Differential transport requirements of HLA and H-2 class I glycoproteins

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Abstract. Transport of human and mouse major histocompatibility complex class I glycoproteins has been examined in a transport deficient B-lymphoblastoid cell line × T-lymphoblastoid cell line (B-LCL × T-LCL) hybrid, 174 × CEM.T2 (T2). This cell line expresses no detectable endogenous HLA-B5 and reduced levels of HLA-A2 on its surface although these molecules are synthesized. In order to study this defect further, either HLA-Bw58 or HLA-B7 genomic clones were transfected into T2. Metabolic labeling and immune precipitation demonstrated biosynthesis of the Bw58 or B7 glycoprotein. However, like the endogenous HLA-B5 molecule, neither HLA-Bw58 nor HLA-B7 was expressed at the cell surface. The cloned genes were properly expressed on the surface of C1R, a control B-LCL. To determine if mouse class I alleles had the same transport requirements as the human class I glycoproteins, either mouse H-2Dp or H-2Kb class I genes were introduced into T2. Surprisingly, the H-2 class I glycoproteins were transported to the cell surface normally. These data suggest a fundamental difference between human and mouse histocompatibility antigens in their requirements for intracellular transport.

Introduction

Human HLA-A, -B, -C antigens have a structure and function similar to the mouse class I major histocompatibility complex (MHC) antigens H-2K, H-2D, H-2L (Klein 1975). The antigens consist of a single transmembrane glycoprotein of relative mass (Mr) 44 000 which is non-covalently associated with a Mr 12 000 nonglycosylated protein, beta-2 microglobulin (B2m) (Cresswell et al. 1974, Ploegh et al. 1981a). The biosynthesis, assembly, and expression of human class I glycoproteins have been studied extensively (Krangel et al. 1979, Owen et al. 1980). Briefly, class I heavy chains are inserted into the rough endoplasmic reticulum, where they are core glycosylated. Within 5–15 min after synthesis, association with B2m occurs. Heavy chain glycans are then converted into their complex form within the Golgi apparatus. Finally, the glycoproteins are transported to and appear on the cell surface approximately 1 h after synthesis. Upon surface expression, these heterodimers are presented to the cells of the immune system. They play a crucial role in T-cell recognition of allogeneic or virus-infected target cells.

Although the basic scheme of class I biosynthesis has been well established, many details of the process, particularly regarding its regulation, remain poorly understood. At least two trans-acting regulatory factors important for class I surface expression have been identified in studies of human lymphoblastoid cell lines (LCL). Lack of one of these factors, as exemplified by the T-LCL CEM, results in diminished levels of class I heavy chain transcripts (Howell et al. 1984). The second factor, encoded within the class II region of the MHC, operates at the post-translational level; it is missing in the mutagenically derived B-LCL.174 (DeMars et al. 1985, Shimizu et al. 1986). Hybrids of CEM and .174 express normal levels of class I as a result of trans-complementation of the two regulatory factors; 174 × CEM T2 (T2), a subline of one of these hybrids lacking both CEM-derived copies of chromosome 6, has a phenotype similar to .174 (Salter et al. 1985a). To better understand the transport defect in T2, various human and mouse class I genes were transfected into T2, and the fate of the resultant class I glycoproteins was examined.
Materials and methods

Cell lines. All cell lines were cultured in Iscove’s modified Dulbecco’s medium (IDMEM) (Gibco, Grand Island, New York) supplemented with 10% fetal bovine serum (FBS). The B-LCL WT49 (HLA-A2; Bw58) (Ways et al. 1985) was provided by Dr. Peter Parham. JY is a B-LCL expressing HLA-A2; B7. C1R (class I reduced) is a derivative of the B-LCL LICR-LON-HMY2 (Edwards et al. 1982). This mutant was made by gamma irradiation followed by antibody and complement (C') selection. It expresses no detectable HLA-A or -B locus protein products. C1R, in contrast to T2, expresses trans-acting factors necessary for class I antigen expression, as determined by somatic cell fusion (C. Lamb and P. Cresswell, unpublished results). T2 is a hybrid of the B-LCL 174 (DeMars 1984a, DeMars et al. 1984b) and the T-LCL CEM (Foiley et al. 1965). It synthesizes HLA-A2 and -B5 encoded by the B-cell parent (.174) but has lost both CEM-derived copies of chromosome 6. It lacks surface expression of HLA-B5 antigen and expresses a decreased amount of HLA-A2 (Saltar et al. 1985a). EL4 (ATCC TIB39), a mouse thymoma, expresses K0 and D0 antigens. F12 is a mouse L cell expressing the product of a transfected H-2Dd class I gene (Dursley et al. 1987).

Plasmids: class I genes and selectable markers. pSVa1 (rat a1 cDNA encoding ovain resistance in pSV2 neo) was constructed by Janet Emanuel (Herrera et al. 1987). This construct was provided by Dr. Robert Levenson. pRMI5 is a genomic H-2Dd clone (Murray et al. 1985). pSV2-neo (ATCC # 37149) is a plasmid which confers resistance to the antibiotic G418. pBw58, WT49, a genomic Bw58 clone in pBR322 (Ways et al. 1985), was provided by Drs. Judy Ways and Peter Parham. JY150, a genomic HLA-B7 clone (Engelhard et al. 1985), was provided by Dr. Victor Engelhard.

