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# Tissue-specific and cell surface expression of human major histocompatibility complex class I heavy (HLA-B7) and light ($\beta_2$-microglobulin) chain genes in transgenic mice

(major histocompatibility complex gene regulation/$\gamma$ interferon/thymocyte differentiation)

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**ABSTRACT** We introduced the human genes *HLA-B7* and *B2M* encoding the heavy (HLA-B7) and light [$\beta_2$-microglobulin ($\beta_2$m)] chains of a human major histocompatibility complex class I antigen into separate lines of transgenic mice. The tissue-specific pattern of HLA-B7 RNA expression was similar to that of endogenous class I *H-2* genes, although the *HLA-B7* gene was about 10-fold underexpressed in liver. Identical patterns of RNA expression were detected whether the *HLA-B7* gene contained 12 or 0.66 kilobase(s) (kb) of 5′ flanking sequence. The level of expression was copy number dependent and as efficient as that of *H-2* genes; $\gamma$ interferon enhanced HLA-B7 RNA expression in parallel to that of H-2. In addition to the mechanism(s) responsible for $\gamma$ interferon-enhanced expression, there must be at least one other tissue-specific mechanism controlling the constitutive levels of class I RNA. Tissue-specific human $\beta_2$m RNA expression was similar to that of mouse $\beta_2$m, including high-level expression in liver. Cell surface HLA-B7 increased 10- to 17-fold on T cells and on a subset of thymocytes from *HLA-B7/B2M* doubly transgenic mice compared to *HLA-B7* singly transgenic mice. The pattern of expression of HLA-B7 on thymocytes resembled that of H-2K as opposed to H-2D. These results confirm that coexpression of both human chains is required for efficient surface expression and that *HLA-B7* may share a regulatory mechanism with *H-2K*, which distinguishes it from *H-2D*.

The class I transplantation antigens encoded in the major histocompatibility complex (MHC) of man (HLA-A, -B, and -C) and mouse (H-2K, -D, and -L) are expressed at the cell surface as heterodimers of a polymorphic $M_r$ 43,000–45,000 glycoprotein heavy chain with a $M_r$ 12,000 light chain called $\beta_2$-microglobulin ($\beta_2$m) (1). Class I gene expression is regulated during development, between tissues and cell types in the adult, and in response to various lymphokines and viruses (1–3). The sequences and factors underlying expression of these genes have recently come under scrutiny (4, 5). We previously reported that HLA-B7 RNA and protein was expressed in transgenic spleen cells and that cell surface HLA-B7 was detectable on T cells from mice expressing HLA-B7 RNA at a level exceeding total H-2 RNA (6). We report here on the tissue-specific expression of *HLA-B7* and *B2M*, the gene encoding human $\beta_2$m (h$\beta_2$m), in transgenic mice and on the role of h$\beta_2$m in the surface expression of HLA-B7.

## MATERIALS AND METHODS

DNA fragments of *HLA-B7* (7) or *B2M* (8) genomic clones were microinjected into male pronuclei of one-cell (B6/SJL)-$F_1$, (B6/SJL)$F_2$, or (B6/D2)$F_2$ mouse embryos and reimplanted into pseudopregnant CD1 females (Charles River Breeding Laboratories) (6, 9, 10). Transgenic mice were mated with C57BL/6J mice to establish transgenic lines, and progeny were named as described (6).

Tail DNA (9) was analyzed for transgene sequences by dot (10) or Southern blotting (11). Tissue RNA was prepared by guanidium isothiocyanate extraction (11). Denatured RNAs (10 $\mu$g) were electrophoresed in formaldehyde/agarose gels and transferred to nitrocellulose (11) or were applied directly by slot-blotting (12). Hybridization of DNA and RNA blots was as described (6, 11, 12). The probes for *HLA-B7* DNA and RNA (probes 1 and 2 in Fig. 1) were as described (6). The probe for H-2 RNA was from exon 8 of a H-2K$^k$ cDNA clone (13). The first 310 nucleotides of this probe correspond to noncoding region 1 conserved among mouse class I genes. The remaining 138 bp correspond to the *H-2K* locus-specific noncoding region 2$\alpha$ (6, 14, 15). The probe for h$\beta_2$m DNA and RNA was a 0.54-kb *Pst* I fragment from a h$\beta_2$m cDNA clone (16). The probe for mouse $\beta_2$m (m$\beta_2$m) RNA was a 0.6-kb *Cfo* I fragment from a m$\beta_2$m cDNA clone containing about 265 bp of m$\beta_2$m sequence (17). Densitometric analyses of autoradiograms were carried out on Bioimage Visage 2000 and Joyce Loebl Chromoscan III densitometers.

