells (Garrido *et al.*, 1995). Human EC cell lines like N-Tera2, and murine EC cell lines such as F9 and P19, like early mammalian embryonic cells, do not express class I MHC antigen (Morello *et al.*, 1982). Undifferentiated EC cells in response to retinoic acid undergo differentiation and this induces expression of

many genes including class I MHC genes (Nagata *et al.* 1992). In general, these differences reflect basal tissue specific regulation of gene expression.

Expression of MHC I genes is regulated by a variety of nuclear factor which bind regulatory elements 5' to the coding sequences. Investigation of HLA-B7 genes showed the presence of *cis*-acting regulatory sequences between -75 to -660, which are responsible for constitutive as well as IFN- γ induced tissue-specific expression of this gene (Chamberlain *et al.*, 1991). Similarly, enhancer A, and B (Figure 1) regions present upstream of class I MHC coding sequence act as a negative element in F9 EC cells. After retinoic acid induced differentiation, this element appears to function as an enhancer for MHC I gene expression (Shirayoshi *et al.*, 1987). Another negative regulatory element, has been demonstrated in the promoter of the miniature swine class I MHC gene, PD1 (Ehrlich *et al.*, 1988). This element reduced the activity of both the heterologous simian virus 40 promoter and the homologous PD1 promoter. *In vivo* competition experiments indicated that the functions of the PD1 positive and negative elements are mediated by distinct cellular *trans*-acting factors called "enhancers" and "silencers" respectively (Weissman *et al.*, 1991). This suggests that the expression of class I MHC genes is developmentally regulated and involves switching from negative to positive control (Miyazaki *et al.*, 1986).

The class I regulatory element (CRE) is critical in determining the level of class I constitutive gene expression (Shirayoshi *et al.*, 1987; Burke *et al.*, 1989). In MHC CRE, there are three discrete sequences (region I, II and III) (Figure 1) that bind different nuclear protein factors. KBF1, NF- κ B, H2TF1 are a few known factors that bind to region I (Kimura *et al.*, 1986; Baldwin *et al.*, 1988; Israel *et al.*, 1989; Potter *et al.*, 1993). Gel mobility shift assay showed that spleen and liver tissues showed

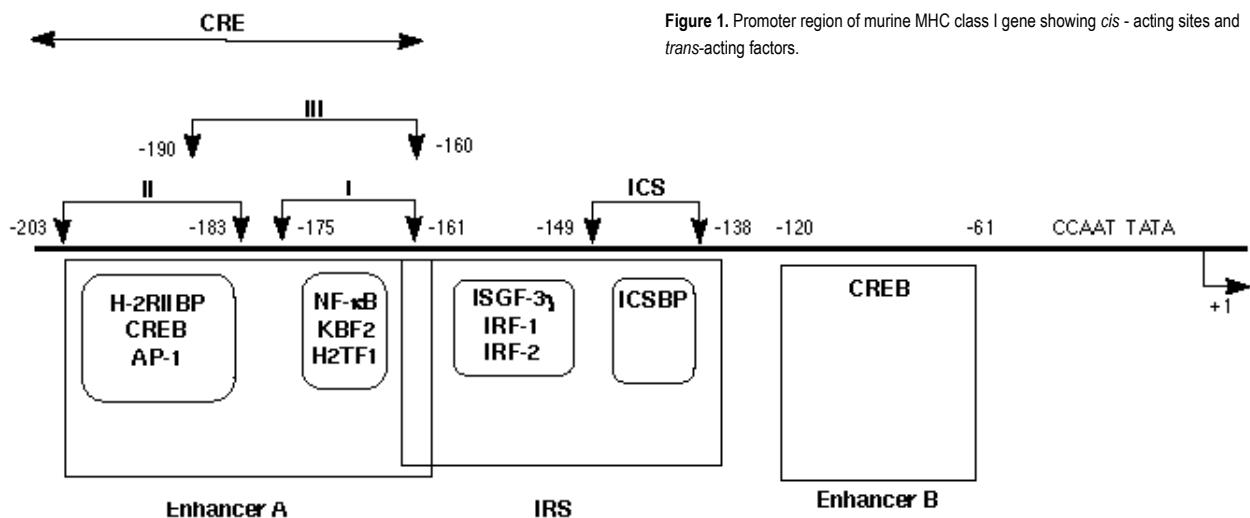


Figure 1. Promoter region of murine MHC class I gene showing *cis*-acting sites and *trans*-acting factors.

