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Transcriptional regulation of *HLA-A* and *-B*: Differential binding of members of the Rel and IRF families of transcription factors

(baculovirus/interferon)

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ABSTRACT **HLA-A and -B transplantation antigens can be expressed differentially at the basal level and in response to interferons (IFNs). To determine which DNA control elements and nuclear factors are responsible for these differences, *HLA-A* and *-B* upstream regulatory regions were used in expression and mobility-shift analyses. The HLA-A enhancer was found to contain two Rel (KBF/NF-κB) binding motifs, while the HLA-B enhancer has only one and is transactivated less well by overexpression of the NF-κB p65 subunit. On the other hand, the HLA-B IFN response element mediates a much stronger induction by IFNs and has a higher affinity for IRF-1 and -2, which are transcription factors implicated in the regulation of major histocompatibility complex class I genes. These results suggest a molecular basis for the way in which *HLA-A* and *-B* loci have adapted to be differentially expressed and to respond to different sets of cytokine signals.**

HLA class I genes (*HLA-A*, *-B*, and *-C*) encode cell surface glycoproteins which are responsible for presenting antigenic peptides to the receptors of cytotoxic T lymphocytes and are also major targets for tissue graft rejection (1). Consistent with their general role in immunosurveillance of pathogens, class I antigens are expressed by most cell types, although basal levels vary substantially (2). Class I protein and mRNA levels can be induced by interferons (IFNs) α, β, and γ (3, 4) and tumor necrosis factor α (5), which is presumed to lead to increased efficiency of recognition by cytotoxic T cells (6, 7). Constitutive class I levels are low in some transformed cells but are induced by IFN treatment (8), and restoration of expression by gene transfection leads to reduced tumorigenicity and metastatic activity (9, 10).

Therefore, the expression of class I loci is subject to cell type-specific controls, as well as modulation by cytokines and viral factors. The main upstream control elements of class I genes include the enhancer (ENH), responsible for basal class I expression (2), and an interferon response element (IRE) which mediates induction by IFNs α, β, and γ (11). Class I ENHs are bound by members of the Rel family of transcription factors such as KBF1, a homodimer of Rel p50, and NF-κB, a heterodimer of Rel p50 and p65 (2, 12). Rel binding sites are found upstream of many immune-response genes and can mediate regulation by a number of cytokines, as well as agents such as phorbol esters (12). The IRE is bound by members of the IFN response factor (IRF) family, which are induced by IFNs, and include both an activator, IRF-1, and repressors, IRF-2 and IFN consensus sequence-binding protein (ICS-BP), of transcription (13, 14). Overexpression of IRF-1 has been shown to increase the basal and IFN-induced levels of class I expression (15, 16).

HLA-A and *-B* genes are highly homologous but exhibit locus-specific differences in both their transcribed (17) and upstream regions (18, 19) and are not tightly coordinated in their regulation. They can differ in basal and induced levels of expression, with cortical thymocytes and related thymomas such as MOLT-4, for example, expressing low levels of *HLA-A* but undetectable *HLA-B* (20), while *HLA-B* loci respond more strongly to IFNs (21, 22) and are subject to stronger suppression by c-*myc* expression (23). We report here that some of the locus-specific differences between the ENH and IRE of *HLA-A* and *-B* have significant effects on the binding of the Rel and IRF families of transcription factors and consequently on the regulation of the two loci.

MATERIALS AND METHODS

Cell Culture, Transfection, and Chloramphenicol Acetyltransferase (CAT) Assays. *MOLT-4* and YHHH (24) cell lines were maintained in Dulbecco's modified Eagle's medium, and JM and Daudi in RPMI 1640 medium, all with 5% fetal bovine serum. Induction of NF-κB in JM cells was achieved by a 2-hr treatment with phytohemagglutinin (PHA, 5 μg/ml; Sigma) and phorbol 12,13-dibutyrate (PBt_2, 50 nM; Sigma). Transfections and CAT assays were performed as described (25). Cells were harvested 20 hr after transfection. IFN-αA (kindly provided by M. Brunda, Hoffmann–La Roche) was added after transfection at 2000 units/ml where indicated.