Thymocytes and splenic T cells were obtained as described (6, 18). Single-color fluorescence-activated cell sorter (FACS) analysis was done as described (6). Dual-color FACS analysis was performed using fluorescein isothiocyanate-labeled goat anti-mouse IgG (HyClone, Logan, UT) and phycoerythrin-conjugated streptavidin (Becton Dickinson) (20). Evaluation of immunofluorescence was done by analyzing 20,000 cells with a Becton Dickinson FACS IV. The anti-HLA monoclonal antibodies (mAbs) used were ME-1 (anti-HLA-B7, -B22, -B27, and -B14) (21) and B9.12.1 (anti-HLA class I heavy chain encoded by most *HLA-A, -B,* and *-C* alleles in association with $\beta_2$m) (22, 23). The epitopes recognized by these mAbs are unaffected by the species of origin of associated $\beta_2$m (23). Other mAbs used were BBM.1 (anti-h$\beta_2$m) (24), Y-3 (anti-H-2K of most haplotypes) (25), B22/249 (anti-H-2D$^b$) (26), and Y-19 (anti-Thy-1) (27).

## RESULTS

Nine transgenic founder mice, representing 11 integration events, were derived from microinjection of a 6.0-kb *Eco*RI–*Bam*HI fragment of *HLA-B7* genomic clone pJY150 (7) (fragment a in Fig. 1). Two founders were derived from a 6.5-kb

Abbreviations: MHC, major histocompatibility complex; $\beta_2$m, $\beta_2$-microglobulin; h$\beta_2$m and m$\beta_2$m, human and murine $\beta_2$m; *B2M*, h$\beta_2$m gene; *B2m*, m$\beta_2$m gene; IFN-$\gamma$, $\gamma$ interferon; FACS, fluorescence-activated cell sorter; mAb, monoclonal antibody.
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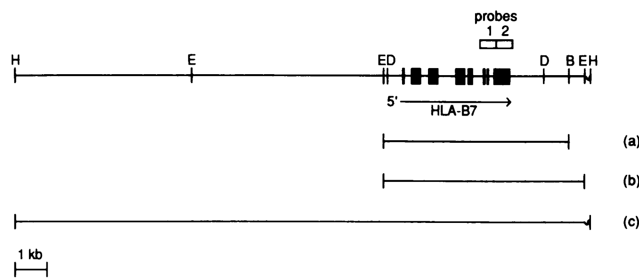


FIG. 1. Structure of *HLA-B7* DNA fragments used for microinjection. The uppermost line is a partial restriction map (B, *Bam*HI; D, *Dra* I; E, *Eco*RI; H, *Hin*dIII). The solid boxes represent exons 1 to 8. The open boxes mark the probes for DNA (probe 1) and RNA (probe 2). Lines a, b, and c represent the fragments microinjected into eggs. a and b were 6.0-kilobase (kb) *Eco*RI–*Bam*HI and 6.5-kb *Eco*RI fragments, respectively, and contained no vector DNA; c was an 18-kb *Hin*dIII fragment and contained 24 bp of vector DNA (represented by a short wavy line, not drawn to scale).

*Eco*RI fragment from pJY150 (fragment b in Fig. 1), and three were derived from an 18-kb *Hin*dIII fragment subcloned from the *HLA-B7* cosmid clone cos30 (7) (fragment c in Fig. 1). Injected fragments a, b, and c contained the intact gene with 0.66 kb (fragments a and b) or 12 kb (fragment c) of 5′ flanking sequence and 2.0 kb (fragment a) or 2.5 kb (fragments b and c) of 3′ flanking sequence. The copy number and intactness of the *HLA-B7* transgene were determined for each line by Southern blot analysis of *Dra* I-digested tail-skin DNA (Table 1).

**Expression of HLA-B7 RNA in Transgenic Mice.** RNA from tissues of transgenic offspring was analyzed by blot hybridization. The autoradiograms in Fig. 2 *A* and *C* were obtained with tissue RNAs from mice of lines 17 and 18A, respectively (see Table 1). A band of the expected size (1.6 kb) varying in intensity between tissues was detected in RNA samples from the transgenic mice and from human spleen (Fig. 2*A*, lane Hu.Sp). The tissue-specific pattern of HLA-B7 RNA expression generated with the fragments containing 12 or 0.66 kb of 5′ flanking sequence was indistinguishable. Lymphoid tissues and lung contained the highest level of B7 RNA. Relative to spleen, small intestine contained 20–30%; liver, heart, and kidney contained 5–10%; skeletal muscle and brain contained 1–3%; and pancreas contained <1% of the amount of B7 RNA. A similar pattern was observed for all mice examined from all lines carrying three or more copies of any of the three microinjected fragments. Corresponding tissues from mice of independent lines carrying a different number of copies of the same fragment contained levels of B7 RNA that varied over a 30- to 50-fold range (Table 1). Multiple offspring within a line (lines 18A, 34, and 65 were examined) expressed similar levels of B7 RNA with a similar tissue-specific distribution.

