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Regulated Expression of a Murine Class I Gene in Transgenic Mice

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The major histocompatibility complex class I genes play an essential role in the immune presentation of aberrant cells. To gain further insight into the regulation of the expression of these class I genes and to better define the functions of their protein products, we made use of the technique of gene transfer into the germ line of inbred mice. With the use of locus-specific DNA probes, we observed that a transgenic class I gene was expressed in a tissue-dependent fashion analogous to that of an endogenous class I gene. In addition, the level of expression of the transgenic gene was substantially higher that that of the endogenous gene. The availability of transgenic mice properly expressing a foreign murine class I gene provides a unique system to further define the role of the class I antigens in the maturation of the immune response and in determining the malignant and metastatic phenotypes of tumor cells.

The murine major histocompatibility complex (MHC) contains 25 to 35 class I genes organized into three clusters, the H-2, the Qa, and the Tla regions (7, 25). The H-2 region which is located on the centromeric side of the MHC encodes the highly polymorphic transplantation antigens, designated K, D, and L, which are found in association with β_2-microglobulin on the surfaces of virtually all nucleated cells. The Qa and Tla regions located on the telomeric side of the MHC encode antigens which are structurally related to the transplantation antigens but are less polymorphic and more restricted in tissue distribution.

The transplantation antigens are known to play an important role in permitting the cytotoxic T cells to recognize foreign antigens in the context of self (27). For that reason, a reduction in the level of class I gene expression may allow tumor cells to escape immune surveillance (18). The recent observation that cells transformed by the highly oncogenic human adenovirus type 12 escape immune recognition by turning off class I gene expression underscores the need to understand the regulation of these genes and the function of their products (19, 22).

The developmental regulation of class I genes has been investigated in embryonal carcinoma cells (21) and in early embryos (15). Studies in embryonal carcinoma cells have shown that expression of class I genes is positively correlated with the extent of DNA methylation (21). Other studes with DNA-mediated gene transfer into embryonal carcinoma cells have shown that an introduced class I gene is regulated appropriately during differentiation in tissue culture (17). A preliminary report has suggested that a porcine class I gene can also be expressed in a transgenic mouse (3).

To gain further insight into the regulation of class I genes and the function of their products, we introduced a D gene cloned from a BALB/c mouse (*H-2*^{d} haplotype) into C57BL/6 embryos (*H-2*^{b} haplotype) by microinjection. The C57BL/6 mouse was chosen as the recipient because it does not produce an RNA transcript that is recognizable by the D-specific DNA probe (10). The use of locus-specific oligonucleotide probes has allowed us to unambiguously detect and quantify the expression of the foreign D gene in transgenic C57BL/6 mice and to compare its expression with that of the endogenous K gene without cross-reactivity from any of the remaining class I transcripts. This approach provides a unique system to examine the regulation of the foreign class I gene in all tissue types.

The entire D^d gene present within an 8.0-kilobase (kb) *Eco*RI fragment was used in this study (11, 20). Approximately 500 copies of this *Eco*RI fragment were microinjected, essentially as previously described (4), into the male pronucleus of single-celled C57BL/6 embryos. Of 441 embryos transferred to CD-1 outbred recipients, 45 developed to term and 24 survived to weaning.

Tail DNA was isolated at 3 weeks of age and screened for the presence of the injected sequences by Southern blot hybridization with a cDNA probe (designated 8D) derived from the 3′ noncoding region of the K transcript (2). This probe has previously been shown to detect selectively only those class I genes which encode the H-2 transplantation antigens but does not discriminate between the K, D, and L genes (9). Transgenic founder mice were identified by the appearance of novel *Eco*RI fragments, particularly an 8.0-kb component which represents the entire microinjected DNA fragment. A total of four animals (D8, D19, D24, D25) carrying from 2 to 20 copies of the foreign gene were identified.

After *Eco*RI digestion, DNA from mice D19, D24, and D25 contained bands whose mobility was indistinguishable from that of the input DNA fragment, whereas DNA from mouse D8 had two bands whose mobility was similar but not indentical to the input fragment (Fig. 1A and B). These data suggest that the injected fragment was intact in the genomes of mice D19, D24, and D25, but not in mouse D8. Since the additional bands detectable in the DNA of mice D19, D24, and D25 did not segregate in the offspring, they must be derived from either a single integration site or from multiple, but closely linked, sites (Fig. 1). Comparison of the intensities of the bands representing the injected DNA to bands derived from endogenous genes, and to plasmid controls, indicated that the number of copies per cell of the transgenic sequence was 2 in mouse D8, 2 to 3 in mouse D19, 10 to 15 in mouse D24, and 15 to 20 in mouse D25.