binding activity to region I but extract from brain did not show region I binding activity. Region II binding factors are namely AP1, CREB, H-2RIIBP (Roesler *et al.*, 1988; Nagata *et al.*, 1992). H-2RIIBP is a member of the nuclear hormone receptor superfamily and activates class I MHC expression in embryonal carcinoma cells in response to retinoic acid. Result of reporter gene CAT assay with mutant CRE constructs showed that the mutation that eliminated region I binding activity greatly impaired enhancer activity. Mutation that eliminated region II binding also caused a lesser but measurable effect. This indicated that both the region I and II are capable of enhancing class I MHC gene. Interestingly, fetal tissue that express very low levels of class I MHC, did not show any region I binding activity though it showed region II binding activity. Binding activity to region I becomes detectable during neonatal period when class I MHC expression sharply increases.

By *in vivo* footprinting of HLA-B7 transgene and the endogenous H-2Kb gene it has been shown that the upstream regions of both the transgene and the endogenous gene were extensively occupied in spleen tissue and no occupancy was detected in brain tissue (Dey *et al.*, 1992). Sites exhibiting *in vivo* protection corresponds to the *cis*-acting elements involved in regulation of class I MHC gene. This kind of negative regulation allows for rapid increases in transcription in response to immunomodulators e.g. IFN- γ induces expression of class I MHC expression. As stated before, cells of central nervous system do not express class I antigens but showed induction of class I MHC expression in response to IFN- γ and TNF (Drew *et al.*, 1993). Gel retardation assays show that these cytokines increased factor binding to distinct sites within regulatory elements of class I MHC gene and independently enhanced the expression of the gene. This suggests that the absence of class I MHC expression in the cells of central nervous system could be either due to absence of positive regulatory factor or due to presence of other factors ("silencer") which prevents the binding of the positive regulatory factor to the corresponding enhancer element in the upstream sequences of class I MHC genes.

Mechanism of regulation of class I MHC gene by IFN- γ

IFN- γ receptors

Both type I (IFN- α and β) and type II (IFN- γ) IFNs influence MHC I expression, but they bind different cell surface receptors (Langer *et al.*, 1988, Bazan *et al.*, 1990). Both murine and human IFN- γ receptors have been cloned and expressed (Auge *et al.*, 1988; Gray *et al.*, 1989; Hemmi *et al.*, 1989; Kumar *et al.*, 1989; Munru *et al.*, 1989). IFN- γ receptors are 90 kDa glycoproteins and are present on nearly all cell types. Based on nucleotide sequence

data, mature human and murine IFN- γ receptors consist of 472 and 451 amino acids, respectively and share 52% sequence homology. IFN- γ receptor as well as MHC I genes are present on the human chromosome 6. However, presence of chromosome 6 in somatic cell hybrids is insufficient to confer sensitivity to IFN- γ as assayed by class I MHC induction. The presence of both human Chromosome 6 and 21 are required in the hybrid to generate a response to human IFN- γ (Jung *et al.*, 1987, Jung *et al.* 1990, Hemmi *et al.*, 1992). Similarly when murine IFN- γ receptor on Chromosome 17 was expressed on human cells, it did not respond to murine IFN- γ . Only the presence of mouse Chromosome 16 exhibits complete concordance with MHC antigen expression in response to murine IFN- γ (Hibino *et al.*, 1991). This shows that both human and murine IFN- γ receptors are themselves not sufficient to generate a biological response and they require an accessory factor(s), which is also responsible for the species-specificity of the receptor.

