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# Syrian Hamsters Express Two Monomorphic Class I Major Histocompatibility Complex Molecules

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**Abstract.** The description of the Syrian hamster major histocompatibility complex (MHC), *Hm-1*, has progressed to the point that multiple class II alloantigens have been defined using structural and functional studies. However, no comparable success has been achieved using allotypic differences to detect class I molecules. We now report that xenoantisera raised in other species against hamster tissues have made it possible to describe class I MHC homologues in the hamster. Evidence which confirms that these molecules exist includes (1) on immunoprecipitation of radiolabeled lymphoid cell lysates, heterodimers of approximate molecular weight 47 000 and 12 000 are identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the latter representing hamster $\beta_2$-microglobulin; (2) by direct immunoprecipitation these molecules are ubiquitously expressed on hamster tissues; (3) partial N-terminal amino acid sequence analysis reveals striking homology with class I molecules described in other species. In addition, the amino acid sequence data reveal that two class I molecules are expressed on the surfaces of hamster cells. On two-dimensional PAGE analysis, these molecules are invariant among the several strains of genetically disparate hamsters available for study. We conclude that (1) hamsters have the capacity to make class I MHC molecules, (2) at least two genetic loci are dedicated to this purpose, and (3) no allelic forms can be detected, suggesting that there is no class I polymorphism.

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*Abbreviations used in this paper:* BM, bone marrow; BSA, bovine serum albumin; BSS, balanced salt solution; Con A, concanavalin A; IEF, isoelectric focusing; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NP-40, nonidet P-40; PBS, phosphate buffered saline; *S. aureus, Staphylococcus aureus;* SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris buffered saline; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis.

## Introduction

A partial description of the Syrian hamster major histocompatibility complex (MHC), *Hm-1*, has been achieved recently. A set of polymorphic class II alloantigens has been described; these determinants are able to elicit acute skin graft rejection, graft-versus-host disease, alloantibody formation, and mixed lymphocyte responses (Duncan and Streilein 1978a, 1978b, 1981). Biochemical analyses of cell surface antigens detected by hamster alloantibodies identify exclusively class II type MHC molecules of approximate molecular weights 29 000 and 39 000 (Phillips et al. 1978). Tissue distribution studies have shown these alloantigenic molecules to be limited in expression to cells present within hamster lymph nodes, spleen, and bone marrow, to macrophages, and to a very small extent within the thymus. Hamster Ia homologues are not detected on normal hamster fibroblasts, epidermal cells, red blood cells or a fibrosarcoma cell line (Phillips et al. 1981a). What is extraordinary is that of 12 different hamster alloantisera that have been studied to date, only class II-like molecules have been observed. Not a single alloantiserum was able to detect molecules that resembled MHC class I molecules (Streilein et al. 1981).

Several hypotheses have been advanced to explain these unexpected results: (a) hamster class I molecules may be serologically "silent"; that is, the relationship among hamster class I molecules may resemble that among the $K^b$ molecule and its mutants ($K^{bm1}$, $K^{bm3}$, etc.) in which alloantibody production among these strains is virtually impossible to achieve (Klein 1978); (b) hamster cells may not express class I molecules on their surfaces, analogous to the situation described for the human cell line Daudi (Fellous et al. 1977); (c) hamster cells may express class I molecules on their surface, but there is no polymorphism at class I loci among the strains of hamsters available for study. It has previously been demonstrated in this laboratory that lymphoid cells from MHC disparate hamster strains fail to generate strain-specific cytotoxic T cells in response to in vivo and/or in vitro alloimmunization (Zinkernagel et al. 1978, Nelles and Streilein 1980). This finding makes it very unlikely that hamster class I molecules could differ from each other in a fashion analogous to the $K^b$ mutants. Moreover, the critical role class I molecules are known to play in T-cell recognition of nominal antigen in other species makes the second suggestion – that hamsters fail to express class I molecules – very unattractive. Therefore, we elected to pursue the third possibility, namely, that hamsters express nonpolymorphic class I molecules.

The approaches taken in this study have utilized properties of class I molecules independent of polymorphism: (a) we have produced and used xenoantisera directed at hamster cell surface molecules; (b) we have employed class-I specific alloantisera from other species that cross-react with hamster cell surface molecules; and (c) we have used cross-reacting anti-$\beta_2$-microglobulin sera which react with hamster molecules. Our findings indicate that Syrian hamsters possess at least two genetic loci encoding class I-like MHC molecules, and that these loci each contain monomorphic genes.

## Materials and Methods

*Animals.* CB, LSH, and MHA inbred Syrian hamsters were purchased from the Charles River Lakeview Co., Newfield, New Jersey. The inbred MIT hamsters and the congenic CB.DFW and CB.SYR hamsters were derived from the progeny of wild hamsters captured in 1970 in Syria (Murphy 1971), and have been bred in our breeder colonies at the University of Texas Health Science Center at Dallas (UTHSCD). The BUN hamster was caught in Syria in 1978 and partially inbred (CB × BUN)$F_7$ animals have been bred in our breeder colonies. At the *Hm-1* locus, BUN has been shown to be heterozygous expressing the *Hm-1*$^a$ allele (similar to MHA and LSH) as well as unique Hm-1 molecules not detected in any other hamsters (unpublished observations).

*Antisera.* Xenoantisera were generated by hyperimmunization of mice, with pooled spleen and lymph node cells (LNC) from inbred Syrian hamsters. In the mouse, an initial intraperitoneal (i. p.) injection of $30 \times 10^6$ lymphoid cells, emulsified in complete Freund's adjuvant, was followed by two i. p. injections of $30 \times 10^6$ lymphoid cells in balanced salt solution (BSS) on days 14 and 28. Mice were subsequently bled and immunized on alternate weeks. Rabbit anti-MHA was prepared as described elsewhere (Duncan and Streilein 1977). Briefly, rabbits were hyperimmunized with $300 \times 10^6$ hamster lymphoid cells. The mouse alloantisera (B10 × 129/J) anti-B10.D2, (B10.D2 × DBA/2) anti-B10.SM, (129/J × B10) anti-B10.K, and (C3H/HeJ × B10.K) anti-B10 were a gift from Dr. Chella S. David, Department of Immunology, Mayo Clinic and Medical School, Rochester, Minnesota, and were used without further testing. The IgG fraction of a rabbit antihuman $\beta_2$-microglobulin serum (Dako Corp., Santa Barbara, California) was used without modification. The IgG fraction of a rabbit antihamster IgG (heavy and light chains) serum (Cappel, Cochranville, Pennsylvania) was used without further modification. All antisera were stored aliquoted at −70 °C.