To compare *HLA-B7* expression to endogenous *H-2* class I genes, the blot of Fig. 2*A* was stripped of the HLA-B7 cDNA probe and rehybridized with a H-2 class I probe (Fig. 2*B*). The highest levels of 1.6-kb H-2 transcripts were detected in lymphoid tissues, liver, lung, and small intestine.

Table 1. Transgene copy numbers and expression in *HLA-B7* transgenic mice

| *HLA-B7* DNA fragment | Line no.* | Copy no.† | Expression of *HLA-B7*: RNA ratio‡ (B7/H-2) | Expression of *HLA-B7*: Surface protein§ |
|---|---|---|---|---|
| a | 18A | 30 | 5.0 | + |
| a | 18B | 25 | 3.6 | + |
| a | 34 | 3 | 0.4 | − |
| a | 35A | 7 | ND | ND |
| a | 35B | 1 | 0.1 | − |
| a | 65 | 1 | 0.2 | − |
| b | 176 | 7 | 1.9 | + |
| b | 179 | 10 | 2.4 | + |
| c | 2 | 10 | 1.5 | + |
| c | 7 | 7 | ND | + |
| c | 17 | 30 | 5.0 | + |

ND, not determined.

*The strains of the founder mice of each line were as follows: 18-(B6/SJL)$F_1$; 34,65,176,179-(B6/D2)$F_2$; 35,2,7,17-(B6/SJL)$F_2$.

†Transgene copy number was determined by dot- and Southern blot analysis of offspring tail DNA. When more than one integration was identified in a founder, the individual integrations were represented as A, B, etc.

‡The ratio of the level of HLA-B7 RNA (B7 RNA) to total H-2 RNA in spleen was determined by quantitating autoradiographic hybridization signal intensities. A ratio of 1 indicates a similar amount of B7 and total H-2 RNA. An estimate of the amount of B7 RNA per gene copy relative to H-2 RNA per gene copy is obtained by multiplying the B7/H-2 ratio in the table by ($X$/the no. of transgene copies), where $X$ represents the number (four, five, or six) of *H-2* class I genes per cell (*H-2K*, *-D*, and *-L* from both chromosomes). Mice of *b* or *s* haplotype have four conventional class I genes expressed, while mice of *d* haplotype have six class I genes expressed.

§Cell surface HLA-B7 protein was detected by indirect immunofluorescence of splenic T cells with the FACS. +, A significant shift in fluorescence intensity with anti-HLA monoclonal antibodies ME-1 and B9.12.1; −, no shift in fluorescence intensity.

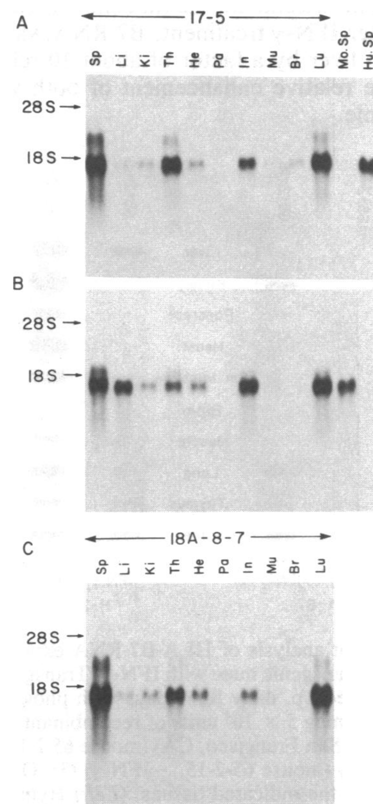


FIG. 2. Blot-hybridization analysis of HLA-B7 RNA in transgenic mice. RNA samples were from tissues of transgenic mice 17-5 (fragment c) (*A* and *B*) and 18A-8-7 (fragment a) (*C*). Lanes: Sp, spleen; Li, liver; Ki, kidney; Th, thymus; He, heart; Pa, pancreas; In, small intestine; Mu, skeletal muscle; Br, brain; and Lu, lung. Nontransgenic mouse spleen (lane Mo.Sp) and human spleen (lane Hu.Sp) RNAs were also analyzed (*A* and *B*). The autoradiograms in *A* and *C* were obtained by hybridization with HLA-B7 cDNA probe 2. The autoradiogram in *B* was obtained by stripping the blot in *A* and rehybridizing with the H-2 class I cDNA probe. The exposure times were 4 hr for all samples in *A* except Hu.Sp. RNA, which was for 10 hr; exposure times were 16 hr for samples in *B* and 4 hr for samples in *C*.