Plasmids. CAT reporters were produced by cloning synthetic oligonucleotides into an *Sph* I site introduced into the *Bgl* II site at the 5′ end of the metallothionein promoter of pMCAT3 (26) and verified by double-stranded sequencing. The Rel p50 and p65 expression plasmids (27) were kindly provided by A. Baldwin (University of North Carolina, Chapel Hill) and the CMV-GalVP16 vector (28) by P. O'Hare (Marie Curie Research Institute, Oxted, Surrey, U.K.).

Electrophoretic Mobility-Shift Assays. Nuclear extracts from cell lines were prepared (29) and mobility-shift assays were performed (25) as described.

Baculovirus Expression of IRF-1 and IRF-2. Human IRF-1 and IRF-2 clones were obtained from the lymphoid lines HUT78 and MOLT-4, respectively, by two rounds of amplification by polymerase chain reaction (PCR) from oligo(dT)-primed cDNA. The first rounds employed primer pairs based on the published sequences for human IRF-1 (30) and IRF-2 (31). The second rounds used primers which introduced *Bam*HI sites immediately 5′ to the initiation codon and 3′ to the termination codon. The PCR products were cloned into the *Bam*HI site of the baculovirus transfer vector p36C (32), verified by sequencing, and used to generate recombinant virus by standard methods (33). Seventy-two hours after infection, *Spodoptera frugiperda* (Sf9) cells ($1–5 \times 10^6$) were

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Abbreviations: CAT, chloramphenicol acetyltransferase; ENH, enhancer; IFN, interferon; ICS, IFN consensus sequence; ICS-BP, ICS-binding protein; IRE, IFN response element; IRF, IFN response factor; MHC, major histocompatibility complex; PBt_2, phorbol 12,13-dibutyrate; PHA, phytohemagglutinin.

washed twice in phosphate-buffered saline, scraped into 10 ml of ice-cold phosphate-buffered saline, and collected by centrifugation. The cell pellet was extracted immediately or frozen on dry ice and stored at −70°C. Cells were extracted by passage through a 26-gauge needle 10 times after suspension in 1 ml of lysis buffer [100 mM KCl/20 mM Hepes, pH 7.4/0.5 mM dithiothreitol/5% (vol/vol) glycerol/0.1% Nonidet P-40/5 mM NaF/10 mM $Na_4P_2O_7$/0.2 mM phenylmethylsulfonyl fluoride with leupeptin (5 μg/ml) and pepstatin (5 μg/ml)]. The homogenate was microcentrifuged for 15 min, and the supernatant was stored in aliquots at −70°C. Proteins were separated from excess insect cell DNA in some preparations by application of the supernatant onto a heparin-agarose column (Sigma) and elution with Dignam buffer C (29).

Measurement of Binding Affinities. Fixed amounts of protein extracts, typically 0.1 μl, were incubated at room temperature with ^{32}P-end-labeled oligonucleotides at 0.15–660 nM in 8 μl of 25 mM KCl/50 μM EDTA/5 mM Hepes, pH 8.0/5% glycerol with poly(dI·dC) (0.06 μg/μl). The complexes were resolved by electrophoretic mobility-shift assay using 5% acrylamide gels (25), and quantified by analysis of exposed films with a Molecular Dynamics 300A densitometer.

RESULTS

Comparison of Major Histocompatibility Complex (MHC) Class I Upstream Control Sequences. Alignment of the ENH/ICS region of MHC class I genes (Fig. 1) shows the conservation of a symmetric Rel recognition motif. However, outside of this motif there are locus-specific differences between *HLA-A* and *-B*, and protein binding and expression studies were performed to determine whether they are functionally significant.

ENH Binding Studies. The Rel-site oligomer yielded a complex presumed to be NF-κB (45) with extract from PBt_2/PHA-induced JM cells (Fig. 2*A*, lanes 1 and 2) and a constitutive band, DC, with Daudi extract (lane 3). On close examination, DC was seen to migrate slightly faster than the JM NF-κB band and may represent another combination of Rel family members. DC (lane 4) formed with the A5′ oligomer, although less strongly than with the Rel site (Fig. 2*B*, lanes 3 and 4), whereas the B5′ oligomer did not produce DC (Fig. 2*A*, lane 6) or compete for the formation of DC (Fig. 2*B*, lanes 5 and 6). Similar results were obtained with the NF-κB in JM cell extract. Therefore, the HLA-A ENH appears to have two Rel recognition motifs, while the HLA-B ENH has only one.