*Preparation of cell suspensions.* Hamster lymph nodes (cervical, axial, inguinal, brachial, mesenteric, and cheek pouch) and thymuses were collected in BSS plus 1% bovine serum albumin (BSA). Single cell suspensions were made and washed two times in BSS at 1000 × g for 10 min. Bone marrow (BM) was obtained by perfusion of cleaned fibula and femur bones with phosphate-buffered saline (PBS). Cells were washed two times in PBS at 1000 × g for 10 min. Primary embryonic fibroblasts, third passage, were obtained from Dr. Sally Atherton, Department of Cell Biology, University of Texas Health Science Center at Dallas, Texas. Briefly, 13–14-day minced embryos were trypsinized and suspended in tissue culture media, and the fibroblasts were grown out in tissue culture flasks. Concanavalin A (Con A) activated lymph node cells (LNC) were generated by incubating $10^8$ cells in 20 ml of RPMI 1640 plus 10% fetal calf serum (FCS), 0.001 mg/ml Con A, 2 m*M* glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) and $5 \times 10^{-5}$ *M* 2-mercaptoethanol (2-ME) at 37 °C with 5% $CO_2$ for 48 h. Lipopolysaccharide (LPS)-activated LNC were generated by incubating $10^6$ cells/ml in RPMI 1640 plus 10% FCS, 15% rat Con A supernatants, 2 m*M* L-glutamine, penicillin (100 units/ml), streptomycin (100 mg/ml), $5 \times 10^{-5}$ 2-ME, and 10 μg/ml LPS, at 37% with 5% $CO_2$ for 72 h.

*Radiolabeling of cells.* The different cell types were all labeled identically. For external labeling with $^{125}$I, single cell suspensions were resuspended to a final concentration of $1.5 \times 10^8$ cells/ml in PBS and 4 mCi/ml carrier-free Na $^{125}$I (Amersham-Searle Corp., Arlington Heights, Illinois), 50 μg/ml lactoperoxidase (LPO), and 25 μl of a 0.003% $H_2O_2$ were added. At 4 and 8 min, additional aliquots of LPO and $H_2O_2$ were added. At 12 min, only $H_2O_2$ was added; at 16 min, the reaction was quenched by the addition of 10 volumes of ice-cold PBS.

For internal labeling with $^3$H-leucine for gel electrophoresis, washed cells were adjusted to a volume of $2 \times 10^7$ cells/ml in RPMI 1640 lacking leucine (Associated Biomedic Systems, Buffalo, New York) supplemented with 2 m*M* L-glutamine (GIBCO, Santa Clara, California). (L-(4, 5-$^3$H) leucine (Amersham) was added to a final concentration of 0.2 mCi/ml and the suspension was incubated for 8 h at 37 °C in a 5% $CO_2$ incubator with continuous rocking. The reaction was quenched by the addition of 10 volumes of cold RPMI 1640. For internal labeling with tritiated amino acids for amino acid sequencing analysis, washed cells were adjusted to a volume of $2 \times 10^7$ cells/ml in RPMI lacking the appropriate amino acid (GIBCO). The appropriate tritiated amino acid (New England Nuclear, Boston, Massachusetts or Amersham) was added to a final concentration of 0.5 mCi/ml and the suspension was incubated for 8 h at 37 °C in a 5% $CO_2$ incubator with continuous rocking. The reaction was quenched by the addition of 10 volumes of cold RPMI. All radiolabeled cells were lysed in 5 ml of Tris-buffered saline

(TBS) containing 0.5% of the non-ionic detergent Nonidet P-40 (NP-40). After 30 min at 4 °C, nuclei and debris were removed by centrifugation at 3000 × g for 15 min. Lysates of radiolabeled cells were subjected to lentil lectin affinity chromatography to isolate the glycoprotein pools, as described previously (Phillips et al. 1978). Glycoprotein pools were concentrated to approximately 1 ml by negative pressure dialysis.

*Immunoprecipitation of radiolabeled cell proteins.* To deplete labeled immunoglobulin from NP-40 lysates of radiolabeled LNC, BM, thymocytes, Con A-activated cells and LPS-activated cells, rabbit antihamster IgG was added initially (30 µl serum/$10^8$ cells) and a second time and incubated for 30 min, at 4 °C each time. Complexes were removed with 200 µl of a 10% *Staphylococcus aureus* solution/30 µl of serum for 20 min at 4 °C (Cullen and Schwartz 1976). Fibroblasts, which do not have any immunoglobulin associated with them, were treated twice with 200 µl of *S. aureus* for 20 min at 4 °C. Complexes were removed by centrifugation at 8000 × g for 2 min. To remove actin, which was suspected of contaminating the lysates, 0.1-1 mg of DNase, which is known to bind actin, was added on the tip of a wooden stick during each of the above incubations (Lindberg and Lazarides 1974). Lysates were divided into $20 \times 10^6$ cell equivalent aliquots, unless otherwise stated, and stored at minus 20 °C until used. Immunoprecipitations were done by adding 20–30 µl of antisera to lysate aliquots at 4 °C for 12–18 h. *S. aureus* (200 µl) was added for 20 min at 4 °C. Pellets were washed three times with TBS plus 0.5% NP-40.

*One-dimensional SDS gel electrophoresis. S. aureus*-bound immunoprecipitates were eluted by boiling for 2 min in 50 ml of sample buffer [0.0625M Tris base, 10% (w/v) glycerol, 5% 2-mercaptoethanol, 2% SDS, 0.0006% bromphenol blue]. *S. aureus* was pelleted by centrifugation at 8000 × g for 5 min. Molecular weight markers (Bio-Rad, Richmond, California) were then added to the samples. Samples were electrophoresed in the discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) through 10% or 13% acrylamide tube gels or 10% slab gels. Tube gels were fractionated and the radioactivity was counted. Slab gels were stained and fixed in 0.0003% Coomassie blue 250R, 12% trichloroacetic acid and 50% methanol for 2–24 h. Gels were subsequently destained in 7% acetic acid and 10% methanol for 24 h and dried by heat and negative pressure. Radioactivity was visualized by exposure of the dried gel to Kodak XAR-5 X-Omat film.

*Two-dimensional gel electrophoresis. S. aureus* bound samples were eluted by incubating in isoelectric focusing (IEF) sample buffer (9.5 *M* urea, 2% (w/v) NP-40, 1.6% pH 5–7 ampholine, 0.4% pH 3.5–10 ampholine, 5.0% 2-ME) for 2 h at room temperature. *S. aureus* was pelleted by centrifugation at 8000 × g for 5 min. Molecular weight markers were then added to sample. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was done as initially described by O'Farrell (1975) and as detailed by Jones (1980). Immunoprecipitated proteins were separated according to charge in first dimension tube gels using isoelectric focusing with a pH range of 4.5 to 7.0. The second dimension size preparation was done on 10% SDS-PAGE slab gels.

*Amino acid sequence determination.* Preparative 13% acrylamide tube gels were fractionated and the fractions were collected in tubes containing 0.05% SDS and incubated overnight at room temperature to allow the radiolabeled protein to elute from the gel matrix. Aliquots of each fraction were counted in a Beckman scintillation counter (Irvine, California). Appropriate peak fractions corresponding to the antigen of interest were pooled, gel pieces were removed by gel filtration through a 0.45 µ millipore filter, and the samples were lyophilized. Samples were resuspended to 1 ml in a 1% ovalbumin solution. Samples were dialyzed against water overnight at 4 °C to remove excess SDS.

