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Genes Regulating HLA Class I Antigen Expression in T-B Lymphoblast Hybrids

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Abstract. Regulation of HLA class I and class II antigen expression was studied in hybrids of human T and B lymphoblastoid cell lines (LCL). The T-LCL CEM^R.3 expresses no HLA class II antigens. It expresses little total HLA class I antigen and no HLA-B antigens. The B-LCL 721.174 is a radiation-induced variant immunoselected for loss of class II antigen expression. In addition to showing a deletion of all *HLA-DR* and *DQ* structural genes, 721.174 expresses no HLA-B antigens and a decreased level of HLA-A antigen compared with the parental cell line. A hybrid of 721.174 and CEM^R.3 expresses class II antigens encoded by CEM^R.3. Increased expression of HLA class I antigens encoded by both 721.174 and CEM^R.3 was also observed. Specifically, the previously undetectable HLA-B5 and HLA-Bw6 antigens encoded by 721.174 and CEM^R.3, respectively, were present on the hybrid. Increased expression of the HLA-A2 antigen encoded by 721.174 was also observed. An immunoselected variant of the hybrid lacking both CEM^R.3-derived copies of chromosome 6 lost expression of the HLA-B5 antigen encoded by 721.174 and expressed a decreased amount of HLA-A2. From these data, we infer that two complementary *trans*-acting factors mediate enhanced expression of HLA class I antigens in the hybrid. One of these factors is provided by a gene located on chromosome 6 derived from CEM^R.3. The second factor, introduced by 721.174, is the gene previously postulated to induce expression of CEM^R.3-encoded class I antigens in hybrids of CEM^R.3 with B-LCL.

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Introduction

The human major histocompatibility complex (MHC) encodes molecules of immunological importance, including class I and class II antigens. Class I antigens are cell-surface proteins recognized in combination with viral determinants by cytotoxic T lymphocytes (Zinkernagel and Doherty 1975, McMichael et al. 1977). Present at the surface of human cells as dimers of HLA-A, B, or C locus-encoded heavy chains and β_2 microglobulin (β_2m) (Berggard and Beam 1968, Springer et al. 1974), these molecules are found in varying amounts on virtually all nucleated cells. Class II antigens serve as restricting elements for immune recognition by helper T lymphocytes on the surface of antigen-presenting cells (Hansen et al. 1978, Rodey et al. 1979). Comprised of dimers at the cell surface of HLA-DR, DQ, or DP-encoded α and β subunits (Bodmer 1984, Strominger et al. 1981) in man, these molecules are expressed on relatively few cell types.

B lymphoblastoid cell lines (B-LCL) normally express high levels of class I and II antigens, while T lymphoblastoid cell lines (T-LCL) express no class II antigens and often express low amounts of class I antigens, especially the HLA-B locus products (Billing and Lucero 1982, Howell et al. 1984). We have produced somatic cell hybrids of B-LCL and T-LCL which express high levels of class I and II antigens encoded by both parent cell lines (Howell and Cresswell 1983, Howell et al. 1984), and have postulated that *trans*-acting B-LCL-derived factors mediate enhanced expression of T-LCL-encoded class I antigens and *de novo* synthesis of T-LCL-encoded class II molecules.

Control of class I antigen expression has also been studied in hybrids of the myeloid-erythroid line K562 with two Burkitt's lymphoma lines. Both of the hybrids, like K562 itself, express little or no class I antigen (Klein et al. 1980, Ziegler et al. 1982). Dominance of the nonexpressing phenotype is therefore opposite to that observed in our B-LCLxT-LCL hybrids.

Evidence for a *cis*-acting regulatory element controlling class I antigen expression was obtained in a mouse embryonal carcinoma (EC) × human B-cell hybrid (Benham et al. 1983). The MCP6 hybrid described in this study lacks all human chromosomes except an X chromosome bearing a portion of translocated chromosome 6. Barely detectable levels of endogenous H-2 mRNA were found in the hybrid and the parental mouse EC cell line. However, HLA class I genes were transcribed at high levels in the hybrid. HLA class II genes present in the hybrid were not transcribed at detectable levels. The authors concluded that a *cis*-acting element linked to the *HLA* region overcame regulation inherent to the EC cell line.

