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Functional Expression of a Heterologous Major Histocompatibility Complex Class I Gene in Transgenic Mice

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The regulated expression of major histocompatibility complex class I antigens is essential for assuring proper cellular immune responses. To study H-2 class I gene regulation, we have transferred a foreign class I gene to inbred mice and have previously shown that the heterologous class I gene was expressed in a tissue-dependent manner. In this report, we demonstrate that these mice expressed the transgenic class I molecule on the cell surface without any alteration in the level of endogenous H-2 class I antigens. Skin grafts from transgenic mice were rapidly rejected by mice of the background strain, indicating that the transgenic antigen was expressed in an immunologically functional form. As with endogenous H-2 class I genes, the class I transgene was inducible by interferon treatment and suppressible by human adenovirus 12 transformation. Linkage analysis indicated that the transgene was not closely linked to endogenous class I loci, suggesting that *trans*-regulation of class I genes can occur for class I genes located outside the major histocompatibility complex.

The major histocompatibility complex (MHC) class I antigens are highly polymorphic cell surface glycoproteins that play an indispensable role in the immune recognition of aberrant cells (for a review, see reference 17). The class I gene family is unique in that the estimated 40 to 45 members are tightly clustered within the MHC on mouse chromosome 17. This characteristic distinguishes it from other multigene families whose members are dispersed throughout the genome. The conservation of tight linkage of class I genes through evolution may have a functional significance with regard to their regulated expression.

The H-2 class I genes which encode transplantation antigens (designated K, D, and L) are coordinately expressed in most cell types, although the level of expression varies from tissue to tissue (13). In recent years, it has become clear that the expression of these genes is not static but is subject to induction and repression in response to a variety of physiologic stimuli (29). Cytotoxic T cells bear receptors that recognize non-self molecules on the surface of target cells only in the context of a self class I antigen (40). It appears that precise control of the level of expression of class I genes is essential for proper functioning of the immune recognition process.

Part of the immune defense strategy against virally infected cells is the production of interferons (IFNs), which have the effect of increasing the level of class I antigens in a wide variety of cell types (6, 9, 21, 27). As a consequence of this augmentation, presentation of foreign antigens to the appropriate immune effector cells may be facilitated. IFN appears to increase the accumulation of class I mRNA primarily by stimulating transcription (15), although post-transcriptional mechanisms also appear to be involved (11). Several in vitro gene transfer studies have been reported which have begun to define the *cis*-acting DNA elements required for IFN induction of class I genes (15, 37). These studies have identified an IFN-responsive sequence 5′ to the coding region of class I genes which, in concert with a specific enhancer, can confer IFN inducibility to heterologous genes. However, the inducibility conferred by these sequences was significantly less than that observed for endogenous class I genes, suggesting the possible need for additional 5′ intragenic or 3′-flanking sequences to achieve full induction. Further evidence for the involvement of non-5′ sequences comes from gene transfer experiments in which human class I genes with deleted 5′ regions maintained partial IFN inducibility when transferred to mouse cells (39).

Other physiologic agents known to increase class I expression have also been identified. They include retinoic acid, which induces the expression of class I genes in embryonal carcinoma cells through a mechanism that appears to involve DNA methylation (34), and tumor necrosis factor, which increases class I expression by a mechanism that is not well understood (8, 22).

While elevation of class I gene expression may augment immune presentation, suppression of these same genes appears to facilitate the escape of certain tumor cells from immune detection. This latter suggestion is supported by studies of different transforming viruses. Human adenovirus types 5 (Ad5) and 12 (Ad12) can efficiently transform cells in culture (2). However, Ad12-transformed cells but not Ad5-transformed cells can induce tumors in immunocompetent syngeneic hosts. One distinguishing feature of the two viruses is the ability of the early 1a (E1a) gene of Ad12 to suppress class I antigens, a property that is not seen with the E1a gene of Ad5 (3, 31). The observation that transfection and expression of a cloned class I gene in Ad12-transformed cells can effectively abrogate tumorigenicity in immunocompetent recipients suggests that suppression of class I antigens is responsible for the Ad12 tumorigenicity (35, 36). Attempts to define 5′ sequences from class I genes that are the target of the E1a product have yielded conflicting results (16, 38).