Isolated 45 000 mol. wt. molecules labeled with a single $^3H$ amino acid were sequenced on a Beckman 890C sequencer (Irvine, California) modified with a cold trap (McCumber et al. 1980) using a 0.1 *M* Quadrol program. Polybrene (Klapper et al. 1978) and occasionally a $^{35}S$-labeled internal standard were added to the sequencer cup. The butyl chloride fractions were dried down and scintillation fluid was added to each fraction. The radioactivity in each fraction was determined using a liquid scintillation counter.

## Results

### *Generation and testing of xenoantisera*

Syrian hamster xenoantisera were generated by immunizing rabbits and mice with Syrian hamster lymph node cells and splenocytes. These xenoantisera were tested in hemagglutination and complement-dependent lymphocytotoxicity assays with cells from the classically inbred strains MHA and LSH, and from a recently wild, inbred strain, MIT. Not surprisingly, all xenoantisera contained strong cytotoxic and hemagglutinating antibodies reactant with molecules on cells of all hamster strains tested (data not shown). While it is expected that some of these antibodies detect MHC-related molecules, it is also likely that antibodies responsible for cytotoxic and hemagglutinating reactions may be directed at cell surface components totally unrelated to the hamster MHC. We turned to immunoprecipitation studies to determine whether molecules in the expected molecular weight ranges of MHC molecules were present.

### *Cell surface molecules detected by unidimensional SDS-PAGE analysis*

*Use of xenoantisera.* Radiolabeled cell surface molecules recognized by these xenoantisera were isolated by immunoprecipitation from MHA Con A-activated cells and analyzed by SDS-PAGE. As seen in Figure 1, cell surface molecules of various sizes were immunoprecipitated by the xenoantisera. Importantly, molecules in the 29 000–39 000 and 45 000–50 000 mol. wt. ranges were observed, and we considered these to represent candidates for MHC encoded class II and class I

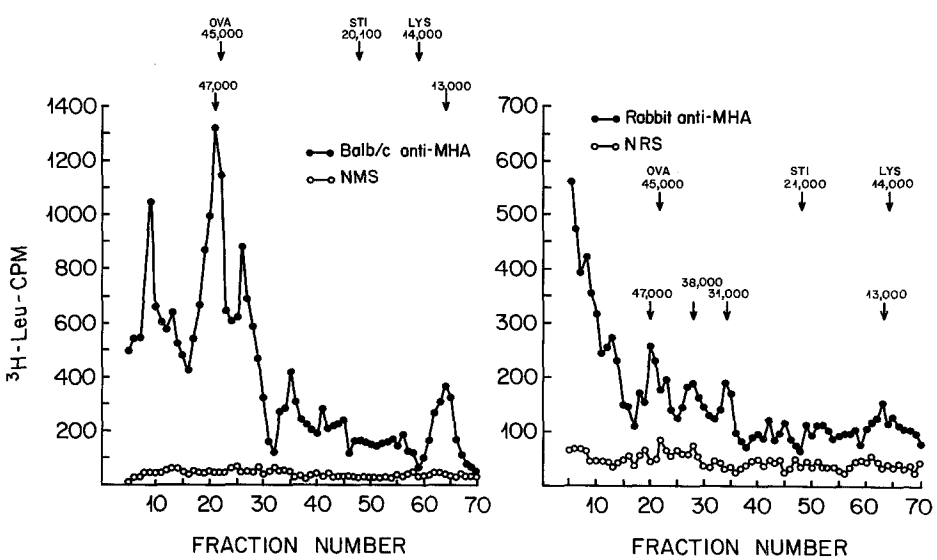


**Fig. 1.** Thirteen percent SDS-PAGE (reducing conditions) of $^3$H-Leu labeled antigens from MHA Con A blasts detected by xenoantisera. Aliquots of $35 \times 10^6$ cell equivalents were precipitated with (●) specific hyperimmune sera, either BALB/c anti-MHA (*left profile*) or rabbit anti-MHA (*right profile*); (○) normal sera, either normal mouse serum (*left profile*) or normal rabbit serum (*right profile*).

molecules, respectively. When hamster cell lysates were first pretreated with hamster alloantisera known to react with hamster class II molecules, subsequent immunoprecipitation with the xenoantiserum BALB/c anti-MHA revealed that the band in the 45 000–50 000 mol. wt. range remained; however, the molecules in the 29 000–39 000 mol. wt. range had been removed (data not shown). These results indicate that the proteins in the 29 000–39 000 mol. wt. range immunoprecipitated by the xenoantisera represent class II molecules encoded within the hamster MHC. We thus felt encouraged that the 45 000–50 000 mol. wt. molecules might represent hamster class I molecules. The molecules in the 45 000–50 000 mol. wt. range that we were interested in were more readily detected, in our system, on Con A-activated lymph node cells than on their unstimulated counterparts. Consequently, all further experiments were performed with Con A-activated lymphocytes. No further characterization was attempted of molecules of greater than 65 000 mol. wt. that these antisera detected.

*Use of anti-$\beta_2$-microglobulin sera.* Class I molecules are expressed on cell surfaces in noncovalent association with $\beta_2$-microglobulin (Grey et al. 1973, Vitetta et al. 1976). Taking advantage of this association, we assayed several heterologous anti-$\beta_2$-microglobulin sera to identify hamster $\beta_2$-microglobulin by cross-reactivity. In so doing, we hoped by co-precipitation to identify hamster class I molecules. Previous work in this laboratory had demonstrated the ability of a rabbit antihuman $\beta_2$-microglobulin reagent to cross-react with molecules on unstimulated hamster LNC and precipitate a 45 000 mol. wt. protein (Phillips et al. 1978). In the present studies, a rabbit antihuman $\beta_2$-microglobulin serum was tested on $^3$H-leucine-labeled MHA Con A-stimulated LNC. As revealed in Figure 2A, 46 000 mol. wt. and 13 000 mol. wt. molecules similar to those identified previously on LNC were identified. The fact that a 46 000 mol. wt. molecule can be detected which appears to be associated with $\beta_2$-microglobulin does not by itself identify hamster K/D-like class I molecules. In the mouse, both Qa and TL molecules are 40 000–45 000 mol. wt. glycoproteins that associate with $\beta_2$-microglobulin (Michaelson et al. 1977, Vitetta et al. 1975). This issue is addressed by tissue distribution studies (vide infra) in which Qa and TL molecules have a restricted tissue distribution, whereas K/D molecules are distributed ubiquitously (Klein 1975, Flaherty 1980).