Relative to spleen, liver contained 80–90%, heart and kidney contained 10–15%, brain and skeletal muscle contained 1–2%, and pancreas contained <1% of the amount of H-2 RNA. Similar results were obtained for the nine lines analyzed (Table 1) and for nontransgenic siblings (not shown). There was less than a 2-fold difference in the ratios of the RNA blot signal intensities for HLA-B7 relative to H-2 (i.e., B7 RNA/H-2 RNA) for most tissues from mice for a given line. However, B7 RNA was about 10-fold under-represented relative to H-2 RNA in liver compared with other tissues and 3-fold under-represented relative to H-2 RNA in small intestine. The B7 RNA/H-2 RNA ratios for spleen RNA for most lines are given in Table 1. There was an almost linear relationship between the *HLA-B7* gene copy number and the level of B7 RNA detected. If all *H-2* class I genes contribute equally to the hybridization signals detected with the H-2 probe, then the level of B7 RNA in spleen from most lines is similar to the level of H-2 RNA on a per gene copy basis.

The effect of $\gamma$ interferon (IFN-$\gamma$) on HLA-B7 and H-2 RNA expression was analyzed for line 65 (Fig. 3). The levels of enhancement of B7 and H-2 RNA after IFN-$\gamma$ treatment were similar within a factor of 2 in each tissue examined except skeletal muscle and brain. The low level of expression of B7 RNA in this single copy line is likely responsible for these apparent exceptions. Similar results were obtained with offspring of line 34, including a similar enhancement of B7 and H-2 RNA in skeletal muscle (not shown) (see the legend to Fig. 3). After IFN-$\gamma$ treatment, B7 RNA was still under-represented in liver by a factor of about 10 relative to H-2 RNA, and the relative enhancement of both was approximately the same.

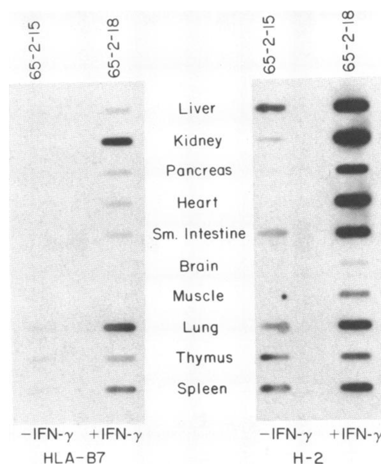


FIG. 3. Slot-blot analysis of HLA-B7 RNA expression after *in vivo* treatment of transgenic mice with IFN-$\gamma$. Transgenic siblings of line 65 were injected i.p. daily for 7 days with phosphate-buffered saline (PBS) containing $5 \times 10^4$ units of recombinant murine IFN-$\gamma$ (Genentech, South San Francisco, CA) (mouse 65-2-18, +IFN-$\gamma$) or PBS without IFN-$\gamma$ (mouse 65-2-15, −IFN-$\gamma$) (3). On day 8, RNA was prepared from the indicated tissues. (*Left*) Hybridization with the HLA-B7 probe 2 (film exposure time 24 hr). (*Right*) Hybridization with the H-2 class I probe (film exposure time 8 hr). The average enhancement of HLA-B7 RNA relative to H-2 RNA caused by IFN-$\gamma$ in various tissues,

$$\left[\frac{\text{B7 RNA } (+\text{ IFN-}\gamma)}{\text{B7 RNA } (-\text{ IFN-}\gamma)} \Big/ \frac{\text{H-2 RNA } (+\text{ IFN-}\gamma)}{\text{H-2 RNA } (-\text{ IFN-}\gamma)}\right],$$

for lines 65 and 34 offspring were as follows: liver (6.5/5.8), kidney (31.7/42.9), pancreas (28.3/32.2), heart (24.5/44.1), small intestine (6.0/8.3), brain (—/4.7), skeletal muscle (13.2/14.0), lung (7.5/5.4), thymus (1.3/1.8), and spleen (2.6/2.8). For RNAs from nontreated mice with no apparent hybridization signal, a value was assigned based on longer exposures of the same blot (H-2), or on the relative level of expression in the same tissue(s) of higher-expressing lines (HLA-B7).