HLA class I antigen regulation has also been uncovered in a group of γ-irradiation-induced B-LCL mutants in which cell-surface expression of class I antigens was simultaneously reduced as a consequence of homozygous mutations in B-LCL 721 (DeMars et al. 1984). In one of these mutants, 721.174, the region of homozygous deletion indicates that the lesion responsible for reduced class I antigen expression is located between the complement gene cluster and the *DP*α*2* locus (DeMars 1984; Ehrlich et al., manuscript in preparation; Whitehead et al. 1984). Class II structural genes other than DPα2 and DPβ2 are absent from this mutant.

The defined homozygous deletion in 721.174 prompted us to fuse 721.174 with the T-LCL CEM^R.3 to determine whether dominant *trans*-acting genetic factors expressed by B-LCL and responsible for enhancing expression of class I and class II antigens encoded by T-LCL upon hybridization are located in the region of the homozygous deletion. In this report, we examine control of class I and class II expression in the resultant hybrids.

Materials and Methods

Cell lines. CEM (Foley et al. 1965), obtained from Dr. R. Metzgar, is a T-LCL. CEM^R.3 is a clone of an 8-azaguanine and ouabain-resistant variant of CEM produced in this laboratory. It expresses low amounts of surface HLA-A1 and HLA-Aw30. It encodes but does not express HLA-B8, HLA-Bw6, HLA-DR3, and HLA-DR7 (Howell et al. 1982, 1984). B-LCL 721.45.1 and 721.174 are immunoselected variants of LCL 721 and have been described previously (DeMars et al. 1983, 1984). Briefly, 721.45.1 was derived by the deletion of one entire *HLA* haplotype after γ-irradiation and complement-mediated selection of LCL 721 with alloantibody against the B8 molecule encoded by that haplotype (Kavathas et al. 1980). 721.174 was derived from 721.45.1 after re-irradiation and selection with an class II-specific monoclonal antibody, CC6.4 (DeMars et al. 1984). No detectable serologically defined class II antigens are present on this cell line (DeMars et al. 1984). It expresses reduced amounts of HLA-A2 relative to 721.45.1, and encodes but does not express HLA-B5 (DeMars 1984). 174 × CEM.T1 is a cloned hybrid of 721.174 and CEM^R.3 which was produced by a PEG-mediated fusion procedure as previously described (Howell et al. 1982). Human foreskin fibroblasts (HFF) were donated by Dr. Kay Singer and were maintained as previously described (Howell and Cresswell 1983). Cells in bulk culture were grown in either Iscove's-modified Dulbecco's medium (IMDM) (Gibco) or Chee's essential medium (B&B/Scott Laboratories). Both of these media were supplemented with 10% fetal bovine serum (FBS) (Gibco). For cloning on HFF feeder layers, cells were grown in IMDM containing 20% FBS and 50 μg/ml gentamicin sulfate (Schering).

Monoclonal antibodies. BB7.2 (anti-HLA-A2), MB40.7 (anti-HLA-Bw6 associated), and w6/32 (anti-HLA-A, B, C monomorphic) (Brodsky et al. 1979, Parham 1983) were provided by Drs. Peter Parham and Frances Brodsky. 4D12 (anti-HLA-B5) (Haynes et al. 1982) and 3A1 (anti-T cell and T-LCL) (Haynes et al. 1979) were provided by Dr. Barton Haynes. Tu48 (anti-HLA-Aw23, Aw24, Aw32, Bw4) (Muller et al. 1982) was provided by Dr. Andreas Ziegler. SFR8-B6 (anti-HLA-Bw6) (Radka et al. 1982), SFR16-DR7G (anti-HLA-DR7) (Radka et al. 1984a), and SFR1-MI.3 (anti-HLA-DR monomorphic) (Radka et al. 1984b) were produced by Dr. Susan Radka in this laboratory. GAP A3 (anti-HLA-A3) was produced by Dr. Ann Berger in this laboratory (Berger et al. 1982). 16.23 (anti-HLA-DR3) was provided by Dr. Judith Johnson (Johnson et al. 1982).