*Use of cross-reacting murine alloantisera.* To characterize further the 45 000–50 000 mol. wt. molecules recognized by xenoantisera and by anti-$\beta_2$-microglobulin sera, mouse alloantisera, known to recognize mouse class I molecules, were assayed on hamster cell lysates by immunoprecipitation followed by SDS-PAGE. Previous work had indicated that the NIH anti-$D^d$ reagent, specific for the private specificity of the $D^d$ molecule, H2.4, reacted with determinants on hamster lymphoid cell surfaces producing cell death in the presence of complement. This antiserum proved to be very weak and difficult to use for immunoprecipitation assays (Phillips et al. 1981b). In the present study, three other mouse alloantisera that are reactive with both public and private specificities of the K, D, and TL molecules were tested (C. S. David, personal communication). The three mouse alloantisera used in this study were: (B10 × 129/J) anti-B10.D2 (b anti-d), (129/J × B10) anti-B10.K (b anti-k), and (C3H/HeJ × B10.K) anti-B10 (k anti-b). These antisera react weakly with hamster

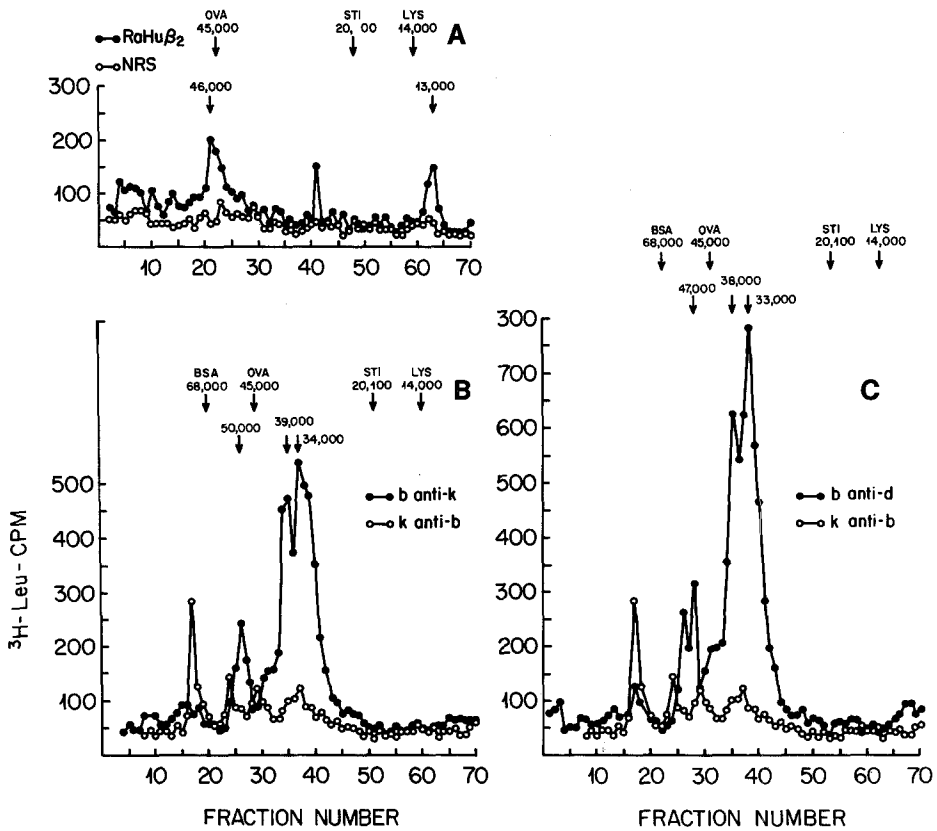


**Fig. 2 A–C.** Thirteen percent SDS-PAGE (reducing conditions) of $^3$H-Leu labeled antigens from MHA Con A blasts detected by cross-reactive mouse alloantisera. Aliquots of $25 \times 10^6$ cell equivalents were precipitated with (●) specific hyperimmune sera, rabbit antihuman $\beta_2$-microglobulin, b anti-k, b anti-d; (○) irrelevant hyperimmune serum, normal rabbit serum, k anti-b.

cells in a lymphocytotoxicity assay (data not shown). However, at least two of them appear to detect hamster class I-like molecules when they are reacted with cell lysates and analyzed on polyacrylamide gels. As can be seen in Figure 3, the b anti-d and b anti-k sera precipitate hamster class I-like molecules, with molecular weights of approximately 49 000. The k anti-b reagent does not.

Note that there are protein bands in the molecular weight range of 29 000 to 39 000 using the b anti-k and b anti-d sera. We believe these to be hamster class II molecules which are homologues of mouse *I-E* since both reagents detect the Ia.7 specificity which has been shown previously to be present in a cross-reactive form on hamster cells (Phillips et al. 1981a).

In the aggregate, the data presented so far, using hamster xenoantisera, cross-reactive anti-$\beta_2$-microglobulin sera, and cross-reacting mouse alloantisera known to detect class I molecules, strongly support the notion that class I-like molecules are expressed on the surface of Syrian hamster cells.

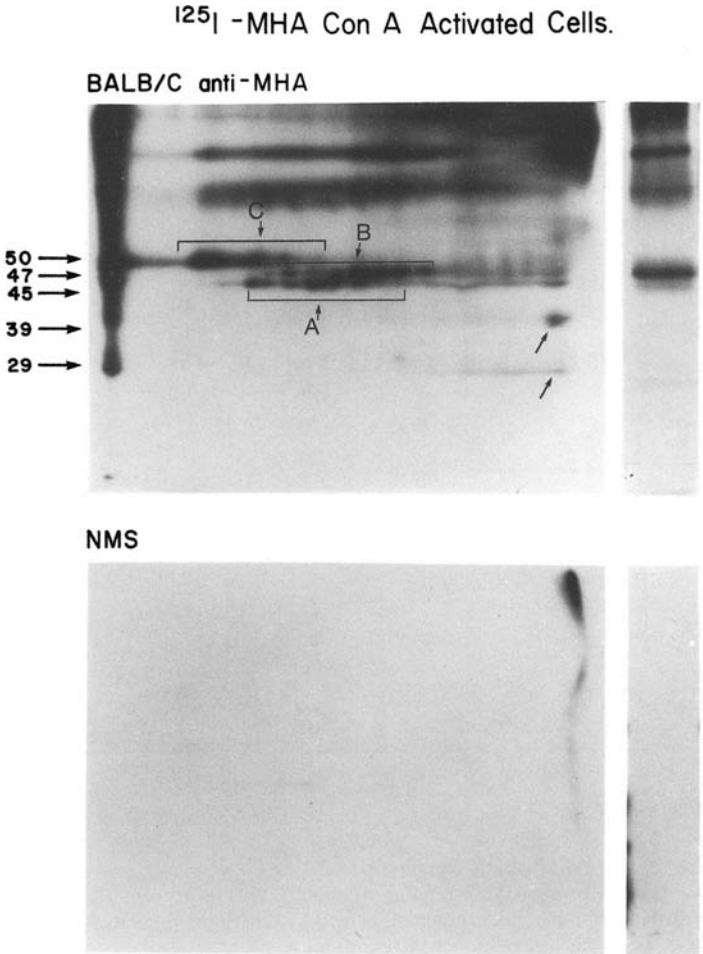


**Fig. 3.** Composite 2D and 1D SDS gel patterns of the proteins immunoprecipitated from $^{125}$I-labeled MHA Con A-activated cells by BALB/c anti-MHA (*upper panel*) and normal mouse serum (*lower panel*). 1D gels were exposed to film for an hour. 2D gels were exposed to film for 4 days. *Arrows* pointing upwards indicate hamster class II molecules. The complexes at 45 000, 47 000, and 50 000 labeled A, B, and C, respectively, indicate hamster class I-like molecules. The basic end of the IEF gel is on the *left* and the direction of SDS electrophoresis was from *top to bottom.*

The xenoantiserum, BALB/c anti-MHA, appeared to react most strongly with hamster class I-like molecules, and it has been used exclusively in the subsequent experiments.