**Generation and Analysis of *B2M* Transgenic Mice.** Cell surface HLA-B7 was detected only on cells from transgenic lines that expressed a 1.5- to 5-fold excess of HLA-B7 compared with total H-2 RNA (Table 1). The level of surface B7 for these lines was estimated to be less than H-2K or H-2D$^b$ by factors of 2–8. This implies that some posttranscriptional event(s) is limiting cell-surface expression of the human heavy chain. To examine whether altered association of HLA-B7 with m$\beta_2$m was in part responsible for this, we generated transgenic mice carrying *B2M*, the gene for h$\beta_2$m, and crossed these with *HLA-B7* mice. Nine *B2M* transgenic mice were produced from microinjection of a 14-kb *Pvu* I–*Sal* I fragment that contained the intact gene with about 2.9 and 1.2 kb of 5′ and 3′ flanking sequence, respectively, and 120 bp of pEMBL9 vector DNA at the 3′ end (8). Mouse 14 carried about 30 copies of the transgene and transmitted this in a Mendelian fashion (not shown). To examine the transcriptional activity of *B2M* in this line, tissue RNAs were examined as above (Fig. 4*A*). A band of about 1 kb was detected in human JY cell RNA (JY) and in transgenic tissue RNAs. The tissue-specific pattern of endogenous *B2m* (the gene encoding m$\beta_2$m) expression was very similar to that of the *B2M* transgene (Fig. 4*B*). Densitometric analysis indicated that there was about 2 times more h$\beta_2$m RNA than m$\beta_2$m RNA in tissues of line 14 mice.

Cell surface expression of h$\beta_2$m was analyzed by flow cytometry (FACS) with anti-h$\beta_2$m mAb BBM.1. Significant shifts in fluorescence intensity were obtained with T cells from mice of line 14 (Fig. 5*A*) and the three other *B2M* lines analyzed (not shown) but not with nontransgenic cells (not shown). The relative staining intensity with line 14 T cells was similar to that for human JY cells (not shown). These data suggest that h$\beta_2$m protein was efficiently expressed at the surface of transgenic cells.

**Cell Surface Expression of HLA-B7 and h$\beta_2$m in Doubly Transgenic Mice.** To determine whether coexpression of h$\beta_2$m would affect cell surface expression of the human heavy chain, T cells from doubly transgenic offspring from

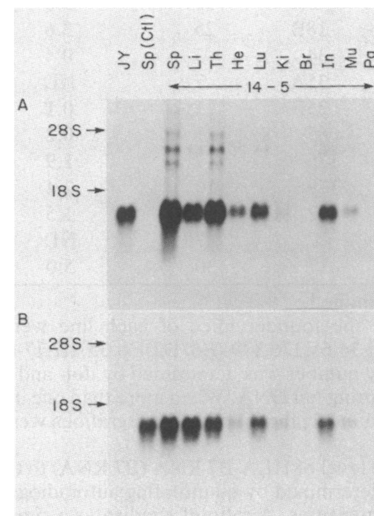


FIG. 4. Blot-hybridization analysis of h$\beta_2$m RNA in transgenic mice. RNA samples were from the following tissues from mouse 14-5: spleen (lane Sp), liver (lane Li), thymus (lane Th), heart (lane He), lung (lane Lu), kidney (lane Ki), brain (lane Br), small intestine (lane In), skeletal muscle (lane Mu), and pancreas (lane Pa). RNA from nontransgenic mouse spleen [lane Sp (Ctl)] and human B lymphoblastoid cell line JY (lane JY) were also analyzed. The autoradiogram in *A* was obtained by hybridization with a h$\beta_2$m cDNA probe. The autoradiogram in *B* was obtained by stripping the blot in *A* and rehybridizing with a m$\beta_2$m cDNA probe. Exposure times were 6 hr for samples in *A* and 12 hr for those in *B*.

crosses of line 18A *HLA-B7* females with line 14 *B2M* males were analyzed by FACS (Fig. 5). The level of HLA-B7 cell surface fluorescence was 10–17 times greater for T cells from doubly transgenic mice (Fig. 5*B*) compared with *HLA-B7* singly transgenic mice (Fig. 5*C*) (compare corresponding curves). The level of h$\beta_2$m on T cells was about 2 times greater for doubly transgenic mice than for *B2M* singly transgenic mice (Fig. 5 *A* and *B*). The levels of H-2K and H-2D$^b$ were similar for T cells from *HLA-B7/B2M* doubly and *B2M* singly transgenic mice (Fig. 5 *A* and *B*). This was 1.8–2.5 times greater than detected on T cells from *HLA-B7* singly transgenic (Fig. 5*C*) and control mice (not shown).

Thymocytes from 4- to 6-week-old transgenic mice were analyzed by FACS (Fig. 6). Thymocytes from *HLA-B7* singly transgenic mice of line 18A gave bimodal distributions when stained with mAbs for HLA-B7, H-2K, or H-2D$^b$ (Fig. 6*A*). About 15% of the population stained positively for HLA-B7 or H-2K at an intensity similar to splenic T cells, while the remaining 85% was negative (Fig. 6*A*). Two positive peaks representing ≈20% and 80% of the total population were obtained for H-2D$^b$ (Fig. 6*A*). Similar distributions for H-2K or H-2D$^b$ were obtained for nontransgenic thymocytes (not shown). Dual-color FACS analysis of thymocytes from *HLA-B7* mice with anti-HLA-B7 and anti-H-2K mAb showed that 15–20% of the population coexpressed both antigens, while the remaining 80–85% expressed neither (Fig. 6*C*).