Immunoselection of 174 × CEM.T2 variant. 174 × CEM.T1 cells (2×10^6) were suspended in 2 ml of SFR1-MI.3 tissue culture supernatant for 1 h at 37 °C. Cells were pelleted, then resuspended in 2 ml of 3- to 4-week-old rabbit serum (Pel-Freez) as a complement source, for 1 h at 37 °C. After the selection process had been repeated three additional times at 3-day intervals, the cells in bulk culture did not bind the selecting antibody, SFR1-MI.3, in an indirect immunofluorescence assay. Cells were then cloned at limiting dilution on HFF feeder layers, and wells positive for growth after 3 weeks screened for negativity with SFR1-MI.3 by indirect immunofluorescence. A large number of DR negative clones were isolated.

Indirect immunofluorescence. Cells were washed once in 2% PBA [2% bovine serum albumin (BSA), 0.02% NaN_3 in phosphate-buffered saline], then incubated with 50 μl of monoclonal antibody (tissue culture supernatant or ascites dilution) per 10^5 cells per tube for 30 min at 4 °C. Cells were washed two times in 2% PBA, then incubated with 30 μl of fluoresceinated goat anti-mouse Ig (Meloy) for 30 min at 4 °C. Cells were washed three times in 2% PBA, then suspended at 10^5 cells/ml in 2% PBA. Samples were analyzed with a fluorescence-activated cell sorter (Cytofluorograf 50H, Ortho). Data were collected by computer (Ortho 2140 data handler) and frequency versus fluorescence intensity plotted with mean fluorescence channels calculated on a linear scale.

Preparation of high molecular weight DNA. DNA was prepared as described (Mears et al. 1978). Cells were lysed in Mears' solution [0.1 M NaCl, 0.001 M EDTA, 0.5% sodium dodecyl sulfate (SDS), 0.05 M Tris, pH 7.5] containing 100 μg/ml proteinase K for 4 h at 55 °C. Additional proteinase K was then added and the incubation continued for 16 h. The preparation was extracted once with phenol, twice with chloroform, and then dialyzed against 1/100 SSE (1 × SSE = 0.15 M NaCl, 7.5 m*M* EDTA) overnight. After ethanol precipitation, the sample was again dialyzed against 1/100 SSE overnight. DNA purity was assessed by measuring the A_{260}-to-A_{280} ratio.

Southern hybridization. High molecular weight DNA was digested with restriction endonucleases according to the manufacturer's guidelines. Enzyme was added at regular intervals until digestion was judged complete on a 0.9% agarose test gel. The digestion mixture was then extracted with phenol, chloroform, and methyl-t-butyl-ether, ethanol precipitated, and resuspended in 1 × TE (10 m*M* Tris, pH 8.0, 1 m*M* EDTA). DNA fragments were separated by size on a 0.9% agarose gel in 1 × TA (40 m*M* Tris, 5 m*M* sodium acetate, 1 m*M* EDTA), then transferred to nitrocellulose paper (Schleicher and Schuell) as described (Southern 1975). Hind III-digested lambda phage DNA was used as a molecular weight standard. The filter was soaked at 65 °C in 5 × SSC (1 × SSC = 150 m*M* NaCl, 15 m*M* sodium citrate), 5 × Denhardt's solution (1 × Denhardt's = 0.01% each of BSA, Ficoll, and polyvinyl pyrolidone), 0.1% SDS, and 10 m*M* sodium pyrophosphate (NaPPi) in 5 m*M* sodium phosphate, pH 7, for 4 h at 65 °C. For 2 h prior to hybridization, the filter was soaked in 3 × SSC, 2 × Denhardt's solution, 0.1% SDS, and 50 μl salmon sperm DNA at 65 °C. A DRα chain DNA probe, ^{32}P-d CTP nick-translated to a specific activity of 7×10^7 cpm per μg of DNA, was then added and allowed to hybridize for 24 h at 65 °C. The filter was then washed with 3 × SSC, 5 × Denhardt's solution, 0.1% SDS, and 0.1% NaPPi at 65 °C for 15 min, and exposed to Kodak XAR-5 film with Cronex Hi-plus intensifying screens at −70 °C.