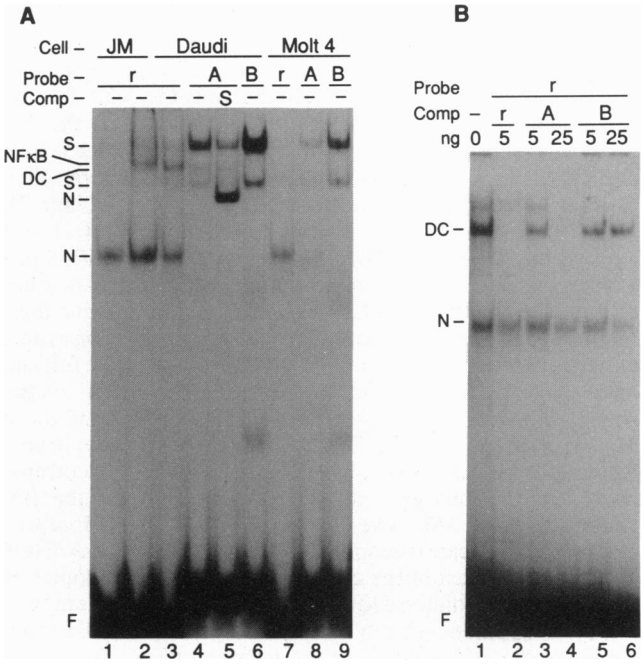

FIG. 2. Binding of nuclear factors to *HLA* class I ENH sequences. Electrophoretic mobility-shift assays were performed with nuclear extracts from human lymphoid cell lines incubated with DNA probes (see Fig. 1; r-Rel, A-A5′, B-B5′). The JM extracts are from uninduced cells (lane 1) and PBt_2/PHA-treated cells (lane 2). A 40-fold excess of nonradioactive Sp1-site DNA (S) was added to the reaction mixture of lane 5. Nonspecific bands are labeled as N, and free probe as F. S, NF-κB, and DC bands are discussed in the text. (*B*) Daudi extract was incubated with Rel probe in the presence of 5 or 25 ng of nonradioactive competitor DNAs.

MOLT-4 nuclear extract did not produce specific complexes with the Rel site (Fig. 2*A*, lane 7), even after PBt_2/PHA stimulation (data not shown), but did produce the bands labeled S with both A5′ and B5′ oligomers (lanes 8 and 9). These bands were also formed with Daudi extract (lanes 4–6), and JM and HeLa extracts (data not shown) and are believed to result from the binding of Sp1 or related proteins (46). The

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A                    ENH                                ICS
                     ___________________________________________________
HLA-A1,2   cATTGGGGAGTCCCAGCCTTGGGGATTCCCCAACTCCGCA***GTTTCTTTTCTCCCTCTCCCgcatg  (34, 35)
    A3     -------------------*-----------------------------------CTG-TC       (36)
    Aw24   ------------------------------*--T-------------------------G----    (37)
    B35,57 ---------G-G----G--------------*----*--CGA-----AC----***------       (38)
    B27    ---------G-G--CAG--------------*----*--CGA-----AC----***------       (39)
 H2-D,L    GG------AG-----GGC-------------T----T----------AC----***G-A--T       (40, 41)
   -K      GG------AG-----GGC-------------T----A----------AC----***G-A--T       (42)
                 5'          Rel                  IRE

B
ENH-A   cTTGGGGAGTCCCAGCCTTGGGGATTCCCCAACTgcatg
ENH-B   cTTGGGGAGGCGCAGCGTTGGGGATTCCCCACTCgcatg
rel     cGTTGGGGATTCCCCACgcatg        ICS-A   AACTCCGCAGTTTCTTTTCTCCCTCTCCC
A5'     cATTGGGGAGTCCCAGCgcatg        ICS-B   CTCCCACGAGTTTCACTTCTTCTCCCAAC
B5'     cATTGGGGAGGCGCAGCgcatg        ICS-BM  ----------A-A------
SVkB      cTGGGGACTTTCCAgcatg         ISRE    GATCCTTCAGTTTCGGTTTCCCTTTCCC

SP1     cAGGGGCGGGGAAgcatg
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FIG. 1. (*A*) Upstream MHC class I sequences containing the ENH and ICS. Dashes indicate identity with the *A1* sequence, and asterisks are spaces introduced to optimize alignment. Lowercase letters indicate residues added for cloning into the CAT reporter plasmid. References are given in parentheses. (*B*) Sequences of oligonucleotides used for electrophoretic mobility-shift assays and expression studies. A5′ and ICS-A are derived from the A1 sequence, and B5′ and ICS-B are derived from the B57 sequence. The IFN-stimulable response element (ISRE) is from ISG15 (43), and the Sp1 oligomer represents a high-affinity binding motif (44). SVκB represents the NF-κB site of the simian virus 40 early promoter.