Each of the founder mice was mated to C57BL/6 mice. Of the founder animals, three (D8, D24, D25) transmitted the D gene to their offspring in a Mendelian fashion, whereas mouse D19 appeared to be germ-line mosaic since its transmission rate was only about 12%.

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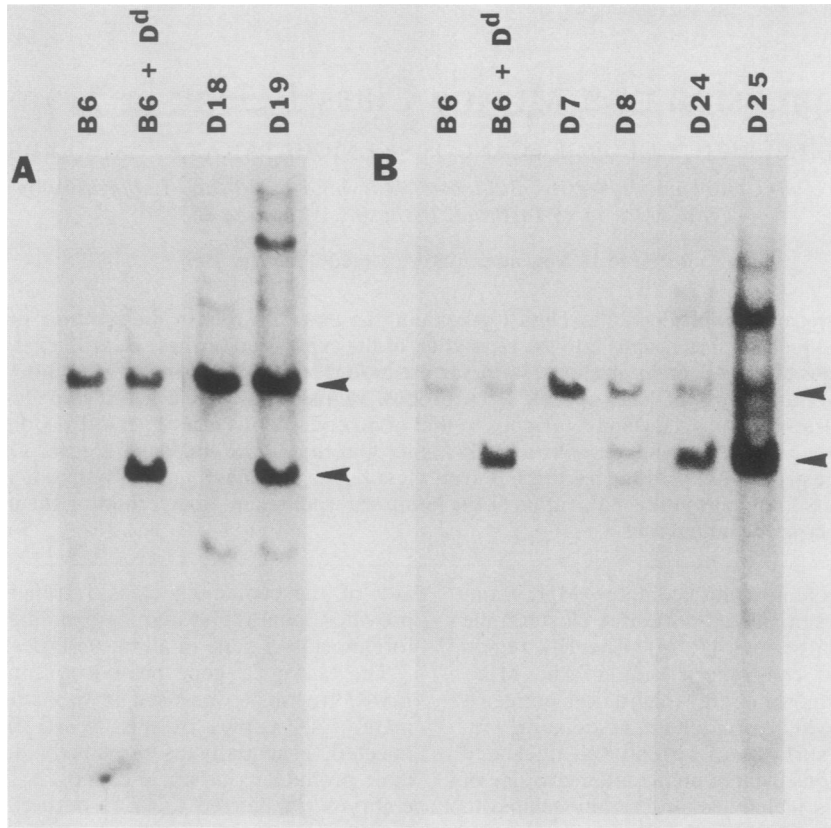

FIG. 1. Southern blot analysis of DNA from transgenic C57BL/6 mice. Tail DNA (10 μg) was digested with *Eco*RI and electrophoresed through an 0.8% agarose gel for 20 h at 50 V. The gel was then irradiated with a shortwave UV light for 10 min, denatured, neutralized, and blotted onto a nitrocellulose membrane. Hybridization was done with a ^{32}P-labeled class I cDNA probe (8D) that was derived from the 3′ noncoding region (2) and is known to detect nondiscriminately the K, D, and L genes (9). Hybridization was done in 40% formamide–4× SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10% dextran sulfate–1X Denhardt solution–0.2% sodium dodecyl sulfate for 16 to 20 h at 45°C. The filter was then washed at 68°C in 0.1X SSC–0.1% sodium dodecyl sulfate and exposed to an X-ray film. *Eco*RI-digested DNA (10 μg) from C57BL/6 (B6) mice, either alone or with 20 pg of the 8.0-kb *Eco*RI fragment containing the D gene (D^d), was analyzed in parallel as controls. The top arrow indicates the hybridizing component present in the C57BL/6 recipient mice, and the bottom arrow identifies the position of the 8.0-kb fragment that was microinjected. Panels A and B represent analyses done on separate days.

Transgenic offspring from each of the founder mice were analyzed for the expression of the foreign D gene. Northern blot analysis of poly(A)$^+$ RNA with a D-specific oligonucleotide probe (9, 10) revealed that three of the four transgenic lines (D8, D19, D24) expressed the D gene in a tissue-specific manner. Transgenic line D25 seemed not to express the transferred gene. The level of the D-specific mRNA in all three expressors was high in liver, lung, spleen, thymus, and kidney but much lower in brain and muscle. A representative Northern blot from a D19 mouse is shown in Fig. 2A. The differential expression of the transgenic D gene was found to parallel faithfully that of the endogenous K gene when a K-specific oligonucleotide probe was used on the same RNA blot (Fig. 2B). A similar distribution for the D^d transcript in BALB/c mice was also observed (data not shown). These RNA data agree well with previous serological studies of the tissue distribution of class I antigens (5).