Results

Class II markers on 174 × CEM.T1. Table 1 shows the results of an indirect immunofluorescence assay with the cell lines 721.174, CEM^R.3, 174 × CEM.T1, and three HLA-DR-specific monoclonal antibodies (MAb). Neither of the parent cell lines expresses DR antigen, as detected by SFR1-MI.3, which binds to a DR monomorphic determinant. The hybrid expresses large amounts of DR antigen, specifically HLA-DR7, as shown by the binding of SFR16-DR7G. We previously demonstrated that both HLA-DR3 and DR7 are encoded by CEM^R.3 (Howell and Cresswell 1983), but are not expressed by that cell line.

The HLA-DR3-specific MAb 16.23 does not bind to 174 × CEM.T1. This is in contrast to other B-LCL × CEM hybrids which express both the DR3 and DR7 antigens. We now believe that early in the production of 174 × CEM, one chromosome 6 of CEM^R.3 origin was spontaneously lost from the hybrid; we have consequently named the cell line 174 × CEM.T1. Two pieces of evidence support

Table 1. Flow cytometry study of class II antigens on 721.174, CEM^R.3, and 174 × CEM.T1

MAb	Specificity	721.174	CEM^R.3	174 × CEM.T1
SFR1-MI.3	HLA-DR	27*	9	281
SFR16-DR7G	HLA-DR7	23	8	237
16.23	HLA-DR3	22	8	11
GAP A3†	HLA-A3	26	9	13

* Mean fluorescence channel.

† Used as control for negative binding in this experiment.

Table 2. Flow cytometry study of class I antigens on 721.174, 721.45.1, CEM^R.3, 174 × CEM.T1, and 174 × CEM.T2

MAb	Specificity	721.174	CEM^R.3	174 × CEM.T1	174 × CEM.T2	721.45.1
SFR8-B6	HLA-Bw6	28*	8	129	18	53
MB40.7	HLA-Bw6	25	9	163	18	32
BB7.2	HLA-A2	130	8	282	78	300
4D12	HLA-B5	26	9	46	18	80
Tu48	HLA-Bw4	35	9	118	19	245
GAP A3†	HLA-A3	26	9	13	20	37

* Mean fluorescence channel.
† Used as control for negative binding in this experiment.

this hypothesis. First, both DR3 and DR7 were detectable on 174 × CEM shortly after hybridization (R. Salter, unpublished observation). Second, tissue typing of current DR3-negative cultures of 174 × CEM.T1 by the microcytotoxicity method (Amos et al. 1980) confirms the absence of HLA-A1 and -B8, two class I antigens known to belong to the same CEM^R.3 haplotype as DR3 (Howell and Cresswell 1983). All known antigens encoded by the remaining CEM^R.3 haplotype are retained in the hybrid (see below).

Class I markers on 174 × CEM.T1. Table 2 summarizes the reactivity of 721.174, CEM^R.3, and 174 × CEM.T1 with several class I antigen-reactive MAb. As we have reported for other B-LCL × CEM hybrids (Howell et al. 1984), the Bw6 marker, not detectable by serological analysis on CEM^R.3, is expressed well above background binding levels on 174 × CEM.T1. Both MB40.7 and SFR8-B6, which recognize HLA-Bw6-associated and HLA-Bw6 determinants, respectively, bind strongly to the hybrid cell line.