Other transforming viruses, including radiation leukemia virus (23) and AKR leukemia virus (30), also down-regulate class I gene expression upon transformation of thymocytes.

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It is interesting that a recent study demonstrated that the tumorigenicity of neuroblastoma cells is correlated with an increase in the expression of the N-*myc* oncogene and a decrease in the expression of class I genes (1).

The importance of defining the mechanisms involved in the up- and down-regulation of class I genes is paramount for our understanding of immune functions. Most of the experiments designed to gain insights into these mechanisms have involved manipulating class I genes in vitro by deleting potential control regions and returning the genes to established cell lines to assess their ability to respond to regulatory agents. While this approach can provide useful information regarding regulatory factors, gene transfer into whole animals allows a more accurate and complete analysis to be performed. Genes transferred to single-cell embryos can be subjected to the entire spectrum of developmental regulatory events in all cell types. It is possible that class I genes that have been developmentally conditioned function differently from genes transferred to cultured cells.

We have now taken the latter approach to examine the regulation of class I genes. We have transferred a foreign H-2 class I D^d gene into C57BL/6 (B6) mice and have shown that it is expressed at the mRNA level in all tissues in a manner that parallels that of the endogenous K^b gene (4). In this report, we determine the state of linkage between the transgene and the mouse MHC, and examine the cell surface expression of the transgenic product and its ability to mediate allograft rejection. We also examine the ability of the class I transgene to respond to IFN treatment and to Ad12 transformation.

MATERIALS AND METHODS

Mice. B6, BALB/c, and SJL strains were purchased from the Jackson Laboratory, Bar Harbor, Maine. The B10.YBR strain was a gift from D. Sachs, National Institutes of Health, Bethesda, Md. Transgenic B6 mice carrying the D^d gene cloned from a BALB/c mouse have been previously described (4).

Immunofluorescence staining and flow microfluorometric analysis. For quantitative analysis of expression of class I gene products on lymphoid cells, spleen cells (10^6) were washed three times with FMF buffer (phosphate-buffered saline containing 0.1% bovine serum albumin and 0.1% NaN_3) and were suspended in 50 μl of the following hybridoma culture supernatants: 28-8-6S (anti-K^bL^b) (26), 34-5-8S (anti-D^d) (25), and 30-5-7S (anti-L^d) (24). After 20 min on ice, the cells were washed three times with FMF buffer and then stained with fluorescein isothiocyanate-conjugated goat anti-mouse heavy-chain 2a of immunoglobulin G (Southern Biotechnology Associates) for 20 min on ice. After three washes with FMF buffer, the cells were suspended in 1 ml of phosphate-buffered saline, and the samples were analyzed in a FACS analyzer (Becton Dickinson Vacutainer Systems, Rutherford, N.J.).

Skin grafts. Skin grafts were performed by using an adaptation of the method of Billingham and Medawar (5). Grafts were scored daily until rejection or until day 100.

Cell lines. Primary cells were prepared from kidneys of newborn mice, essentially as described previously (10), and were maintained in a humidified 5% CO_2 incubator in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (GIBCO). Recombinant mouse gamma interferon (IFN-γ), obtained from Taidiro Komeno (Shionogi Seiyaku Institute, Osaka, Japan), was added to the medium at the indicated concentrations. Twenty-four hours after the addition of IFN-γ, cells were harvested by mechanical disruption and cell pellets were stored at −20°C. Transfection of plasmids pAd12RIC and pAd5XhoC (2), containing the E1a and E1b regions of Ad12 and Ad5, respectively, was carried out by calcium phosphate precipitation as described previously (14).

Northern blot (RNA blot) analyses. Total RNA extracted from cell lines by the method of Chirgwin et al. (7) was subjected to oligo(dT)-cellulose chromatography. Aliquots of approximately 1 μg of poly(A)$^+$ RNA were adjusted with 50% formamide–20 mM MOPS (morpholine propanesulfonic acid) (pH 7.0)–5 mM sodium acetate–1 mM EDTA and fractionated in a 1.0% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred from the gel to a nitrocellulose membrane and hybridized to ^{32}P-labeled oligonucleotide probes in a solution of 6× standard saline citrate (SSC) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt, and 10% dextran sulfate for 16 h at 42°C (20). Nitrocellulose filters were then washed in 6× SSC for 50 min at 42°C and exposed to X-ray films with intensifying screens at −80°C. Stripping of oligonucleotide probes was carried out by incubating the nitrocellulose filter in 0.1× SSC at 68°C for 15 min. A single-stranded actin probe was derived by primer extension with ^{32}P-labeled dCTP (36). Hybridization with the single-stranded probe was carried out in 50% formamide–5× SSC–10% dextran sulfate for 16 h at 45°C, and washes were done for 60 min in 3× SSC at 45°C.