S bands, but not DC, were competed for by an Sp1-site oligomer (Fig. 2*A*, lane 5), although in the experiment shown this competition was incomplete and intensified a nonspecific band. Soong and Hui (46) reported that only the *HLA-B* ENH was bound by Sp1-like proteins, but we found that the A5′ motif was recognized, albeit with lower efficiency than is B5′.

ENH Activity. MOLT-4 cells have no nuclear factors which can bind specifically to the ENH Rel site (Fig. 2*A*, lane 7), which correlates with their low basal level of *HLA* class I expression. Therefore, MOLT-4 cells were used with a p65 expression vector to determine whether the different number of Rel sites between *HLA-A* and *-B* ENHs affects their relative transcriptional potential. CAT reporter constructs were prepared with oligonucleotides representing the full and half ENHs of *HLA-A* and *-B* and a tandem repeat of the simian virus 40 NF-κB site (see Fig. 1). None of these reporters produced significant CAT activity when transfected into MOLT-4 cells, but all were activated by cotransfection with a p65 expression vector, except for the B5′ construct (Fig. 3*A*). The simian virus 40 NF-κB repeat mediated the greatest response, with the full HLA-A ENH being the strongest of the class I sequences. Three copies of the A5′ motif mediated a low level of activity, consistent with its lower apparent affinity for the Rel factors, while three copies of the B5′ motif were not transactivated. Therefore, the presence of a second Rel site in the *HLA-A* ENH can contribute to its increased transcriptional activity *in vivo*.

In contrast to p65, expression of Rel p50 in MOLT-4 cells did not transactivate any of the reporters (data not shown), although it did result in the formation of several nuclear factors which bound to the Rel site (Fig. 3*B*, lane 2). This apparent transcriptional inactivity of p50 is consistent with findings with other cell types and reporters (27, 47). p65 expression produced Rel-site complexes with mobilities which, although very similar to those induced by p50, were seen in the original autoradiograms not to be identical (lane 3). Coexpression of p50 and p65 resulted in levels of transactivation similar to those with p65 alone (data not shown) and production of a nuclear complex (lane 4) which comigrated with the NF-κB complex induced in JM cells (data not shown).

We were not able to detect transcripts for p50 by Northern blot or PCR analysis of MOLT-4 RNA but did find evidence for expression of mRNAs for MAD3 (IκB), c-Rel, Rel p50B, and, indeed, Rel-p65 (data not shown). Since p50 overexpression did not transactivate any of the class I ENH reporters, the absence of p50 does not appear to account for the low *HLA* class I expression in MOLT-4 cells. Overexpression of p65 does result in strong transactivation of the class I ENHs, which indicates that the endogenous p65 is present in insufficient amounts or is repressed by inhibitors such as the IκB proteins (48).

ICS Binding Studies. Oligomers representing the ICS regions of *HLA-A* and *-B* each bound several constitutive nuclear factors, some of which are locus-specific (Fig. 4). The band AC was seen only with the *HLA-A* ICS (lanes 1 and 2), whereas BC and R were unique to *HLA-B* (lanes 3 and 4). The band BC was found with Daudi, but not MOLT-4 extract (data not shown), and was competed for by the IFN-stimulable response element (ISRE) oligomer but not ICS-BM, which is mutant for the IRE site (lanes 5 and 6). As for the band R, it was specific for the R site of *HLA-B*, CACGAG, which overlaps the 5′ end of the IRE and is bound by the helix–loop–helix/leucine zipper protein USF (25).