IFN induced protein kinases

Binding of IFNs with their respective receptors activates the Jak-Stat signal transduction pathway. Jak1, Jak2 and Tyk2 are the three members of the JAK (Janus Kinase) family of kinases which are activated in response to IFN. Jak1 (Wilks *et al.*, 1991, Muller *et al.*, 1993) and Jak2 (Silvennoinen *et al.*, 1993; Wating *et al.*, 1993) are associated with the IFN- α receptor and Jak1 and Tyk2 (Velazquez *et al.*, 1992) are associated with type II IFN receptors. On binding of the ligand to its receptor, these kinases are activated, which in turn brings about activation of Stat (signal transducers and activators of transcription) family of proteins. Stats are the latent cytoplasmic proteins which are activated as a result of phosphorylation of their tyrosine residues.

Stat1 (both a and b) and Stat2 are the two major signal transducers of Stat family involved in the activation of IFN inducible genes. After stimulation of cells by IFN- γ , a multiprotein transcription factor ISGF-3 (IFN stimulated gene factor-3) is quickly activated in cytoplasm (Kessler *et al.*, 1990). This multiprotein complex is made up of four proteins, which are 91 kDa (Stat 1a), 84 kDa (Stat 1b), 113 kDa (Stat 2) and 48 kDa (ISGF3- γ) (Fu *et al.*, 1992; Schindler *et al.*, 1992). ISGF-3 complex translocates to the nucleus and binds the ISRE (IFN stimulated response element) present in the promoter region of class I MHC genes.

In case of IFN- α , only the 91 kDa (Stat 1a) is phosphorylated (Shuai *et al.* 1992, Pearse *et al.*, 1993). Phosphorylated Stat1 dimerizes, translocates to the nucleus and binds the IFN- α activation sites (GAS) in the promoter regions of IFN responsive genes, like class I MHC gene. GAS site was first identified in guanylate binding protein (GBP) gene (Lew *et al.*, 1991). IFN- α causes transcriptional activation of GBP gene and a mutation in