Southern blot analyses. Mouse tail DNA was extracted using the proteinase K-sodium dodecyl sulfate (SDS) protocol as described previously (32). Aliquots (15 μg) of DNA digested with *Eco*RI (Pharmacia, Inc., Piscataway, N.J.) by recommended conditions of the manufacturers were fractionated in a 0.8% agarose gel for 20 h at 45 V, 100 to 150 mA. The gels were then irradiated with a shortwave UV light for 10 min, denatured, neutralized, and transferred to a nitrocellulose membrane. Hybridization was carried out in a solution of 40% formamide, 4× SSC, 10% dextran sulfate, 1× Denhardt, 0.05% SDS, and 10 mM Tris (pH 7.5) for 16 h at 45°C with a ^{32}P-labeled single-stranded class I cDNA probe (designated 8D) derived from the 3′ noncoding region which is known to detect the *K*, *D*, and *L* genes (19). Nitrocellulose filters were washed in 3× SSC–0.1% SDS at 45°C for 50 min, followed by a 5-min wash in 0.1× SSC–0.1% SDS at 68°C, and exposed to X-ray films with intensifying screens at −80°C.

RESULTS

We have previously described three lines of transgenic B6 mice carrying the D^d class I gene derived from a BALB/c mouse. The transgenic lines were termed D8, D19, and D24. From analysis of steady-state mRNA, we showed that these mice expressed the transgene in a tissue-dependent manner which paralleled that of the endogenous K^b class I gene (4).

Cell surface expression. To analyze the level of cell surface expression of the D^d transgene product and of endogenous class I molecules in the D8 and D24 transgenic lines, we stained spleen cells with a panel of monoclonal antibodies and measured specific antibody binding by flow cytometry (Fig. 1). Cells from B6 mice, the background strain into which the D^d gene was introduced, and from BALB/c mice, the original strain from which the D^d gene was cloned, served as controls. B6 cells showed a high level of specific staining with an anti-K^bL^b antibody (cf. panels a and b). BALB/c cells were not stained with the same antibody (cf.