Three bands (I1–3) were induced by IFN-α and were formed with both *HLA-A* and *-B* ICS oligomers (Fig. 4, lanes 1–4). I1 was undetectable before induction and is believed to represent ISGF3 (49). I2, I3, and the AC band were present before stimulation and were induced severalfold. The I3 band is presumed to be IRF-1, since it comigrated with human IRF-1 produced by expression from a baculovirus vector (hbvIRF-1) (lane 7). hbvIRF-2 gave a broader band which mostly migrated more slowly than hbvIRF-1 (lane 10). A distinct IRF-2 band was not resolved with the Daudi extract in Fig. 4 but was evident in nuclear extract from IFN-α-treated YHHH, a MOLT-4 derivative (24) (lane 13). The I1 and I3 bands and hbvIRF-1 and -2 were not competed for by the *HLA-B* ICS mutant, BM (lanes 5, 8, and 11) but were competed for by the ISRE oligomer (lanes 6, 9, and 12). Several faster migrating bands seen with the hbvIRF-2 extract (lane 10) were also competed for specifically (lanes 11

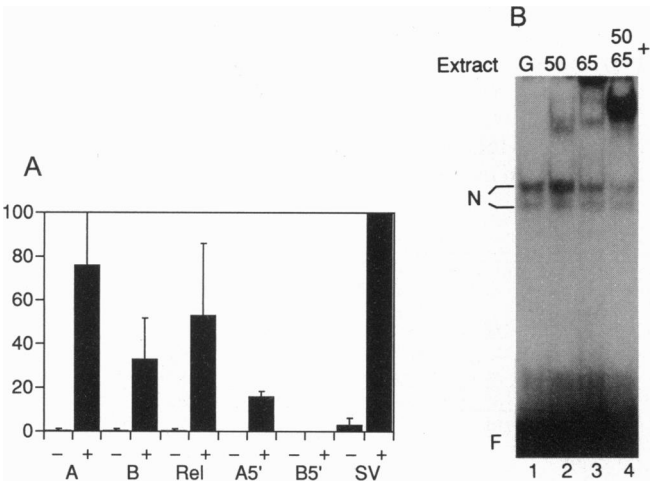

FIG. 3. (*A*) p65 transactivation of HLA class I ENH elements. The ENH-A (A), ENH-B (B), and Rel CAT reporter constructs contained a single copy of the oligomers shown in Fig. 1. The A5′ and B5′ oligomers were present in three copies, and the SVκB motif in two copies (SV). The + bars indicate cotransfection of the CAT reporter plasmids with 2 μg of a p65 expression plasmid. For comparison of CAT activity, transfections were performed in parallel and the SV+p65 value for chloramphenicol conversion was assigned as 100. Bars represent means with standard deviations from six experiments. (*B*) Nuclear extracts from MOLT-4 cells transfected with p50 and/or p65 expression plasmids, or a control Gal–VP16 (G) expression plasmid, were used in mobility-shift assays with the Rel-site probe.

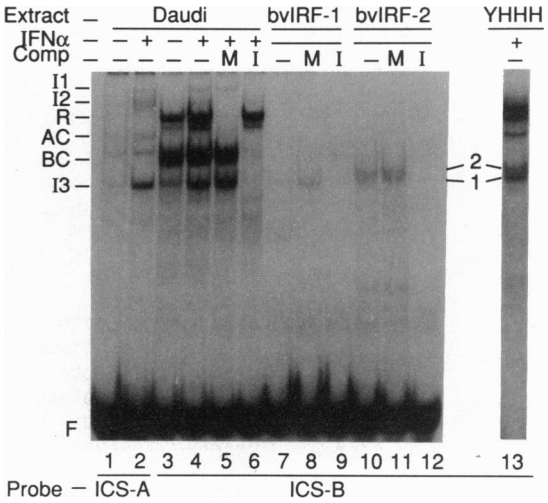

FIG. 4. Nuclear factors binding to the ICS regions. Nuclear extracts from Daudi and YHHH cells that were treated with (+) or without (−) IFNα for 2 hr, and IRF-1 and -2 produced by baculovirus expression (bv), were incubated with ICS-A or -B probes. Nonradioactive competitor DNAs (M, ICS-BM; I, ISRE) were present at 100-fold excess. Indicated bands are discussed in the text.

Table 1. Binding affinities of IRF-1 and -2 for *HLA* class I ICSs

	K_d, nM	
Extract	ICS-A	ICS-B
bvIRF-1	90 ± 17	34 ± 11.5
bvIRF-2	36 ± 10	13 ± 2.5

IRF-1 and -2 baculovirus extracts were titrated with the ICS-A and -B oligomers (Fig. 1) and resolved by mobility-shift assay. The concentrations of active IRF-1 and IRF-2 proteins in the incubations were estimated to be 1.5×10^{-10} M from the amount of DNA in the complexes at saturation, if the proteins bind as monomers. K_d values were defined as the DNA concentrations at half-maximal binding of protein and are given as means with standard deviations from at least three measurements.

and 12) and probably represented breakdown products which retained binding activity.