To confirm that equivalent amounts of RNA from all tissues were analyzed, a cross-reactive actin DNA probe was used (Fig. 2C). Actin was selected as a control because it is expressed at high levels in a cell-cycle independent fashion in all tissues (23). In agreement with previous findings that actin transcripts in muscle cells are shorter that those in other tissues, we observed a faster-migrating component in the muscle lane (Fig. 2C).

From the specific activity of the oligonucleotide probes and the exposure times for the autoradiograms, it was estimated that the transgenic D gene in mice D8, D19, and D24 was expressed at a level significantly higher than that of the endogenous K gene. As has been shown with other genes transferred to mice, there was no correlation between the level of expression and the gene copy number (16). Comparable levels of the D-specific mRNA were detected in tissues of D8 and D19 mice, which contained 2 to 3 copies of the D gene per cell, as well as in D24 mice, which contained 10 to 15 copies per cell. Furthermore, D25 mice did not express the transgenic gene at a detectable level, despite its presence at 15 to 20 copies per cell.

Cell surface expression of the product of the D gene was analyzed in spleen and thymus cells from an offspring of mouse D8 by using a monoclonal antibody specific for the D^d antigen (Table 1). Flow microfluorometric analysis indicated that the transgenic D antigen was expressed on the surfaces of spleen and thymus cells at a level comparable to that found in the corresponding cells of BALB/c mice from which the D gene was cloned. The level of total class I antigen expression was also examined by using a monoclonal antibody which detects all class I molecules. In mouse D8, the overall level of surface class I expression was roughly 1.5 times that of a negative littermate (Table 1).