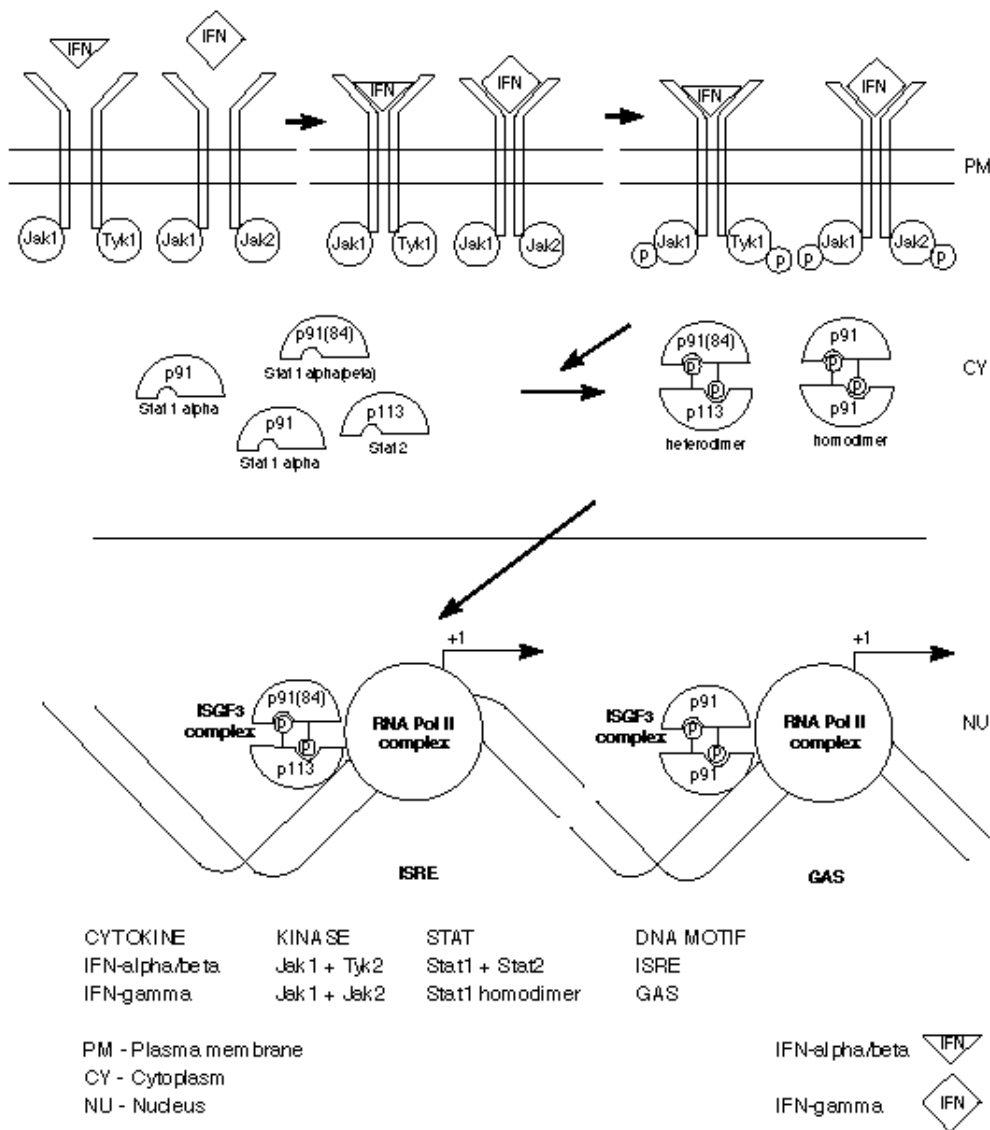


Figure 2. Molecular mechanism of MHC gene activation in response to alpha, beta and gamma interferons. Dimerization of receptors results in the activation of Janus (Jak) kinases which phosphorylate Stat proteins. Phosphorylated and dimerized Stat proteins migrate to nucleus and bind ISRE and GAS DNA motifs.

the GAS region blocks the activation.

Jak 1 is activated by both types of IFNs and is responsible for the phosphorylation of Stat 1. Tyk 2 activated by type I IFN, is the kinase for Stat 2. Although, phosphorylation of Stat1a occurs in response to both IFN- and IFN- , the specificity of the signalling is maintained because the Stat 2 is phosphorylated in response to IFN- alone and not in response to IFN- . At the cytoplasmic level therefore, Stat 1 homodimers and Stat 1-Stat 2 heterodimers determine the specificity of the generation of type II and type I IFN response respectively.

Cis-acting regulatory sequences

Detailed analyses of the mouse MHC class I promoter has revealed several regions that are important for the

expression and regulation of this gene through specific DNA-binding proteins (Friedman *et al.*, 1985, Israel *et al.*, 1986, 1987, Shirayoshi *et al.*, 1987, Sugita *et al.*, 1987, David-Watine *et al.*, 1990) (Figure 1, Table1). Mainly there are two enhancer like sequences, A (-203 to -161) and B (-120 to -61). Enhancer A overlaps with an IFN response sequence, IRS (-165 to -139, also called ISRE). Both enhancer sequences are conserved within the promoter regions of several genes coding class I MHC antigens. A strong homology has been found in the 5' promoter sequences of H-2Kb, H-2Ld, H-2Dd as well as HLA-B7 genes indicating that regulation of class I MHC expression could be similar in both mouse and humans. Using deletion analysis of murine H-2Dd promoter, it was found that in general both enhancer A and IRS regions are required

for gene activation in response to type I IFNs, whereas IRS region appears to be sufficient for upregulating MHC I expression in response to type II IFN (Korber *et al.*, 1987). However, there are differences in different experimental systems. In L cells and BLK SV cells, the action of type I IFN requires the presence of both enhancer A and IRS but in BL5 cells, IRS region appears to be sufficient by itself (Korber *et al.*, 1988). Enhancer B is involved in tissue specific expression of class I MHC genes (Chamberlain *et al.*, 1991). Another enhancer element, IFN consensus sequence (-149 to -138) is present within the IRS and is found in a number of IFN-inducible genes (Shirayoshi *et al.*, 1988, Driggers *et al.*, 1990). There are some reports that regions located 3' to the transcription start site are also involved in the regulation of class I MHC genes (Korber *et al.*, 1987, Chamberlain *et al.*, 1991). The 3' regulatory sequences could be involved in posttranscriptional events such as an increase in RNA stability (Friedman *et al.*, 1984) or in the rate of MHC class I mRNA translation. Region I of CRE, which overlaps with IRS, binds a TNF- α induced transcription factor belonging to the NF- κ B/rel family, inducing MHC I expression (Drew *et al.*, 1993). Thus IFN- γ and TNF- α induce transcription factors which bind to distinct *cis*-elements regulating MHC I gene expression. This explains the synergistic effect of IFN- γ and TNF- α on the expression of MHC class I antigens.