All thymocytes (100%) from *HLA-B7/B2M* doubly or *B2M* singly transgenic mice (not shown) stained positively for h$\beta_2$m (Fig. 6*B*). About 15% of the cells stained as intensely as T cells from *B2M* mice, while the remaining 85% stained less intensely. Thymocytes from doubly transgenic mice stained for HLA-B7 generated a large negative peak representing ≈85% of the population. However, 10–15% of the cells were stained for HLA-B7 at an average level of intensity 6–7 times greater than that detected on thymocytes from *HLA-B7* singly transgenic mice (compare curves in Fig. 6 *A* and *B*). The level of fluorescence intensity of the most positive peak of B7-expressing thymocytes was similar to that for T cells from doubly transgenic mice (Fig. 5*B*). The level of expression of Thy-1 was similar for T cells (Fig. 5) or thymocytes (not shown) from all of the above mice.

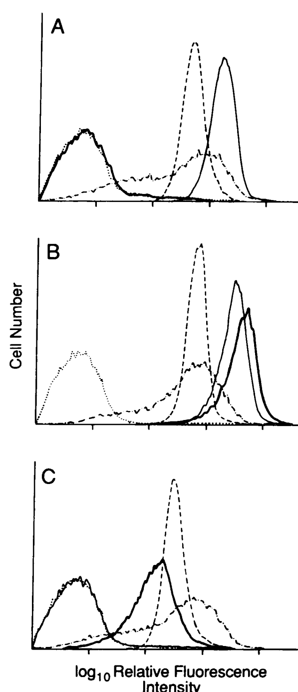


FIG. 5. FACS analysis of cell surface expression of h$\beta_2$m and HLA-B7 in transgenic mice. Splenic T cells from *B2M* singly (*A*), *HLA-B7/B2M* doubly (*B*), and *HLA-B7* singly (*C*) transgenic mice were analyzed by staining with specific mAb by FACS. The three panels show the results obtained with T cells stained with anti-HLA mAb B9.12.1 (━), anti-h$\beta_2$m mAb BBM.1 (—), anti-H-2K mAb Y-3 (- - - -), anti-Thy-1 mAb Y-19 (-·-·-·-) and with no primary antibody (·······). The *x* axes are divided into 256 channels spanning 5 $\log_{10}$ units.

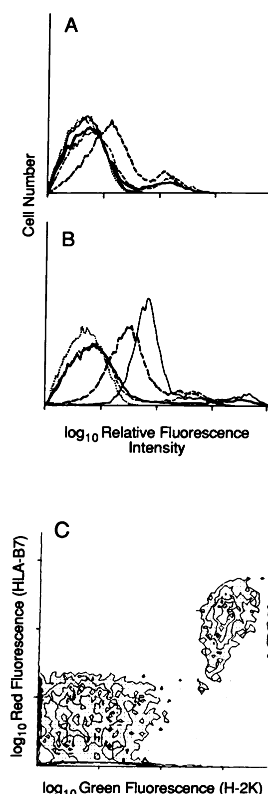


FIG. 6. FACS analysis with specific mAbs of surface expression of HLA-B7 on thymocytes from singly *HLA-B7* (*A* and *C*) and doubly *HLA-B7/B2M* (*B*) transgenic mice. (*A* and *B*) Results obtained with thymocytes stained with anti-HLA mAb B9.12.1 (━), anti-h$\beta_2$m mAb BBM.1 (—), anti-H-2K mAb Y-3 (- - - -), anti-H-2D$^b$ mAb B22/249 (━ ━), and no primary antibody (·······). The *x* axis is divided into 256 channels spanning 5 $\log_{10}$ units. (*C*) Results obtained with thymocytes dual-labeled with biotin-conjugated anti-HLA-B7 mAb ME-1 (detected with phycoerythrin-conjugated streptavidin) and anti-H-2K mAb Y-3 [detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG]. The axes labeled red (phycoerythrin) fluorescence and green (FITC) fluorescence correspond to ME-1 and Y-3 staining, respectively. The *x* and *y* axes are divided into 64 channels, spanning 3.8 $\log_{10}$ units. Contour lines were drawn around areas containing 7, 14, 21, and 28 cells, respectively.

## DISCUSSION

A DNA fragment encoding the human MHC class I antigen HLA-B7, with as little as 660 bp of 5′ flanking sequence, was expressed in transgenic mice with a tissue specificity similar to that of endogenous murine MHC class I genes. Independent lines varied in the level of HLA-B7 RNA in a given tissue but not in the qualitative pattern of expression between tissues. The most obvious exception to this parallel pattern of tissue-specific expression was in liver. In all *HLA-B7* lines analyzed, including two with 12 kb of 5′ flanking sequence, HLA-B7 RNA was under-expressed by about a factor of 10 relative to H-2 class I RNA in liver compared to most other tissues. In contrast, the relative tissue-specific pattern of h$\beta_2$m RNA expression was similar to m$\beta_2$m for all tissues.