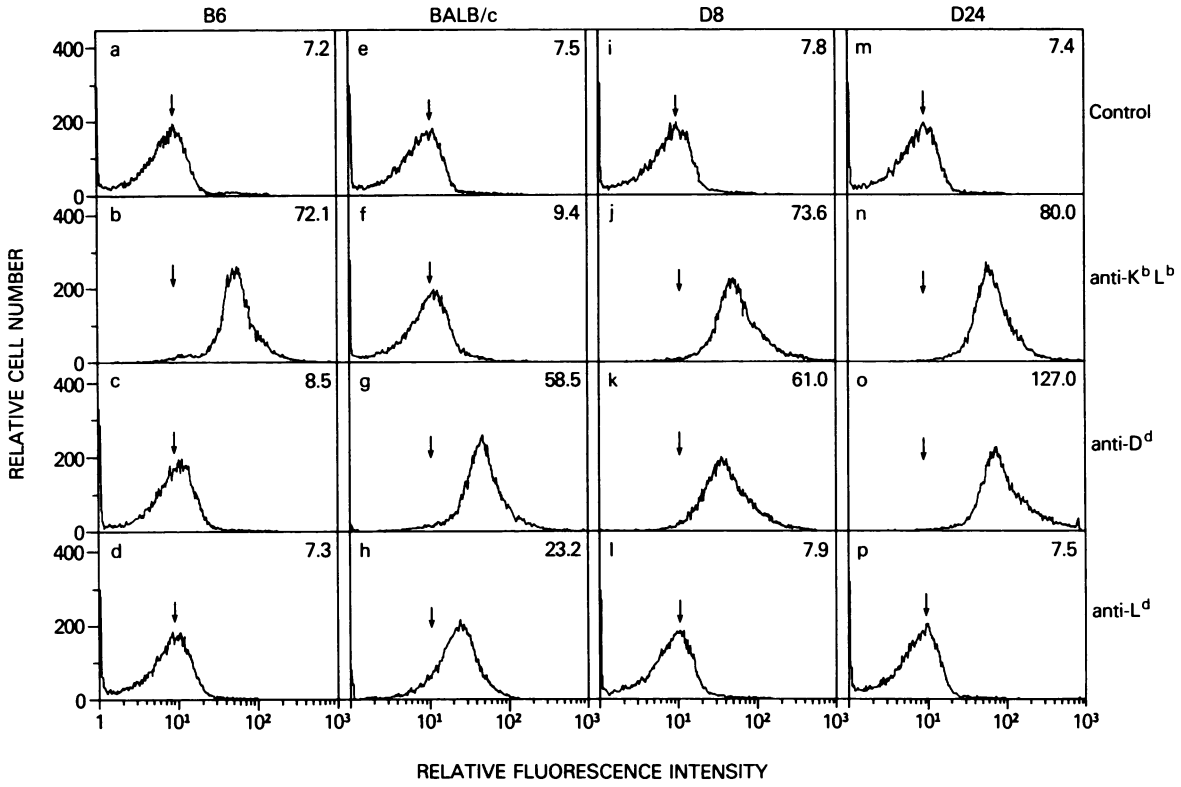

FIG. 1. Expression of class I antigens on the surface of spleen cells from D^d transgenic mice. Spleen cells from transgenic strains D8 and D24 and from control strains B6 and BALB/c were incubated with monoclonal antibody 28-8-6S (anti-K^{b}L^b), 34-5-8S (anti-D^d), or 30-5-7S (anti-L^d) and then stained with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin γ2a. Cells were subsequently analyzed on a FACS Analyzer (Becton-Dickinson). The background binding for each cell type was determined by staining cells with the secondary antibody alone and is indicated by an arrow. The number in each panel refers to the mean fluorescence intensity.

panels e and f), indicating its specificity for *b* haplotype antigens. BALB/c cells showed positive staining with an anti-D^d antibody (cf. panels e and g), whereas B6 cells were negative (cf. panels a and c). This result confirmed the *d* haplotype specificity of this antibody. In contrast, spleen cells from both D8 (panels j and k) and D24 (panels n and o) transgenic lines showed positive staining with both antibodies. The level of anti-K^{b}L^b-specific staining in cells from the transgenic mice (panels j and n) was not significantly different from that observed on B6 spleen cells (panel b); the level of D^d-specific staining in cells from D8 mice (panel k) was similar to that seen on BALB/c cells (panel g), while the level of D^d-specific staining on D24 cells (panel o) was consistently twofold higher than on BALB/c (panel g). A third D^d transgenic line, D19, showed a level of D^d staining intermediate between those of D8 and D24 (data not shown). A similar pattern of expression was also observed in cells from lymph node and from thymus. In all three transgenic lines, the level of combined K^{b}L^b antigen expression was not altered compared with the level seen in B6 mice, despite the efficient expression of the *D^d* transgene.

Allograft rejection. To determine whether the transgenic D^d product in D8 mice was expressed in a form that could be detected by the immune system of a B6 mouse, we carried out skin graft experiments. Recipient B6 mice were engrafted with tail skin from syngeneic B6 mice as a negative control for rejection, from congenic B10.YBR mice mismatched with B6 at both *D* and *L* loci as a positive control for rejection, and from transgenic D8 mice. Figure 2 shows that no B6 isografts were rejected within the 100-day observation period. However, B10.YBR allografts were rejected rapidly, with a median survival time of 11 days. All D8 allografts were also rejected, although the rate of rejection was somewhat slower (19 days) than that observed for B10.YBR allografts. The 100% rejection of D8 skin by B6 mice strongly suggests that the transgenic D^d product was expressed in tail skin in a form that was immunologically functional.