Mutant 721.174 contains the *HLA-A2* and *B5* genes (DeMars 1984). Nevertheless, cell-surface expression of A2 antigen is reduced by 75–80% compared with the parent cell line, 721.45.1, as measured by binding of A2-specific MAb BB7.2 in ELISA tests (DeMars et al. 1984). MAbs 4D12 and Tu48 recognize separate determinants present on the B5 molecule, namely, a B5-B35 cross-reactive determinant and the Bw4 determinant, respectively, and neither Mab binds to 721.174 (Table 2, DeMars et al. 1984). HLA-B private specificities and the HLA-Bw4/w6 specificities are separate determinants on the same molecule (Ayres and Cresswell 1976). In contrast to 721.174, 174 × CEM.T1 expresses the B5 antigen, as detected by 4D12 and Tu48 binding (Table 2). HLA-A2 expression is also enhanced in the hybrid. Neither CEM^R.3 nor hybrids of CEM^R.3 with HLA-A2, B5-negative B-LCL react with BB7.2, 4D12, or Tu48 (unpublished data). The binding of w6/32 to 174 × CEM.T1 in Table 3 shows that fusion increases total class I antigen expression to levels well above those present in either parental cell line. In summary, both CEM^R.3 and 721.174 class I molecules are displayed in greater amounts in 174 × CEM.T1 than in either of the respective parental cell lines, and *de novo* expression of the HLA-B antigens encoded by both parents is observed.

Table 3. Flow cytometry study of 721.174, $CEM^R.3$, and 174 × CEM.T1 with w6/32

MAb	Specificity	721.174	$CEM^R.3$	174 × CEM.T1
w6/32	HLA-A, B, C	192*	43	479
GAP A3†	HLA-A3	26	9	13

* Mean fluorescence channel.
† Used as control for negative binding in this experiment.

Table 4. Flow cytometry study of a T-LCL marker and class II antigen on 174 × CEM.T1 and 174 × CEM.T2

MAb	Specificity	174 × CEM.T1	174 × CEM.T2
SFR1-MI.3	HLA-DR	281*	17
16.23	HLA-DR3	11	17
SFR16-DR7G	HLA-DR7	237	17
3A1	CD-7	162	129
GAP A3†	HLA-A3	13	20

* Mean fluorescence channel.
† Used as control for negative binding in this experiment.

Comparison of class II and T-cell markers on 174 × CEM.T1 and 174 × CEM.T2. A variant of 174 × CEM.T1 was selected with the anti-HLA-DR monomorphic antibody SFR1-MI.3, and complement. This cell line, 174 × CEM.T2, does not bind SFR1-MI.3 or SFR16-DR7G, as shown by indirect immunofluorescence (Table 4) and is apparently HLA-DR-negative. The T-LCL-binding MAb, 3A1, binds to both 174 × CEM.T2 and 174 × CEM.T1. Combined with cytogenetic evidence that 174 × CEM.T2 and 174 × CEM.T1 are approximately tetraploid (R. Salter, unpublished observation), these data suggest that 174 × CEM.T2 is derived from 174 × CEM.T1, and is not a contaminating 721.174 cell inadvertently cloned out during the selection procedure. 174 × CEM.T2 appears to be a hybrid which has lost both copies of $CEM^R.3$-derived chromosome 6 (see below).