*Cell surface molecules detected by two-dimensional page analysis*

In all mammalian species so far examined, there are at least two loci which encode class I molecules, having molecular weights within the range 40 000–48 000 (Götze 1977). All of the antisera used in this study have the potential for recognizing more than one class I molecule in any lysate and there is no reason to believe that the

hamster does not possess multiple loci encoding hamster class I molecules. In fact, close scrutiny of the unidimensional gels suggests that multiple bands exist in the 45 000–50 000 mol. wt. range (unpublished observations). To examine the possibility that hamster cells express multiple class I-like molecules, the molecules immunoprecipitated by BALB/c anti-MHA have been analyzed by 2D-PAGE.

For the first dimension of the 2D-PAGE, an isoelectric focusing system with a pH gradient from pH 4.5 to pH 7.0 was used. Figure 3 compares the 1D- and 2D-PAGE patterns of the molecules immunoprecipitated by BALB/c anti-MHA from MHA Con A-activated cells. Arrows pointing upwards indicate those spots which represent hamster class II molecules. They are very faint because Con A activates predominantly T lymphocytes and hamster class II molecules are found predominantly on resting B lymphocytes (Witte and Streilein 1983). Based on internal molecular weight standards, the three complexes of spots which are potential candidates for class I MHC antigens have been labeled A, B, and C and have molecular weights of 45 000, 47 000, and 50 000, respectively. It is not known how many proteins these complexes represent, but it is assumed, for reasons which will be discussed below, that they represent the products of at least two genetic loci. Due to the weak cross-reactivity of rabbit antihuman $\beta_2$-microglobulin sera, as well as the cross-reacting mouse alloantisera, we elected not to examine the class I-like molecules they identify by 2D-PAGE.

*N-terminal sequence analysis of hamster class I-like molecules*

Comparison of the N-terminal amino acid sequences of class I molecules obtained from other species indicates that certain residues are invariant at certain positions (Ploegh et al. 1981). These highly conserved residues can be used to identify newly sequenced molecules that are class I candidates. Partial N-terminal amino acid sequencing of hamster class I-like molecules was performed by radiolabeling MHA Con A-activated cells with a single amino acid and isolating the 45 000–50 000 mol. wt. peak detected by BALB/c anti-MHA serum.

Three different tritiated amino acids have been utilized in these studies: tyrosine, leucine, and phenylalanine. Two different $^3$H-tyrosine- and $^3$H-leucine-labeled protein preparations have been examined by this amino acid approach; one $^3$H-phenylalanine-labeled protein preparation was also examined. These amino acids were chosen because they represent some of the most highly conserved residues within the N-terminal 30 residues of class I molecules studied in other species. Three representative sequence profiles for hamster class I-like molecules are shown in Figure 4. As seen in Table 1, which summarizes the results of the hamster sequence data and compares them with sequences known for mouse and human class I molecules, all seven assignments correspond with known human or mouse class I sequences. We take these results as proof that the molecules we are detecting on hamster cell surfaces are in fact class I-like, i. e., products of homologous genes.

Further analysis of the sequence results indicates that at least two molecules are being sequenced. They share the same amino acids at positions 7, 8, 17, and 27, but possess different amino acids at positions 5 and 22. For example, the yield of $^3$H-tyrosine at position 22 is half of that expected. This suggests that of two molecules which share tyrosine residues at positions 7 and 27, only one of them possesses

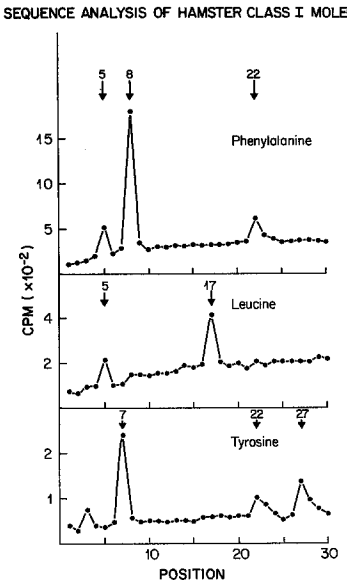


**Fig. 4.** Sequence analysis of hamster class I molecules. Three representative profiles from single amino acid labeling experiments are presented. Hamster class I molecules were isolated by immunoprecipitation with BALB/c anti-MHA from $^{3}$H-leucine, $^{3}$H-tyrosine, or $^{3}$H-phenylalanine labeled MHA Con A-activated cells.

tyrosine at position 22. A similar decrease in counts is also observed for leucine at position 5 and phenylalanine at position 22. Positions 5 and 22 are not highly conserved residues and comparison of several class I molecule sequences from other species indicates that several different amino acids can be assigned at these positions. In fact, position 22 is a moderately conserved position and only two different amino acids have been assigned to this position in other species, namely, tyrosine and phenylalanine. It is interesting that these are the same two amino acids that have been assigned to this position in the hamster molecules. The phenylalanine-tyrosine amino acid interchange represents a single base change in the DNA, which may account for the assignment of either of these two amino acids to position 22. The minor peaks of tyrosine at position 3 and phenylalanine at position 5 are due to a minor population of unknown proteins that contaminate our samples. They may or may not be related to the class I molecules being sequenced.

In summary, comparison of the amino acid sequences obtained for the hamster 45 000–50 000 mol. wt. molecules isolated by BALB/c anti-MHA from MHA Con A-activated cells with known class I sequences from other species indicates possesses class I molecules. The observation that two amino acids have been assigned to position 22 and that the yield of these amino acids at this position is one-half of that expected indicates the presence of two hamster class I molecules.

### *Tissue distribution studies*

Ubiquitous tissue distribution is generally believed to be a hallmark and defining characteristic of class I transplantation antigens. Since the sequencing data indicated the presence of at least two class I molecules and two-dimensional gel analysis indicated a minimum of two proteins in the class I region, it became of

interest to determine (a) whether different tissues had a 2D-PAGE pattern similar to that of MHA Con A-activated cells and therefore all were likely to be K/D like, or (b) whether one of the class I molecules was a Qa or TL-homologue, in which case one molecule might have a limited tissue distribution. BALB/c anti-MHA serum has been used in the present tissue distribution assays because the sequencing studies demonstrated this antiserum to be capable of recognizing hamster class I molecules.

Two-dimensional PAGE analysis was performed on radiolabeled lysates from several lymphoid and nonlymphoid tissues, including LNC, thymocytes, LPS-activated cells, bone marrow cells, and fibroblasts. A comparison of the 2D gels is shown in Figure 5. It would appear that all tissues have very similar 2D-PAGE patterns in the class I region. They all seem to express at least one, and usually two or three, of the same three complexes of spots at 45 000, 47 000, and 50 000 mol. wt. However, these three complexes seem to be present in different amounts on different tissues. Con A-activated cells appear to have relatively equal amounts of the 45 000, 47 000, and 50 000 mol. wt. complexes whereas fibroblasts appear to have more of the 50 000 mol. wt. C complex relative to the 45 000 and 47 000 mol. wt. A and B complexes. The relative amounts of the 45 000, 47 000, and 50 000 mol. wt. complexes seem to vary in other tissues as well. It is not clear whether these are significant differences. Variations in the ability to label cell surface molecules on different tissues could account for the observed differences. The observation that at least one of the complexes appears to be present on all tissues examined and that the electrophoresis mobility of the complexes is very similar, if not identical, indicates that hamster class I molecules have a tissue distribution that is not restricted to lymphoid cells.