IFN induction. To examine the response of the transgenic

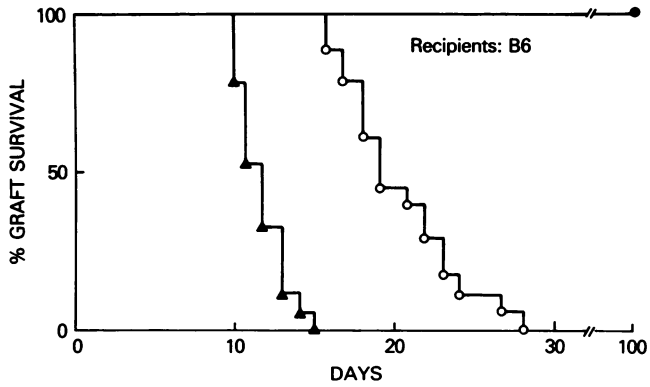

FIG. 2. Rejection of skin grafts from D^d transgenic mice by B6 mice. Tail skin from B6 (●), B10.YBR (▲), and D8 (○) strains was grafted onto B6 mice. The numbers of recipients were 17 for B6, 19 for B10.YBR, and 17 for D8 grafts. Grafts were scored daily until rejection or for 100 days.

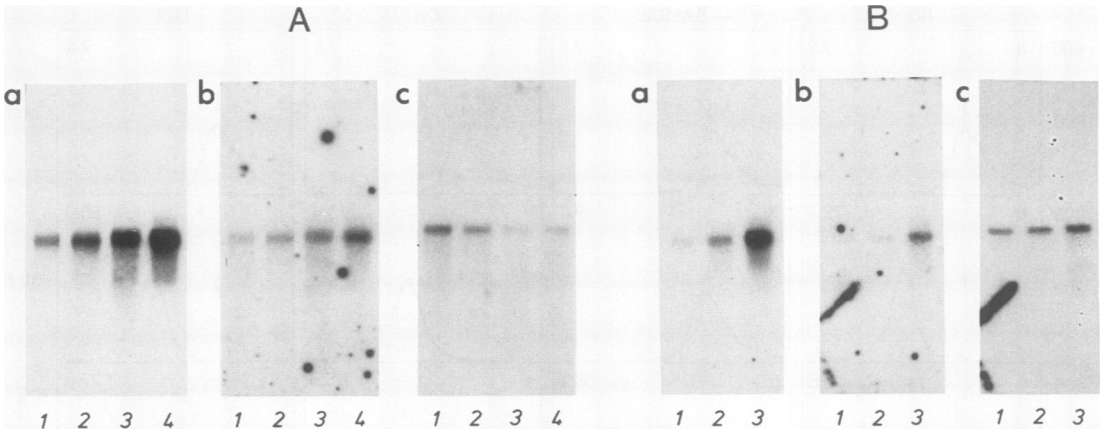

FIG. 3. Accumulation of class I mRNA in IFN-treated cells. Northern blot analysis of poly(A)$^+$ RNA isolated from untreated and IFN-treated primary kidney fibroblasts derived from D8 and D24 mice. Cells from D8 mice (A) were untreated (lanes 1) or treated with 100 (lanes 2), 300 (lanes 3), or 2,000 (lanes 4) U of IFN per ml. Cells from D24 mice (B) were untreated (lanes 1) or treated with 300 (lanes 2), or 2,000 (lanes 3) U of IFN per ml. The RNA blots were successively hybridized with ^{32}P-labeled D-specific oligonucleotide probe (panels a), K-specific oligonucleotide probe (panels b), and β-actin genomic probe (panels c). Densitometric analysis of autoradiograms was done with a laser scanning densitometer.

D^d gene to IFN, we treated primary kidney fibroblasts with various doses of IFN-γ for 24 h. The results of a Northern blot analysis of poly(A)$^+$ RNA extracted from IFN-treated and untreated cells from both D8 (Fig. 3A) and D24 (Fig. 3B) are shown. Panels a show hybridization of mRNA from D8 and D24 cells with a D-specific octadecanucleotide probe which detects mRNA from the D^d transgene. RNAs from untreated cells appear in lanes 1; an ~18S species, characteristic of class I transcripts, was detected. As the concentration of IFN in the culture medium was increased, the steady-state level of the D^d mRNA also increased (lanes 2 through 4). We estimated the extent of increase of the steady-state level of D^d-specific transcripts in D8- and D24-derived cell lines to be at least 20-fold on the basis of a densitometric analysis of autoradiograms.