Southern blotting analysis of 174 × CEM.T1 and 174 × CEM.T2. We have previously selected chromosome loss variants of T-LCL × B-LCL hybrids, suggesting that they can spontaneously lose chromosomes (Howell and Cresswell 1983). We therefore postulated that the DR-negative variant of 174 × CEM.T1 had lost both copies of $CEM^R.3$-derived chromosome 6. Since 174 × CEM.T1 should contain *DR* genes derived from $CEM^R.3$ only, as 721.174 has been shown previously by Southern analysis to contain no *DR* or *DQ* genes (Erlich et al., manuscript in preparation; DeMars 1984), we used Southern analysis to determine whether 174 × CEM.T2 contains no DRα chain genes, and by inference, no $CEM^R.3$-derived copies of chromosome 6. Figure 1 shows an autoradiograph of restriction enzyme-digested 174 × CEM.T1 DNA and 174 × CEM.T2 DNA hybridized to a DRα gene probe, DRα-1 (Das et al. 1983). Hybridizing bands are present in 174 × CEM.T1 DNA digested with three different restriction enzymes, while no bands are apparent

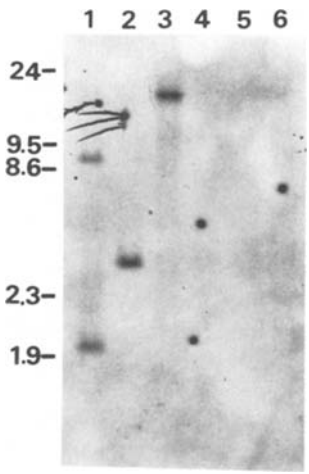

Fig. 1. Southern analysis of 174 × CEM.T1 (lanes 1–3) and 174 × CEM.T2 (lanes 4–6) DNA. Samples were digested with Pst I (lanes 1 and 4), Msp I (lanes 2 and 5), and PvuII (lanes 3 and 6). The nitrocellulose filter was hybridized with the HLA-DR α genomic probe pDRα-1 as described in *Materials and Methods*. Bands are present in 174 × CEM.T1 DNA digested with all three enzymes, whereas no hybridizing bands are apparent in 174 × CEM.T2 DNA.

in 174 × CEM.T2 DNA identically digested. Supporting evidence that 174 × CEM.T2 has lost both T-cell-derived copies of chromosome 6 was obtained from *HLA* typing data and cell sorter studies which demonstrated that the cell is negative for both HLA-A1 and HLA-Aw30, encoded by CEM^R.3 (data not shown), while it remains positive for HLA-A2, encoded by 721.174 (see below).

Comparison of class I markers on 174 × CEM.T1, 174 × CEM.T2, and 721.174. Table 2 summarizes the binding of class I-specific MAb to 174 × CEM.T1, 174 × CEM.T2, and 721.174. The binding pattern for 721.174 and 174 × CEM.T2 is identical. 4D12 and Tu48 react with neither 721.174 nor 174 × CEM.T2, indicating that the B5 antigen is not expressed in these lines. HLA-A2 expression is retained by 174 × CEM.T2, indicating that the HLA-B5-bearing chromosome derived from 721.174 is still present in the hybrid. However, the level of expression is decreased relative to 174 × CEM.T1, to a level similar to that found in 721.174. The Bw6 marker is not expressed in174 × CEM.T2, as measured by the binding of SFR8-B6 and MB40.7. Since the T2 variant of 174 × CEM.T1 has lost both T-cell-derived copies of chromosome 6, we conclude that high HLA-A2 and -B5 antigen expression in 174 × CEM.T1 is controlled by a gene on chromosome 6 supplied by CEM^R.3.

Discussion

We previously reported (Howell et al. 1984) that hybrids of several B-LCL with sublines of the T-LCL CEM express high levels of CEM-encoded class I antigens either not expressed at all by the CEM sublines (B-locus antigens) or expressed by the sublines only at low levels (A-locus antigens). The subline CEM^R.3 was found to express easily detectable levels of intracellular β_2m. It was thus considered unlikely that β_2m was a limiting factor in class I antigen expression in this case, as has been