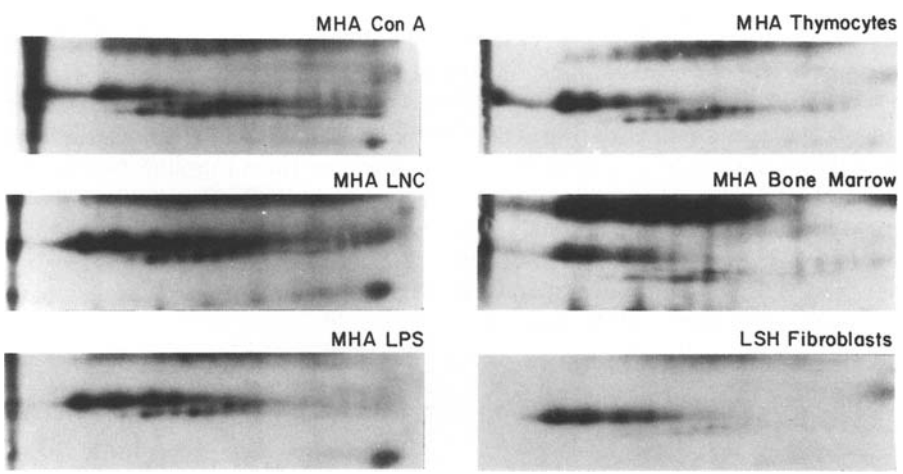


**Fig. 5.** 2D-PAGE analysis of BALB/c anti-MHA immunoprecipitation from various hamster cell types. $^{125}$I-labeled hamster class I molecules were immunoprecipitated by BALB/c anti-MHA from 20–25 $\times$ $10^6$ cell equivalents of various hamster tissues. Cells were exposed to film as follows: MHA Con A gel for 4 days, MHA LNC gel for 6 days, MHA LPS gel for 15 days, MHA thymocyte gel for 8 days, MHA bone marrow gel for 9 days, and LSH fibroblast gel for 19 days. All gels were run under reducing conditions.

Using 2D-PAGE analysis, it is evident that hamster class I molecules are present on a variety of tissues, both lymphoid and nonlymphoid. By 2D-PAGE analysis, it is evident that at least one complex of spots is strongly represented on all tissues, indicating that at least one of the hamster class I molecules may represent the hamster homologue of the mouse K/D class I molecules. Additionally, since all three spots are not equally represented on all the tissues, it is possible that at least one complex of spots represents hamster homologues of the mouse Qa and/or TL class I molecules.

To this point, the results of these studies strongly endorse the notion that Syrian hamsters possess MHC-like genetic loci which encode class I molecules. What remains to be determined is whether the genes present at these loci in disparate hamster strains contain monomorphic or polymorphic sequences. It is essential at this point to make a brief summary of the genetically disparate hamsters available for this series of studies.

Based on the genomic structure of the MHC in other species, one could theorize that hamster class I genes would be linked to hamster class II genes. However, conventional linkage studies cannot be done in hamsters since polymorphism has only been detected among class II genes. Included in this study are the classically inbred strains MHA and LSH, which are both *Hm-1*$^a$, but are known to differ in their background genes as demonstrated by disparity at minor histocompatibility loci (Billingham et al. 1960). The ancestors of these strains were captured in Syria in 1930 (Yerganian 1972). MIT is an inbred strain derived from the 1970 catch of hamsters, possesses the *Hm-1*$^c$ allele, and differs from MHA and LSH at background genes. CB.DFW is a recently produced congenic line that possesses the *Hm-1*$^a$ allele and background genes of the classically inbred CB hamster (*Hm-1*$^b$). CB.SYR is another *Hm-1* congenic strain that possesses the background genes of the CB hamster but the *Hm-1* haplotype of the recently wild hamster SYR (*Hm-1*$^f$) (Duncan and Streilein 1981). The SYR MHC haplotype is different from the *Hm-1* haplotype of any of the other hamsters used in this study. The BUN animal was the most recently caught wild hamster (1978). BUN is heterozygous at *Hm-1;* it expresses molecules similar to those encoded by *Hm-1*$^a$ (MHA and LSH), as well as unique Hm-1 molecules not detected in any other hamsters (unpublished observations). BUN was bred to a CB animal to generate $F_1$ animals. These animals were then randomly inbred to produce a partially inbred strain of (CB × BUN)$F_7$ animals possessing a random mixture of 50% of the genes from BUN and 50% of the genes from CB. By examining two (CB × BUN)$F_7$ animals, we have sampled theoretically 75% of the BUN genome.

*2D-page comparison of class I molecules isolated from Hm-1 disparate strains of hamster*

To determine the extent of polymorphism in the 45 000, 47 000, and 50 000 mol. wt. A, B, and C complexes defined by 2D-PAGE analysis of hamster class I molecules, lymphoid cells from selected strains of hamsters were radiolabeled, lysed, immunoprecipitated with BALB/c anti-MHA and analyzed by 2D-PAGE.

Figure 6 represents immunoprecipitates from MHA, CB.SYR, CB.DFW, and (CB × BUN)$F_7$ lymphoid cell lysates. Essentially, all of these patterns are very

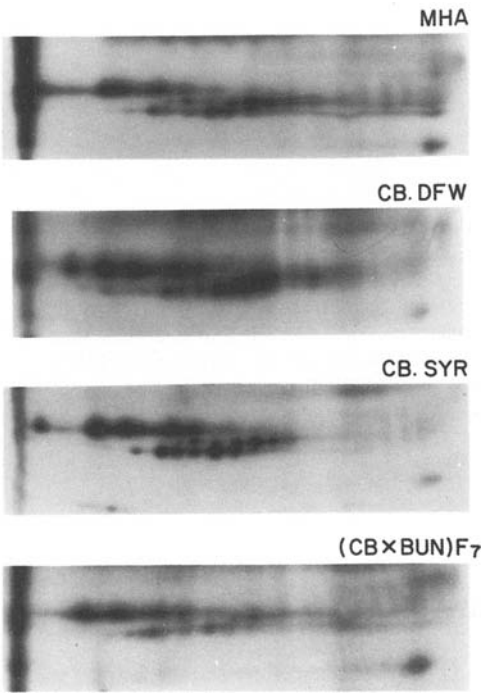


**Fig. 6.** 2D PAGE analysis of hamster class I molecules from MHA, CB.DFW, CB.SYR, and $(CB \times BUN)F_7$ hamsters. $^{125}I$-labeled antigens from MHA, CB.DFW, CB.SYR, and $(CB \times BUN)F_7$ hamsters (*top to bottom*) Con A activated cells were immunoprecipitated by BALB/c anti-MHA. Immunoprecipitations were done from $20 \times 10^6$ cell equivalents of each strain of hamster. All gels were run under reducing conditions. Gels were exposed to film as follows: MHA gel for 4 days, CB.DFW and CB.SYR gels for 9 days, and $(CB \times BUN)F_7$ gel for 15 days.

nearly identical. However, due to variation from gel to gel and experiment to experiment, it is impossible to determine whether the patterns are truly identical, which could be the case if these animals were monomorphic for class I genes.