The same nitrocellulose filter was then stripped of the D-specific probe and hybridized a second time with a K-specific probe to monitor transcription from the endogenous K^b gene. The steady-state level of the K^b mRNA also increased with increasing IFN dosage (panels b). Panels c show the same filters stripped of the K-specific probe and hybridized a third time with a β-actin probe to provide a reference for normalization of amounts of mRNA loaded in each lane. An ~20S species was detected in all lanes.

Ad12 suppression. Poly(A)$^+$ RNA extracted from D8 (Fig. 4A) and D24 (Fig. 4B) primary kidney fibroblasts transformed with the E1a from either Ad5 or Ad12 was analyzed by Northern blot analysis. Lanes 1 contain RNA from an Ad5 E1a-transformed cell line; the remaining lanes contain RNA from different Ad12 E1a-transformed cell lines. The expression of the D^d transgene in these cells was examined with a D-specific probe (panels a). An ~18S species characteristic of class I transcripts was detected in RNA from Ad5 E1a-transformed cell lines (lanes 1); greatly reduced levels of hybridization were observed in RNA from each of the Ad12 E1a-transformed cell lines (lanes 2 through 4). These data indicate that accumulation of the transgenic D^d transcript was suppressed by Ad12 E1a transformation but not by Ad5 E1a transformation.

The same RNA blot was then stripped of the D-specific

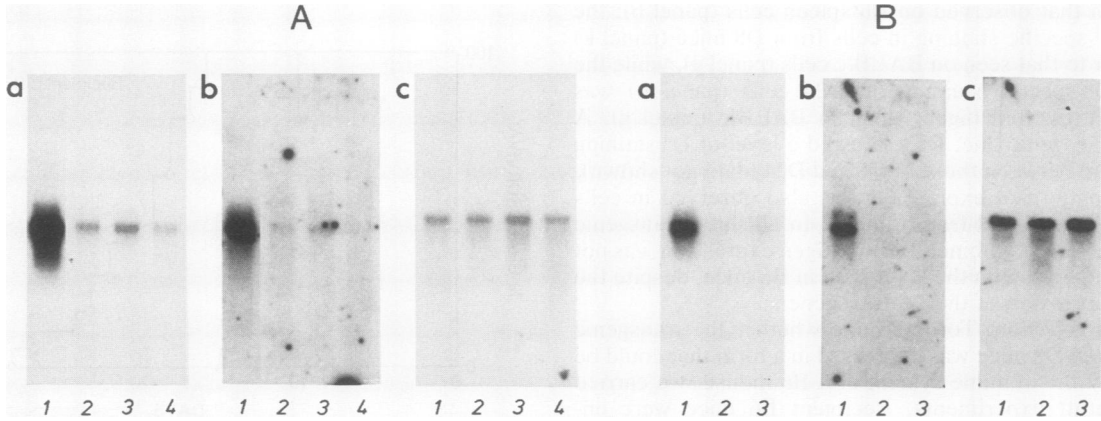

FIG. 4. Suppression of class I mRNA accumulation in Ad12 E1a-transformed cells. Northern blot analysis of poly(A)$^+$ RNA isolated from primary kidney fibroblasts transformed with either the Ad5 E1a or Ad12 E1a region. Cells from D8 mice (A) were transformed by Ad5 (lanes 1) or Ad12 (lanes 2 through 4). Cells from D24 mice (B) were transformed by Ad5 (lanes 1) or Ad12 (lanes 2 and 3). The RNA blots were successively hybridized with ^{32}P-labeled D-specific oligonucleotide probe (panels a), K-specific oligonucleotide probe (panels b), and β-actin genomic probe (panels c).

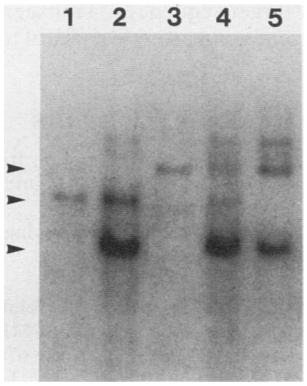

FIG. 5. Southern blot analysis of (D24 × SJL)F_1 × SJL offspring. *Eco*RI digests of genomic DNA (15 μg) were analyzed by Southern blot. The class I 3′ noncoding region probe (designated 8D) detects *K*, *D*, and *L* genes nondiscriminantly. DNA from a B6 mouse (lane 1), a D24 transgenic mouse (lane 2), and an SJL mouse (lane 3) were run as controls. DNA from two (D24 × SJL F_1) × SJL backcross progeny was run in lanes 4 and 5. The top arrow (12.5 kilobases) indicates the position of the major hybridizing component from SJL genomic DNA. The middle arrow (10 kilobases) marks the position of the major hybridizing component from B6 DNA. The lower arrow (8 kilobases) indicates the position of the band representing the transgenic locus. Lane 4 contains all three hybridizing bands. Lane 5 shows hybridization to the SJL (top arrow) and transgenic (lower arrow) bands.