Trans-acting regulating factors

A number of transcription factors bind to the *cis*-acting elements described above, and regulate transcription of class I MHC gene. Amongst these, IFN regulatory factors (IRF) family of proteins plays an important role as they bind to the upstream regulatory sequences of the MHC I genes on treatment with IFNs. IRF-1 (37.3 kDa) and IRF-2 (39.5 kDa) are the first IRF members to be isolated (Miyamoto *et al.*, 1988, Nelson *et al.*, 1993). ISGF-3, which is a constituent of ISGF-3 and activated in response to only type I IFN, is also a member of the IRF family of transcription factors. Yet another important member of the IRF family is the IFN consensus binding protein (ICSBP) (Driggers *et al.*, 1990), which is a 48 kDa protein 45-47% sequence homologous to IRF-1 and IRF-2 over the N-terminal 115 amino acid residues, which constitutes the DNA binding domain.

DNA sequence motifs in ISRE bind members of IRF family of transcription factors. Type I and type II IFN induced transcription factors bind overlapping yet distinct sites of ISRE. IRF-1 and IRF-2 bind the IRS (-165 to -139) region of the MHC I genes in response to IFN- γ stimulation. IRF-1 activates the transcription of class I MHC genes, whereas IRF-2 represses IRF-1 mediated induction of class I MHC genes (Fugita *et al.*, 1989, Harada *et al.*, 1990). ICSBP binds to ICS region present within IRS and

represses the transcription of MHC I genes (Weisz *et al.*, 1992, Kanno *et al.*, 1993, Nelson *et al.*, 1993). While both IRF-2 and ICSBP represses the expression of MHC I genes, they differ in their tissue distribution. ICSBP is predominantly present in the cells of lymphocyte and macrophage lineage (Driggers *et al.*, 1990, Politis *et al.*, 1992, Nelson *et al.*, 1996), whereas IRF-2 appears to be expressed more broadly (Harada *et al.*, 1989, Harada *et al.*, 1990). Furthermore, ICSBP expression is predominantly induced by IFN- α and not by type I IFN (Driggers *et al.*, 1990, Politis *et al.*, 1992) while IRF-1 and IRF-2 are generated in response to both type I and type II IFN.

Constitutive expression of ICSBP in promonocytic cells and B cells may be responsible for the low level of expression of class I MHC gene. Stimulation with IFN- γ overrides this negative regulation and results in the enhanced expression of class I MHC antigens. The attenuation of repression can be explained by two possible mechanisms. IFN- γ may induce transcription activators which either displace ICSBP, or associates with ICSBP through a protein-protein interaction which now acts as an activator. Association of IRF family of proteins with each other has been demonstrated (Sharf *et al.*, 1995). ICSBP was shown to tightly associate with IRF-2, conferring a cooperative DNA-binding activity upon both the proteins. The association of ICSBP with IRF-1 is less stable and does not result in cooperative binding.

The picture that emerges from various studies is that the expression of class I MHC gene can be regulated through several pathways. Different transcription factors bind to different *cis*-acting sites depending on the kind of signal and the type of cell. Various *trans*-acting factors regulating class I MHC expression are induced by different types of IFN. How these different factors contribute to the fine tuning of class I MHC gene under physiological condition has yet to be fully understood.

Acknowledgement

Work in the authors' laboratory supported by a DBT grant. AR and NP got UGC fellowships.

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