demonstrated for the Burkitt's lymphoma line Daudi (Jones et al. 1975, Klein et al. 1977, Arce-Gomez et al. 1978). However, $CEM^R.3$ was found to contain negligible quantities of class I heavy chain mRNA compared with B-LCL or B-LCL × CEM hybrids. It was concluded that B-LCL produce a *trans*-acting regulatory molecule, normally lacking (or present at reduced levels) in the CEM sublines, which induces transcription of normally quiescent CEM class I genes upon hybridization. This communication describes the production of 174 × CEM.T1, a hybrid of $CEM^R.3$ with the mutant B-LCL 721.174. The HLA class I phenotype of 721.174 is similar to that of $CEM^R.3$. It expresses measurable levels of HLA-A2 but no detectable HLA-B antigens (DeMars et al. 1984, Table 2). 174 × CEM.T1 expresses HLA-Bw6, encoded but not expressed by $CEM^R.3$, and HLA-B5, encoded but not expressed by 721.174 (Table 2). Total class I antigen expression on the hybrid, measured by binding of the monomorphic antibody w6/32, is enhanced compared with that of both parents (Table 3). From these data we infer that class I antigen expression in T-LCL × B-LCL hybrids is in fact regulated by a minimum of two *trans*-acting regulatory genes, which act in complementary fashion in 174 × CEM.T1.

The deletion of one of these genes from 721.174 is presumably responsible for the reduced level of class I antigen expression by this cell relative to the parental cell line, 721.45.1 (DeMars et al. 1984, Table 2). Studies by the DeMars group have shown that the *HLA-A2* and *B5* structural genes are present in 721.174, that all class II genes except those encoding DPα2 and DPβ2 are deleted, and that the complement gene cluster (C2, C4, Bf) is intact (DeMars 1984; DeMars et al., manuscript in preparation; Ehrlich et al., manuscript in preparation; Whitehead et al. 1984). From these data DeMars and co-workers have inferred that a regulatory gene controlling class I antigen expression resides within the homozygous deletion introduced into 721.174, i.e., between the complement gene cluster and DPα2. They have preliminary evidence that this gene acts posttranscriptionally and have tentatively named it *EpI-1* (R. DeMars, personal communication).

Our data confirm the presence of a *trans*-acting regulatory gene controlling class I expression in the *HLA* region, and extend the studies of DeMars and co-workers in several ways. First, a gene capable of replacing the function of *EpI-1* must be present in $CEM^R.3$. This gene is either active in $CEM^R.3$ or is activated by fusion with B-LCL, as evidenced by the enhanced expression of HLA-A2 and B5 in 174 × CEM.T1. Second, additional evidence for the linkage of such a gene to chromosome 6 is provided by the cell line 174 × CEM.T2. This cell line lacks both T-LCL-derived copies of chromosome 6 as determined by Southern blot analysis with a DRα chain genomic probe (Fig. 1) and by its lack of reactivity with monoclonal antibodies specific for HLA-DR3 and HLA-DR7, the $CEM^R.3$-encoded HLA-DR products (Table 4). This variant expresses HLA-A2 but not HLA-B5 or Bw6, and its *HLA* phenotype is in fact indistinguishable from that of 721.174 (Table 2). The reversion of the HLA class I phenotype to that of 721.174 in 174 × CEM.T2 is consistent with the linkage of a regulatory gene to chromosome 6, reducing the probability that it is located in unknown chromosomal lesions in 721.174. This gene is presumably identical with that designated *EpI-1* by DeMars.

The expression of class I antigens at a high level by 174 × CEM.T1 argues for the existence of at least one gene in addition to *EpI-1* which regulates class I expression. This gene is presumably that which is responsible for inducing $CEM^R.3$ class I

antigens in hybrids of CEM^R.3 with normal B-LCL. For reasons outlined in the opening paragraph of the *Discussion* and substantiated in a previous publication (Howell et al. 1984), we believe that this gene (or genes) acts at the level of transcription of *HLA* class I genes. To simplify discussion, we will here assume that it is a single gene and refer to it as *HLA* class I regulatory gene 1, or *HC1.ReG.1*. Clearly this gene remains functional in 721.174, differentiating it from *EpI-1* and placing it outside the well-characterized MHC deletions in 721.174. It may or may not be MHC-linked, and is inactive in CEM^R.3.