The *HLA-A* ICS is bound by IRF proteins and can mediate transcriptional activation by IRF-1 (15), although its IRE motif differs from the AGTTTCACTT sequence found with *HLA-B* and *H-2* loci. To determine whether this divergence results in a lower affinity for the IRF proteins, binding studies were performed with hbvIRF-1 and -2 (Table 1). Indeed, both proteins bound the *HLA-B* ICS with about 3-fold higher affinity, and hbvIRF-2 exhibited higher affinity than hbvIRF-1.

IFN-α Induction of *HLA-A* and *-B* Expression. *HLA-A* loci respond less well to IFNs than do *HLA-B* loci. To test whether this is due to the sequence differences which result in lower affinity of IRF proteins for the *HLA-A* IRE, CAT reporter constructs were made with the ENH/ICS regions of *HLA-A1* and *HLA-Bw57* (see Fig. 1) and with variants where the *HLA-A* IRE sequence was modified to that of HLA-B and vice versa (see Fig. 5). The constructs were transfected into the YHHH line, in which class I expression is low at the basal level but is strongly induced by IFN-α (24). The *HLA-A* and *-B* reporters did not show significant basal activity above background, but both were induced by IFN-α (Fig. 5). These constructs reflected the relative activities of the endogenous loci, with the HLA-B sequence mediating a significantly higher response. The TT → AC change in the IRE of our *HLA-A/B* construct (Fig. 5) produced an elevated basal expression over the *HLA-A* reporter and a level of induction even higher than that of *HLA-B*. The converse AC → TT change of the *HLA-B* sequence in the *HLA-B/A* construct reduced its inducibility, although the response was still somewhat greater than that of *HLA-A*. The reason why these results are so pronounced, while similar experiments performed by Hakem *et al.* (22) with Jurkat cells showed no IFN effect on *HLA-A* constructs and only a 2-fold increase on *HLA-B* constructs, is probably due to the substantially lower

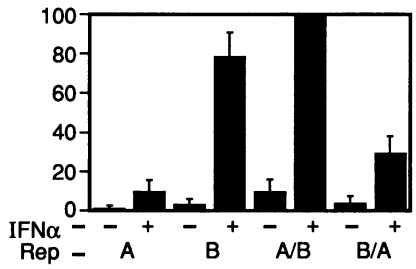

FIG. 5. IFN-α responses of *HLA* class I regulatory elements. YHHH cells were transfected with CAT reporter plasmids (REP) with the full ENH/ICS region (see Fig. 1) of *HLA-A1* (*A*) or *-B57* (*B*) and with variants in which the *A1* IRE was converted to GTTTCACTT (A/B) and the *B57* IRE to GTTTCTTTT (B/A). Activities were compared as in Fig. 3, with the value for the A/B construct assigned as 100. The bars represent means with standard deviations from six experiments.

basal *HLA* class I levels in YHHH cells than in Jurkat cells. Therefore, the difference in the magnitudes of the IFN-α responses of *HLA-A* and *-B* is determined primarily by the conserved changes in the IRE motif, but residues outside of the IRE core influence induction to some degree.

DISCUSSION

We have shown that several locus-specific differences between the upstream control regions of *HLA-A* and *-B* have significant effects on interactions with members of the Rel and IRF transcription factor families. The *HLA-A* ENH, in addition to the symmetric Rel site shared by all the major class I loci, has a second, weaker site. This results in the *HLA-A* ENH being transactivated more strongly than the *HLA-B* ENH by the Rel p65 subunit. On the other hand, *HLA-B* genes are induced more strongly by IFNs α and γ (21), due to conserved differences between the IRE sequences of *HLA-A* and *-B* loci (ref. 22 and this paper). Furthermore, we have shown that this differential induction correlates with a higher affinity of the transcription factors IRF-1 and -2 for the *HLA-B* IRE. Interestingly, the murine *H-2K*, *H-2D*, and *H-2L* genes all have ENHs most like HLA-A in that they have two Rel sites (2) and IREs with the same sequence as HLA-B, an arrangement similar to our *HLA-A/B* CAT reporter. The ENH and IRE of *H-2* genes act synergistically (11), and indeed, our *A/B* construct mediates the strongest response to IFN-α among those tested.