Antisera. Serum F was produced by immunization of a rabbit as previously described (Cresswell and Ayres 1976). It reacts with free or B2m-associated HLA-A,-B, and -C heavy chains and also with B2m alone.

Monoclonal antibodies. BB7.1 (anti-HLA-B7), BB7.2 (anti-HLA-A2; Brodsky et al. 1979), and MA2.1 (anti-HLA-A2, anti-HLA-Bw58; McMichael et al. 1980) were provided by Drs. Peter Parham and Frances Brodsky. GAP.A3 (anti-HLA-A3; Berger et al. 1982) and 7-16.10 (anti-H-2Dd; Harmon et al. 1983) have been previously described. 4E (anti-HLA-B locus; Yang et al. 1984) was provided by Dr. Soo Young Yang.

Metabolic radiolabeling, immune precipitations, and neuraminidase treatment. Radiolabeling with [35S]methionine, immune precipitations, and neuraminidase treatment were performed as described (Machamer and Cresswell 1982). Briefly, 5 x 10^6 cells were labeled with 0.25 mCi [35S]methionine for 4 h. Cells were washed once in 10 mM Tris, 0.15 M NaCl, pH 7.4 (TS), followed by lysis in 0.5 ml 1% Triton X-100 in TS. The supernatant was cleared with normal rabbit serum (NRS), followed by precipitation of antigen-antibody (Ag-Ab) complexes with Panisorbin (Calbiocem, Picataway, New Jersey). The resulting supernatant was reacted with the appropriate monoclonal antibody (mAb) or antisem, followed by precipitation of Ag-Ab complexes with Protein A-Sepharose CL-4B (Sigma, St. Louis, Missouri). The beads were washed twice with 0.05% Triton X-100 in TS, once with H2O, and once with neuraminidase buffer (0.05 M sodium acetate, 9 mM CaCl2, pH 5.5). Following resuspension in 50 ml of neuraminidase buffer, 10 units of neuraminidase (Calbiocem) were added and the beads incubated at 37°C for 1 h.

Two-dimensional (2-D) polyacrylamide gel electrophoresis. Two-dimensional gels with isoelectric focusing (IEF) in the first dimension and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension were run as described (O’Farrell 1977). Biolyte ampholytes 5/7 and 3/10 (Biorad, Richmond, Virginia) mixed in a 10:1 ratio were used.

Indirect immunofluorescence. Indirect immunofluorescence was performed as described (Saltar et al. 1985b). Cells (10^5) were washed twice in PBA (2% bovine serum albumin (BSA), 0.02% NaNO3 in phosphate-buffered saline (PBS)), followed by incubation with 50 µl of monoclonal antibody (tissue culture supernatant or ascites dilution) for 30 min at 4°C. Cells were washed twice in PBA, then incubated with 50 µl of fluoresceinated goat anti-mouse Ig (Chemicon, El Secundo, California) for 30 min at 4°C. Cells were washed again three times in PBA, then suspended at 10^5 cells/ml in PBA. Samples were analyzed with a fluorescence-activated cell sorter (Cytofluorograf 50H, Ortho, Braintree, Massachusetts; or an EPICS 755, Coulter Electronics, Hialeah, Florida). Fluorescence intensity was measured using a linear scale, and data are reported as relative mean fluorescence channels. Signals from single cells were gated on forward and orthogonal light scattering. Control antibodies were used to define the positive and negative regions such that no more than 1% of negative cells fell in the positive region. Positive cell lines exhibited significant heterogeneity in cell surface expression with coefficients of variation ranging from 40% to 70%. Transfectants exhibited no greater heterogeneity than control cells expressing endogenous class I genes and were clearly either negative or positive.

Electroporation. Electroporations were performed using a Biorad Gene Pulser and Capacitance Extender. Cells (10^5) were resuspended in 0.5 ml serum-free medium. Twenty micrograms of class I DNA, linearized with Eco RI, were added with 2 µg of one of two linearized selectable markers ([pSV2a1 (Pvu I) or pSV2-neo (Bam HI)]). Two sets of conditions were used: either 250 volts, 500 µFarads or 210 volts, 960 µFarads. Both resulted in 50–90% cell death. Following incubation on ice for 10 min, electroporated cells were cultured in 10 ml of IDMEM containing 10% FBS for 24 h. Cells were then pelleted and resuspended in selective medium, either in 2 x 10^-6 M ouabain (Sigma) or in G418 (Gibco) at 1800 µg/ml (C1R) or 1200 µg/ml (T2). Cells were plated at a density of 10^6 cells/well in a 96-well plate containing gamma-irradiated feeder layers (5400 rads; normal rat kidney cells expressing a transfected Neo gene). Transfectant clones were macroscopically visible 3–5 weeks following transfer.

Results

Cell-surface expression of class I antigens. In initial experiments, expression of class I genomic clones transfected into C1R and T2 was assessed by indirect immunofluorescence. The successful nature of the transfection was verified by immunoprecipitation from [35S]methionine-labeled cells of the specific class I products encoded by the introduced genes (see below). Figure 1 shows the results of an indirect immunofluorescence assay testing for surface expression of HLA-B7. The two recipient cell lines C1R and T2 do not encode HLA-B7. A genomic HLA-B7 clone was transfected by electroporation into both cell lines. The resultant C1R/B7.1 clone, representative of four C1R/B7 clones tested, expressed surface levels of B7 comparable to JY, a control B7-positive B-LCL. In contrast, T2/B7.3, representative of six T2/B7 clones tested, expressed no detectable B7.