probe and hybridized a second time with a K-specific probe to monitor the accumulation of transcripts from the endogenous K^b gene (panels b). Suppression of the K^b gene was also observed in all Ad12-transformed cell lines but not in Ad5-transformed cell lines. The RNA blot was then stripped of the K-specific probe and hybridized a third time with a probe which detects β-actin transcripts to determine the relative amounts of RNA loaded in each lane (panels c). An ~20S transcript was again detected at nearly equivalent levels in all lanes.

Linkage analysis. To determine if the site of integration of the D^d transgene in both the D8 and D24 strains was linked to the MHC on chromosome 17, we performed simple backcross analyses. D8 and D24 mice were separately bred to SJL mice, and the resulting F_1 progeny were then backcrossed to the SJL parent.

Using the restriction enzyme *Eco*RI in conjunction with a probe (designated 8D) which detects the *K*, *D*, and *L* class I genes, we have identified a restriction fragment-length polymorphism that allows us to unambiguously distinguish between B6, SJL, and transgenic MHC genotypes on the basis of the molecular weights of major hybridizing components. *Eco*RI digests of DNA from offspring of the (D8 × SJL)F_1 × SJL and (D24 × SJL)F_1 × SJL crosses were analyzed by Southern blot analysis, along with DNA from B6, SJL, and D8 or D24 mice as controls.

In Fig. 5, control DNA from B6 (lane 1), D24 (lane 2), and SJL (lane 3) mice are shown. The fragments detected by the 8D probe in B6 DNA (middle arrow) and SJL DNA (upper arrow) are derived from chromosome 17 and are clearly distinguishable from each other on the basis of gel mobility. The D^d transgene integration locus, represented by the lowest-molecular-weight band (lower arrow) can be seen in addition to the B6 band in DNA from D24 mice (lane 2). Lanes 4 and 5 contain DNA from backcross generation mice. If the D^d transgene had integrated into the genome close to the B6 MHC in the D24 founder mouse, the B6 chromosome 17 marker band (middle arrow) should cosegregate with the transgene marker band (lower arrow) in the backcross generation. Independent segregation would indicate a lack of linkage between the D^d transgene and the B6 MHC. Of 20 (D24 × SJL)F_1 × SJL backcross progeny screened, 8 have inherited the D24 integration locus. Of these eight mice, four have inherited the D^d transgene along with the B6 MHC from the F_1 parent (lane 4) and four have inherited the D^d transgene and the SJL MHC from the F_1 parent (lane 5). Similarly, of 29 (D8 × SJL)F_1 × SJL progeny that have inherited the D8 transgenic locus, 13 have also inherited the B6 MHC and 16 carry only the SJL MHC. These data demonstrate independent segregation of the transgene integration sites and the B6 MHC and allow us to conclude that the transgene is not closely linked to the B6 MHC in either the D8 or the D24 line.

DISCUSSION

Since regulated expression of class I antigens is indispensable for proper function of the immune system, it is important to understand the mechanisms which govern their expression. Germ line transformation of mice provides a powerful system to study gene regulation. Transgenic B6 mice carrying the D^d gene cloned from a BALB/c mouse can express the D^d antigen in a tissue-dependent manner (4). In this report, we have examined the level of cell surface expression of the transgenic class I antigen and of the endogenous K^b and L^b antigens on the same cells. These levels were compared with those seen in normal B6 and BALB/c mice. While the level of D^d antigen on spleen cells from the D8 transgenic line was similar to that seen on BALB/c spleen cells, the level on cells from the D24 line was twofold higher. In both D8 and D24 lines, the level of expression of the endogenous K^b and L^b antigens on spleen cells was the same as in B6 control cells. Although we cannot rule out the possibility that the level of either K^b or L^b antigen or both varied whereas the combined level of K^bL^b antigen remained the same, these data strongly suggest that the efficient expression of a transgenic class I antigen does not alter the level of expression of the endogenous class I genes. The number of class I molecules on a resting spleen cell must not be fixed, and β_2-microglobulin appears not to be limiting.