To obtain critical information bearing on this important issue, lymphoid cells from inbred strains LSH and MIT and the $(LSH \times MIT)F_1$ hybrid were assayed. These two inbred strains are known to be *Hm-1* disparate for a class II-like locus; in addition they display numerous disparities among their background genes. If their class I molecules are polymorphic, then co-dominant expression of both parental sets of molecules should be detected upon 2D-PAGE analysis of the $F_1$ hybrid.

Figure 7 represents immunoprecipitates from LSH, MIT, and $(LSH \times MIT)F_1$ lymph node cell lysates. Although the three complexes in the class I region appear identical in the LSH and MIT immunoprecipitates, without running them both on the same gel, it is difficult to say that they are identical. However, it is evident in the gel from the $F_1$ hybrid that the 2D-PAGE patterns of LSH and MIT precipitates are identical, since there do not appear to be any additional spots immunoprecipitated from the $(LSH \times MIT)F_1$ cell lysates that are not present on either the LSH or MIT 2D-PAGE profile.

Thus, by two-dimensional gel analysis the patterns of class I-like molecular complexes are similar for all hamster strains tested. Moreover, the $F_1$ hybrid derived from *Hm-1* disparate parents displays a gel pattern indistinguishable from either parent. These data strongly support the contention that class I molecules in the Syrian hamster are nonpolymorphic.

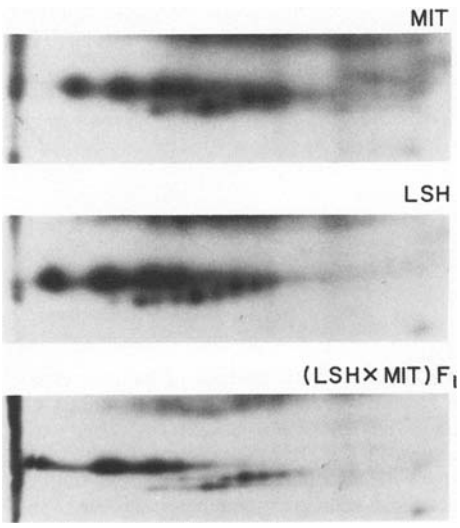


**Fig. 7.** 2D-PAGE analysis of hamster class I molecules immunoprecipitated from MIT, LSH, and (LSH × MIT)$F_1$ hamsters. $^{125}$I-labeled antigens from MIT, LSH, and (LSH × MIT)$F_1$ (*top to bottom*) Con A activated cells were immunoprecipitated by BALB/c anti-MHA. Immunoprecipitations were done from $20 \times 10^6$ cell equivalents of the respective strain of hamster. All gels were run under reducing conditions. Gels were exposed to film as follows: MIT gel for 6 days, LSH gel for 4 days, and (LSH × MIT)$F_1$ gel for 9 days.

## Discussion

Class I MHC molecules have become one of the most carefully studied sets of molecules in contemporary immunobiology. A lexicon of features can be used to describe them: they are transmembrane cell surface glycoproteins of approximate mol. wt. 45 000 that associate noncovalently with $B_2$-microglobulin. They are encoded by genes within the major histocompatibility complex and are expressed ubiquitously on somatic tissues. Class I molecules play a central role in the process by which cytotoxic T cells recognize nominal antigens whether on presenting cells or target cells. One dominant feature of class I molecules that is characteristic in all species studied to date is extensive polymorphism. Until very recently, with the advent of molecular biologic techniques, identification and study of class I MHC molecules have been based on the fact that these molecules are polymorphic, function as transplantation alloantigens, and as a consequence are recognized through their allotypic variations. Consequently, extensive polymorphism has come to be regarded as central to the biologic meaning of class I molecules. It is against this background that the studies reported in this communication must be considered. The results of our experiments in Syrian hamsters demonstrate that class I molecules are present in Syrian hamsters and that they appear to be monomorphic. This conclusion raises two important questions: (1) How confident can we be that our assertion concerning class I monomorphism is correct? and, if it is correct, (2) what is the meaning of monomorphism in this mammalian species?

The assertion that Syrian hamsters possess only monomorphic class I MHC molecules rests on the following evidence which is summarized in Table 1. (a) The cell surface of hamster cells contains transmembrane glycoproteins (in the molecular weight range 45 000–50 000) that can be identified by xenoantisera generated against hamster cells (Fig. 1), by cross-reactive mouse alloantisera known to react with class I molecules, and by co-precipitation with cross-reacting antisera

**Table 1.** Properties of class I molecules in mouse, man, and hamster

| | Man/Mouse | Hamster |
|---|---|---|
| 45 000 mol. wt. glycoprotein | + | + |
| $\beta_2$-microglobulin associated | + | + |
| Ubiquitous tissue distribution | + | + |
| Multiple loci | + | + |
| Conserved N-terminal amino acid sequence | + | + |
| Elicit production of alloantibodies | + | − |
| A restricting element in cytotoxic viral assay | + | − |
| Structural polymorphism detected by 2D gel analysis | + | − |

that detect the hamster homologue of $\beta_2$-microglobulin (Fig. 2). These cell surface molecules have a ubiquitous tissue expression; and this expression is increased on lymphoid cells activated by Con A (Fig. 5). Partial N-terminal amino acid sequence confirms that amino acid residues that are highly conserved at certain positions in class I molecules in other species are also present in hamster class I homologues (Table 2). (b) Alloantisera generated among *Hm-1* disparate hamster strains fail to detect class I molecules on radiolabeled hamster cell lysates via SDS-PAGE analysis (Streilein et al. 1981). (c) Cross-immunization of allodisparate hamsters, either in vivo or in vitro, fails to generate cytotoxic effector T cells (Duncan and Streilein 1977). (d) 2D gels of lysates from (LSH × MIT)$F_1$ hybrid hamster lymphoid cells reveal identical patterns of class I-like complexes to those derived from either parent alone (Fig. 7). Since these hamster strains are as genetically disparate as can be