Expression of the *HLA-B7* transgene was enhanced *in vivo* by murine IFN-$\gamma$ with a tissue specificity similar to that of *H-2*. Tissues that constitutively expressed higher levels of HLA-B7 and H-2 RNA were affected only slightly (i.e., 1.5- to 6-fold enhancement), while tissues that constitutively expressed lower levels of B7 and H-2 were affected to a much greater degree (i.e., 10- to 40-fold enhancement). Therefore, with the possible exception of liver and small intestine, the 6.0-kb *HLA-B7* transgene fragment contained the cis-active sequences required for appropriate tissue-specific constitutive and IFN-$\gamma$-enhanced expression.

Transcriptional enhancers have been identified within a few hundred base pairs of the 5′ ends of murine *H-2* class I and *B2m* genes (4). A short stretch of nucleotides in this region is conserved among genes responsive to IFNs (28). Sequences similar to the H-2 5′ enhancers and IFN-responsive elements are found 5′ of the *HLA-B7* gene. Regulatory mechanisms mediating IFN enhancement of class I gene expression may also involve sequences 3′ of the transcription start site (29, 30).

One possible explanation for the low *HLA-B7* expression in liver is that an additional regulatory element(s) is located more than 12 kb upstream and/or 2.5 kb downstream of the gene. However, an *H-2D$^d$* gene with 2.5 kb of 5′ sequence and

a hybrid *H-2K^b^*–growth hormone gene with 2.0 kb of 5′ sequence gave tissue-specific expression in transgenic mice, including significant expression in liver (31, 32). Alternatively, sequence divergence between cis-active human and murine regulatory elements may impair recognition of the human element by murine liver-specific trans-acting factors, in contrast to the cis elements and trans factors regulating the *HLA-B7* transgene in most other tissues and the *B2M* transgene in liver. Finally, some or all HLA class I genes may not be expressed at the same relative level in adult human liver as *H-2* class I genes in adult mouse liver. Species-specific tissue differences in class I expression have been reported (1, 2). In particular, human liver has been shown to express HLA class I antigens at ≈9% of the level detected in spleen (2). Therefore, our results may reflect appropriate cis and trans regulation of *HLA-B7* expression. Further, as both HLA-B7 and H-2 RNA levels were enhanced ≈6-fold by IFN-$\gamma$, in addition to the mechanism(s) responsible for IFN-$\gamma$-enhanced expression, there must be at least one other tissue-specific mechanism controlling the constitutive levels of class I RNA in different tissues.

In doubly transgenic mice coexpressing HLA-B7 and h$\beta_2$m, the level of cell surface B7 increased 10- to 17-fold on 100% of T cells. This indicates that inefficient cell surface representation of B7 in singly transgenic mice is probably caused by altered association with m$\beta_2$m. We estimate that the amount of cell surface B7 exceeds H-2K or H-2D$^b$ by 5- to 10-fold in *HLA-B7* (line 18A)/*B2M* (line 14) doubly transgenic mice. There was no evidence of any dosage compensation leading to decreased production of H-2 RNA or surface protein. As the level of B7 RNA was proportional to the gene copy number, there was no indication of autoregulation of the *HLA-B7* gene. Quantitative variation in class I expression may play a role in determining the degree of T-cell activity in various types of immune responses (1, 2). It will be of interest to determine whether these doubly transgenic mice display a heightened immune response to particular foreign antigens, immune tolerance to conserved human class I epitopes, or altered thymic selection of the T-cell receptor repertoire.

Subpopulations of thymocytes display a cell surface antigenic phenotype that reflects their stage of differentiation (33). One antigen that appears to be regulated in this regard is H-2K (33). The majority of cortical thymocytes are H-2K$^-$/H-2D$^+$, while the remaining 15% of the total population, which is found in the medulla, are H-2K$^+$/H-2D$^+$. The human *HLA-B7* gene is expressed at the surface of thymocytes with a pattern that closely resembles that of *H-2K*. Cell surface coexpression of *HLA-B7* and *H-2K* on a subpopulation of thymocytes suggests that these two genes share a regulatory mechanism that distinguishes them from *H-2D*.

Variations in expression levels of introduced genes in transgenic animals have been interpreted to be due to position effects (19). One exception to this has been with a human $\beta$-globin construct containing distal erythroid-specific DNase I-hypersensitive sites (34). Our results show that the level of HLA-B7 RNA correlates with transgene copy number and is similar to endogenous class I RNA on a per-copy basis. It is unknown whether the dominant regulatory elements conferring position-independent expression of $\beta$-globin are fundamentally different from conventional tissue-specific enhancers, and if so, whether such elements are also present in a gene such as *HLA-B7* that is expressed at comparatively low levels.