In order for HLA class I antigens to be present at high levels, we hypothesize that *EpI-1* and *HC1.ReG.1* must both be expressed. This situation exists in normal B-LCL and their hybrids with CEM^R.3. As described above, class I antigen expression in 721.174 is low because *EpI-1* has been deleted. The simplest explanation for the high expression of CEM^R.3 and 721.174-encoded class I antigens on 174 × CEM.T1 is that CEM^R.3 expresses *EpI-1* but not *HC1.ReG.1*. Introduction of *HC1.ReG.1* by the 721.174 parent and *EpI-1* by the CEM^R.3 parent results in enhanced expression of class I antigens by the hybrid. While this would be a reasonable explanation in classical Mendelian genetics, it is probably far too simple an interpretation in the case of somatic hybridization of two phenotypically different cell types. Clearly expression of HLA-A and B antigens could be governed by multiple interacting genes. An alternative possibility is that both *EpI-1* and *HC1.ReG.1* could be inactive in CEM^R.3, and the *EpI-1* function induced in the CEM^R.3-derived genome in 174 × CEM.T1, perhaps by the action of *HC1.ReG.1* itself.

A trivial explanation of the class I antigen enhancement observed in 174 × CEM.T1 is that both 721.174 and CEM^R.3 are deficient in β_2m production, and that the hybrid expresses large amounts of cell-surface class I antigen because of increased production of β_2m. Using inhibition of binding assays, we have ruled out this possibility. We previously showed that CEM^R.3 synthesizes easily detectable levels of β_2m (Howell et al. 1984). By inhibition radioimmunoassay, we have demonstrated that 721.174 and its parent 721.45.1 synthesize approximately equal amounts of β_2m, while 174 × CEM.T2 produces more β_2m than either 721.174 or 721.45.1 (R. Salter, data not shown).

A second goal of the studies reported here was to determine whether regulatory genes governing HLA class II antigen expression were deleted from 721.174. We have previously described the induction of T-LCL-encoded HLA-DR, DP, and DQ antigens in hybrids of the class II antigen-negative T-LCL CEM^R.3 and HSB^R with B-LCL (Howell and Cresswell 1983, Howell et al. 1983) and have postulated the existence of a *trans*-acting regulatory gene governing class II antigen expression. This gene may be inactivated in the class II-negative B-LCL variants isolated by Accolla and co-workers (Long et al. 1984) and Pious and co-workers (Gladstone and Pious 1980, Loosmore et al. 1982). However, 174 × CEM.T1 expresses the HLA-DR7 antigen encoded by CEM^R.3. Thus, we cannot conclude that a class II regulatory gene maps to the MHC-linked homozygous lesion of 721.174.

Control of class I and II antigen expression has been studied by methods other than somatic cell hybridization and by isolation of variants lacking regulatory elements. Gamma interferon (IFN-γ) treatment of a variety of cell types has been shown to cause increased class I antigen expression and induce *de novo* synthesis of class II antigens (Pober et al. 1983). Several human B-LCL tested contained

increased levels of class I mRNA when treated with human IFN-α, IFN-β, or IFN-γ (Fellous et al. 1982). Both B- and T-cell subsets of peripheral blood mononuclear cells showed enhanced class I antigen expression after treatment with human IFN-α, while levels of surface IgM and T-cell marker antigens were not affected (Heron et al. 1978). Preliminary studies show that expression of class I antigen in 721.174 is not increased after treatment with recombinant IFN-γ (R. Salter, unpublished observations). Similar observations have been made with 721.174-like mutants 721.61 and 721.134, which have class I phenotypes and genotypes similar to 721.174 (R. DeMars and M. Fellous, personal communication). However, the role of the regulatory genes described in this publication in modulating surface HLA class I antigen expression in response to external stimuli such as IFN is currently unknown.

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