Israël *et al.* (5) showed that both Rel sites of the *H-2K* ENH were required for induction by TNF-α, which appeared to be mediated by NF-κB. NF-κB is also activated by viral and double-stranded RNA, interleukin 1, and tumor-promoting phorbol esters (12), suggesting that a number of agents may induce the *HLA-A* ENH to a greater extent than the *HLA-B* ENH. Furthermore, in addition to the p50 and p65 subunits of NF-κB, the mammalian Rel family has three other identified members (c-Rel, RelB, 50B) which can bind to DNA as homodimers, or as heterodimers with other family members (12). This large combinational diversity has functional consequences, since the different dimers have distinct DNA recognition specificities, as well as different transcriptional activities (50). Relevant to the IFN response, Rel proteins have been shown to interact functionally with other types of transcription factors, such as helix–loop–helix proteins (51), serum response factor (52), and human immunodeficiency virus Tat protein (47), again with differences seen between the family members. Since the ENH and IRE function synergistically (11), it will be important to determine which combinations of Rel proteins can cooperate with IRE-binding factors to mediate IFN-induced expression.

IRF-1 is a good candidate for mediating IFN induction of *HLA* class I genes, since it is present in nuclear extracts from IFN-treated cells and binds to class I IREs, and overexpression of IRF-1 has been shown to transactivate reporters driven by *HLA-B7* (16) as well as *HLA-A2* control sequences (15). Our finding that IRF-1 binds to the *HLA-A* IRE with lower affinity than to the *HLA-B* IRE correlates with the differential induction of *HLA-A* and *-B* genes by IFNs (21, 22). We find that the IFN-α-activated transcription factor ISGF3 (49) also binds to the class I IREs, but it does so with severalfold lower affinity than to the ISG15 ISRE (data not shown) and is not detected in cell lines such as YHHH which show a strong *HLA* class I response to IFN-α. Moreover, ISGF3 is activated rapidly (49), which does not correlate with the relatively slow induction of *HLA* class I genes (53).

Binding studies with baculovirus-produced IRF-1 and -2 showed that IRF-2, a repressor of transcription (13), bound the *HLA* IRE motifs with about 3-fold higher affinity than did IRF-1. Harada *et al.* (13) also found that IRF-2 bound with higher affinity than did IRF-1 to a synthetic multimer of

recognition sites. However, their bacterially produced IRF-1 and -2 bound with much lower affinity to the IFN-β virus-inducible ENH region (IRF-1, 327 nM; IRF-2, 370 nM) than did the baculovirus-produced proteins to the class I IREs. It is not known whether this is due to sequence differences between the binding sites or to differences between the proteins produced in bacteria and insect cells.

There is increasing evidence that HLA-A and -B proteins serve overlapping but distinct purposes, as exemplified by the matching for *HLA-B* alleles being more important for survival of kidney transplants (54, 55). This may be due to locus-specific differences between the class I proteins (17). However, quantitative differences in expression which lead to a higher probability of CTL recognition may also be an important factor. Increased expression can have deleterious consequences, as shown by the onset of diabetes (56) and thyroiditis (57) in transgenic mice overexpressing *H-2* class I genes in pancreatic β cells and thyrocytes, respectively, and spondyloarthropathies in transgenic rats expressing high levels of *HLA-B27* (58). Therefore, the differential regulation of *HLA-A* and *-B* expression by cell type-specific and cytokine-induced transcription factors is likely to have important consequences for immune function and pathology.

The divergence of the regulation of class I antigen expression is common to mouse and man (59). However, the way in which this has been achieved differs between the two species. Whereas differential regulation of *HLA-A* and *-B* is due to a small number of locus-specific differences in their ENH and IRE sequences, *H-2K* and *H-2D* loci have identical ENH and IRE sequences. In the case of mouse, however, the differential regulation of H-2 class I antigens appears to be controlled at the posttranslational level (60). It appears, therefore, that the gene duplication of the class I loci, with its associated increase in the gene pool, gives also the advantage of the possibility of differential regulation. The strategy for such regulation, however, differs between species.

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