The rapid rejection of skin grafts from D^d transgenic mice on the background B6 mice indicates that the D^d product expressed in tail skin can be recognized as foreign and can stimulate a vigorous immune response. The median survival time of the D8 to B6 grafts was similar to that observed for K^b mutant skin allografts (28), which also differ from B6 at a single class I locus. B10.YBR allografts were rejected by B6 at a faster rate than D8 grafts were, perhaps because they were mismatched at both the *D* and *L* genes or because of a difference in the antigenicity of D^d and L^d antigens in this system.

Although recombination between a transgene and a homologous gene in the host genome has not been demonstrated in transgenic mice, transfection studies in cultured cells have suggested that such events may occur (12, 33, 37). The large number of endogenous class I genes, their high degree of homology, and the presence of recombinational hotspots in the MHC (18) could facilitate homologous recombination and led us to test for linkage between the D^d transgene and the B6 MHC in our transgenic lines. Southern blot analyses of backcross generation progeny clearly demonstrated that the transgene and the endogenous H-2 class I

genes are not linked in either the D8 or D24 strain. It appears that the mechanisms which regulate the expression of class I genes are not chromosomal site specific.

Transactivation of class I genes has been demonstrated in vitro, and recent experiments have begun to define the *cis*-acting DNA elements important for the IFN-mediated stimulation and Ad12-mediated suppression of class I mRNA accumulation. The general approach of these experiments has been to modify class I genes in vitro by deletion of putative control regions, followed by DNA-mediated gene transfer into established cell lines to determine the effects of the modifications. While a great deal of insight into regulation of class I gene expression can be learned through this approach, gene transfer into cultured cells has many inherent limitations. In particular, genes transferred to cultured cells circumvent the normal developmental process, which can clearly involve heritable changes in DNA, which may be critical for proper regulation of gene expression. By approaching the problem with in vivo gene transfer, we have been able to overcome this limitation imposed by cultured cells and have provided clear evidence that class I genes located outside of the MHC are regulated by IFN-γ and by Ad12.

The results presented here demonstrated that induction of MHC class I gene expression by treatment with IFN-γ and its suppression by transformation with Ad12 occurred for class I genes located outside of the MHC in primary cells from transgenic mice. Furthermore, the induction was similar to that seen for endogenous class I antigens. In Northern blot analyses of mRNA extracted from IFN-treated or Ad12-transformed cells, induction and suppression of the transgene were evident. It has been suggested that the tightly clustered arrangement of class I genes within the MHC may have some functional significance with regard to gene regulation (17). Initial experiments to elucidate the mechanism of Ad12 suppression of class I genes lent some credence to this hypothesis (36). Primary mouse cells transformed by Ad12 showed a dramatic decrease in class I gene expression. Since transfected class I genes were capable of escaping Ad12 suppression, it was possible that the Ad12 activity was specific for class I genes located within the MHC on chromosome 17. An obvious test of this hypothesis would be to first transfect a class I gene into primary cells, then ask if subsequent Ad12 transformation can suppress the introduced class I gene now located outside of the MHC. However it is not possible to carry out such an experiment because Ad12 suppression of class I genes occurs only in primary cells; in establishing class I transfectants, the cells become resistant to Ad12 modulation. The use of class I transgenic mice provides the only alternative approach to address this problem. In addition, our observations regarding Ad12 modulation of transfected genes underscores the need to interpret results from cell culture transfection studies with caution. Certain genes may be modified during development in a manner that renders them responsive or unresponsive to regulatory factors. Accurate assessment of *cis*- and *trans*-acting elements may sometimes require returning genes to animals.

We present evidence that class I genes located outside the context of the MHC can be regulated by IFN treatment and Ad12 transformation to the same degree as class I genes residing within the MHC. These observations argue strongly for mechanisms of class I gene regulation that involve *trans*-acting factors. Given that class I antigen expression is essential for the immune presentation of aberrant cells, it will be of utmost importance to define the nature of these factors and their target sequences. Transgenic mice carrying modified class I genes can provide a useful tool for approaching this problem.

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