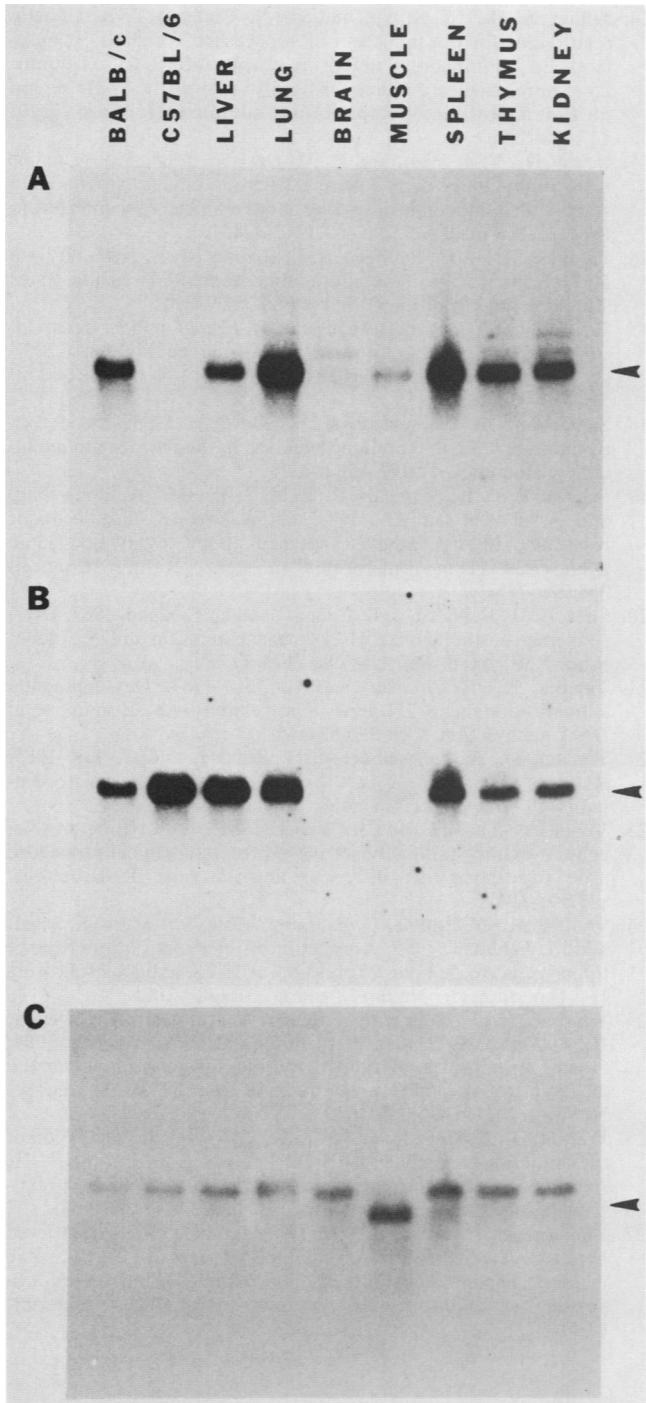

FIG. 2. Northern blot analysis of poly(A)$^+$ RNA from different tissues of a transgenic offspring of mouse D19. Total RNA was prepared by the method of Chirgwin et al. (1) and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. The poly(A)$^+$ RNA (5 μg) was fractionated on a 0.9% agarose gel in the presence of formaldehyde and transferred to a nitrocellulose membrane. The RNA blot was then hybridized successively to one of several ^{32}P-labeled probes as previously described (10). (A) End-labeled oligonucleotide probe specific to a region located within the transmembrane domain of the D gene (6-h exposure). (B) End-labeled oligonucleotide probe specific to a region located within the transmembrane domain of the K gene (18-h exposure). (C) Nick-translation probe derived from a human β-actin gene. The arrow indicates the position of 18S rRNA.

TABLE 1. Expression of the D^d antigen in tissues of a transgenic mouse[a]

Cells	Monoclonal antibody	Antibody dilution	Mean fluorescence[b]	
			B6	D8
Spleen	34.5.8	1:1	0.44	22.89
Thymus	34.5.8	1:1	1.26	14.51
Spleen	43.3.9.8	1:1	45.94	77.01
Spleen	43.3.9.8	1:5	52.30	80.27
Spleen	43.3.9.8	1:25	46.70	65.86
Spleen	43.3.9.8	1:125	10.29	16.96

[a] Cells from the spleen and thymus of a C57BL6 mouse (designated B67) and an offspring of mouse D8 were incubated with either a mouse monoclonal antibody specific to D^d (34.5.8) or a rat monoclonal antibody that has broad reactivity to class I antigens (43.3.9.8), stained with fluorescein-conjugated rabbit anti-mouse or anti-rat immunoglobulin G, and analyzed by flow microfluorometry with a FACS analyzer (Becton Dickinson and Co., Paramus, N.J.).

[b] The values represent the mean fluorescence observed with the appropriate antibody minus that observed with an irrelevant antibody.

The technique of gene transfer into inbred mice, combined with the use of locus-specific DNA probes, makes it possible to further examine two important aspects of class I genes: regulation of mRNA expression and, ultimately, the functions of the antigens produced. In this study, we focused on the former by comparing the expression of an exogenous class I gene with that of an endogenous one. Our results show that the foreign D gene, when introduced into the genome of C57BL/6 mice, was subject to the same tissue-dependent regulation that controls the level of expression of the endogenous K gene. We also show that the transgenic D gene was expressed more efficiently than the endogenous K gene. Because these transgenic genes probably are integrated outside of the MHC, our data suggest that the factors which regulate their tissue-specific expression were able to act in *trans*. In addition, the sequences which are important for regulation must lie within the 8.0-kb fragment which was transferred. It includes approximately 2.5-kb 5′-flanking and 2.0-kb 3′-flanking sequences.

The availability of transgenic mice expressing foreign murine class I genes provides a unique system for answering several important questions regarding the functions of class I antigens, particularly with regard to their role in the maturation of the immune response and in the malignant and metastatic phenotypes of tumor cells. By using these mice, we should be able to analyze the developmental regulation of the transgenic class I gene and its ability to induce self-tolerance. A previous observation that elevated expression of the D gene is correlated with resistance to leukemia (12, 13) can now be directly tested to determine whether any causal relationship exists. The suggestion that the D antigen may exhibit suppressive effects on the immune response of the host (6, 14, 26) and hence allow the escape of tumor cells from immunosurveillance can also be ascertained with these transgenic mice. If the nonexpression of class I genes is responsible for the malignant and metastatic phenotypes of tumor cells (8, 22, 24), it will be of utmost importance to determine whether the expression of the transgenic class I gene can be suppressed concommitently with the endogenous class I genes upon transformation by human adenovirus type 12 (19). A class I gene integrated outside of the MHC may be resistant to suppression and hence render a transformed cell immunogenic and nontumorigenic. Many such biological questions can now be approached with the use of transgenic animals.

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