In summary, the present results argue that *HLA-B7* is an autonomous expression unit, relatively independent of the flanking sequences in which it is inserted and not subject to feedback regulation either by competition for scarce transcription factors or by other, more specific, mechanisms. At least with respect to this gene, it appears unlikely that its expression is significantly modified by more remote cis-active MHC sequences; therefore, its position in the MHC is either a reflection only of evolutionary history or of selection mechanisms that do not operate by effects on the levels of expression of the gene.

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1. Klein, J. (1986) *Natural History of the Major Histocompatibility Complex* (Wiley, New York).
2. Harris, H. & Gill, T. (1986) *Transplantation* **42,** 109–117.
3. Skoskiewicz, M., Colvin, R., Schneeberger, E. & Russell, P. (1985) *J. Exp. Med.* **162,** 1645–1664.
4. Kimura, A., Israel, A., LeBail, O. & Kourilsky, P. (1986) *Cell* **44,** 261–272.
5. Baldwin, A. & Sharp, P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 723–727.
6. Chamberlain, J. W., Nolan, J. A., Gromkowski, S. H., Kelley, K., Eisenstadt, J., Herrup, K., Janeway, C. A. & Weissman, S. M. (1988) *J. Immunol.* **140,** 1285–1292.
7. Srivastava, R., Duceman, B., Biro, P., Sood, A. & Weissman, S. M. (1985) *Immunol. Rev.* **84,** 93–121.
8. Krimpenfort, P., Rudenko, G., Hochstenbach, F., Guessow, D., Berns, A. & Ploegh, H. (1987) *EMBO J.* **6,** 1673–1676.
9. Hogan, B. F., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
10. Brinster, R., Chen, H., Trumbauer, M., Yagle, M. & Palmiter, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4438–4442.
11. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Meinkoth, J. & Wahl, G. (1984) *Anal. Biochem.* **138,** 267–284.
13. Arnold, B., Burgert, H., Archibald, A. & Kvist, S. (1984) *Nucleic Acids Res.* **12,** 9473–9487.
14. Lalanne, J., Transy, C., Guerun, S., Darche, S., Meulen, P. & Kourilsky, P. (1985) *Cell* **41,** 469–478.
15. Lew, A., Lillehoj, E., Cowan, E., Maloy, W., VanSchravendijk, M. & Coligan, J. (1986) *Immunology* **57,** 3–18.
16. Suggs, S., Wallace, R., Hirose, T., Kawashima, E. & Itakura, K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6613–6617.
17. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2253–2257.
18. Mishell, B. B. & Shiigi, S. M. (1980) *Selected Methods in Cellular Immunology* (Freeman, San Francisco).
19. Palmiter, R. & Brinster, R. (1986) *Annu. Rev. Genet.* **20,** 465–499.
20. Ceredig, R., Lynch, F. & Newan, P. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8578–8582.
21. Ellis, S., Taylor, C. & McMichael, A. (1982) *Hum. Immunol.* **5,** 49–59.
22. Malissen, B., Rebai, N., Liabeuf, A. & Mawas, C. (1982) *Eur. J. Immunol.* **12,** 739–747.
23. Ferrier, P., Fontecilla-Camps, J., Bucchini, D., Caillot, D., Jordan, B. R. & Lemonnier, F. A. (1985) *Immunogenetics* **21,** 321–331.
24. Brodsky, F., Bodmer, W. F. & Parham, P. (1979) *Eur. J. Immunol.* **9,** 536–545.
25. Jones, B. & Janeway, C. A. (1981) *Nature (London)* **292,** 547–549.
26. Lemke, H., Hammerling, G. & Hammerling, U. (1979) *Immunol. Rev.* **47,** 175–206.
27. Jones, B. (1983) *J. Immunol.* **13,** 678–684.
28. Friedman, R. & Stark, G. (1985) *Nature (London)* **314,** 637–639.
29. Yoshie, O., Schmidt, H., Lengyel, P., Reddy, E., Morgan, W. & Weissman, S. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 649–653.
30. Korber, B., Hood, L. & Stroynowski, I. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3380–3384.
31. Bieberich, C., Scangos, G., Tanaka, K. & Jay, G. (1986) *Mol. Cell Biol.* **6,** 1339–1342.
32. Morello, D., Moore, G., Salmon, A., Yaniv, M. & Babinet, C. (1986) *EMBO J.* **5,** 1877–1883.
33. Scollay, R. & Shortman, K. (1983) *Thymus* **5,** 245–295.
34. Grosveld, F., van Assendelft, G., Greaves, D. & Kollias, G. (1987) *Cell* **51,** 975–985.