**Table 2.** Comparison of N-terminal amino acid sequences of hamster, mouse, and human class I molecules

| Species | 1* | 2 | 3* | 4* | 5 | 6* | 7* | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| $K^{b}$[†] | Gly- | Pro- | His- | Ser- | Leu- | Arg- | Tyr- | Phe- | Val- | Thr- |
| Qa-2[‡] | | | His | | Leu | | Tyr- | Phe | | |
| HLA-B7[‖] | Gly- | Ser- | His- | Ser- | Met- | Arg- | Tyr- | Phe- | Tyr- | Thr- |
| Hamster | | | | | Leu | | Tyr- | Phe | | |
| | 11 | 12 | 13 | 14* | 15* | 16* | 17 | 18* | 19 | 20 |
| $K^{b}$ | Ala- | Val- | Ser- | Arg- | Pro- | Gly- | Leu- | Gly- | Glu- | Pro |
| Qa-2 | | Val | | Arg | | | Leu | | | |
| HLA-B7 | Ser- | Val- | Ser- | Arg- | Pro- | Gly- | Arg- | Gly- | Glu- | Pro- |
| Hamster | | | | | | | Leu | | | |
| | 21 | 22 | 23 | 24 | 25* | 26* | 27* | 28* | 29 | 30 |
| $K^{b}$ | Arg- | Tyr- | Met- | Glu- | Val- | Gly- | Tyr- | Val- | Asp- | Asp |
| Qa-2 | | Phe | | | Val- | Leu- | Tyr- | Val | | |
| HLA-B7 | Arg- | Phe- | Ile- | Ser- | Val- | Gly- | Tyr- | Val- | Asp- | Asp |
| Hamster | | Tyr Phe | | | | | Tyr | | | |

[†] Coligan et al. 1981.
[‡] Soloski et al. 1982.
[‖] Orr et al. 1979.

currently arranged, identical patterns for both parental strains and the $F_1$ hybrid strongly support the contention that hamsters lack class I polymorphism.

Experimental approaches which "fail" to demonstrate an expected result must always receive special scrutiny. While we believe that these data are compatible with and supportive of the monomorphism hypothesis, there remain other approaches which might yet detect minor differences. Limited proteolysis followed by peptide map analysis has not yet been accomplished. Peptide maps can distinguish among $K^b$ and its mutant molecules (Nairn et al. 1980). These molecules resemble hamster molecules to the extent that they are serologically "silent"; however, they induce CTLs, which putative hamster class I determinants do not (Klein 1978, Duncan and Streilein 1977). To our knowledge the $K^b$ mutant molecules have not been subjected to analysis by 2D gels as we have examined the hamster molecules. It is possible that the mutant molecules would also appear to be monomorphic in this assay. P. P. Jones (personal communication) claims that 2D gel analysis of I-$A^b$ and the bm12 mutant molecules yield identical patterns. Another approach would be to conduct partial N-terminal sequence analysis of class I molecules from several *Hm-1* disparate hamster strains, especially at positions known in other species to be highly polymorphic. Experiments of these types are currently in progress. However, it is extremely unlikely that these more discriminating approaches will detect a degree of polymorphism among class I hamster molecules that is equivalent in extent to that described in other species; i.e., in mouse, man and other species, 20–30% nonhomology has been the typical result when two or more class I allelic products are sequenced (Ploegh et al. 1981). We are confident that if allelic differences are revealed among hamster class I molecules, the number of disparate residues will be much smaller.

Until these potentially more discriminating studies are completed, we cannot say for certain that hamsters have *no* class I polymorphism. However, we are confident that the degree of polymorphism, if present, must be exceedingly low and significantly different from that described in other mammalian species.

The domestic cat has been reported to be monomorphic at its MHC (Pollack et al. 1982). However, since the cheetah, a close relative of the domestic cat, is monomorphic at a wide variety of loci (O'Brien et al. 1983), the monomorphism seen at class I loci in the cat may be due, perhaps, to a population bottleneck which has eliminated polymorphism at most loci in the cat.

But if polymorphism is the central feature of class I molecules, how could a mammalian species (hamsters) be evolutionarily successful with a set of virtually monomorphic class I genes? One level of responses to this question is essentially mechanistic, couched in genetic terms. The following possible explanations can be advanced: First, the hamsters we have studied may not constitute a sufficiently diverse sample of the wild, native hamster gene pool. We consider this to be unlikely since the animals we have studied represent the descendants of three independent captures of wild hamsters over a span of 40 years and from geographically distant sites. Moreover, these inbred strains display considerable polymorphism at hamster loci encoding class II MHC molecules (Duncan and Streilein 1981). On the presumption that hamster class I and class II loci are chromosomally linked (as in other species), a rather diverse spectrum of hamster haplotypes, and therefore potential class I variants, should be represented in our current stocks. Additionally,

the hamster has been shown to express a degree of heterozygosity at non-MHC loci which is similar to that reported in other species (Csaikl 1984). Second, hamsters as a species have experienced passage through a "genetic bottleneck" from which has emerged only a small proportion of the original hamster gene pool. The diversity of class II alleles among our inbred strains argues against this explanation. Third, hamsters lack the genetic endowment necessary to generate class I polymorphism. Ohno and Wallace (1983) have suggested that tandemly arranged, duplicated genes possess the inherent property of generating polymorphism. If we had found that hamsters possessed a unitary species of class I cell surface molecules, this proposal would be acceptable. However, instead it was found that the hamster genome contains at least two (and probably more) class I-like loci. Therefore, by Ohno's hypothesis, this species has the genetic material with which to generate polymorphism, yet it does not. Fourth, extreme polymorphism and monomorphism are polar positions that class I loci can adopt depending upon selective pressures exerted upon individual species. By implication there are pressures which favor monomorphism as well as pressures which favor polymorphism.

A highly favored, current hypothesis proposes that extreme polymorphism of class I MHC molecules results from the requirement for association of viral antigens with class I molecules in order for cytotoxic T cell recognition to occur (Zinkernagel and Doherty 1974). This hypothesis proposes that there is a direct relationship between the diversity of class I molecules in a species and the diversity of the T cell repertoire. It is presumed that a great variety of class I alleles in a population ensures that at least some members will possess the appropriate determinants necessary for T cells to recognize any virus; therefore, the species can survive successive waves of epizootics with many different viruses.

If this hypothesis is correct, then epizootics will exert their effects only on those species in which epizootics are a realistic threat to survival. Syrian hamsters differ from virtually all other species whose MHC has been extensively studied in that their habitat and the way they have accomodated to it mitigate strongly against horizontally transmitted epizootics (Lerwill and Makings 1971, Johnston 1975). In the Syrian desert, hamsters live as solitary creatures; each adult is responsible for digging and surviving within his/her own burrow, deep beneath the surface. There is no deme structure. Social interactions among hamsters are limited exclusively to the act of procreation, which takes place in the female's burrow. Immediately thereafter the socially dominant, aggressive female chases the male away. The mother and her litter interact until weaning is accomplished, at which time the young are driven from the burrow. Neither the population density nor the social habits of hamsters are appropriate to support epizootics with infectious agents. We would propose that it is this adaptation of hamsters to their environment which relieves them of the selective pressure of epizootics and allows their class I loci to adopt a monomorphic configuration.

If true, this realization raises a final point. The hamster genome encodes not one, but two or more class I products. The fact that this species dedicates more than one locus to class I molecules suggests that the physiologic role of these molecules in monomorphic form may be unrelated to immunology and T cell recognition. Within the past few years, numerous investigators have reported that there is a poorly understood relationship between class I molecules and unexpected ligands:

insulin receptor (Chvatchko et al. 1984, Simonsen and Olsson 1984), epidermal growth factor receptor (Schreiber et al. 1983), penicillin (Edidin 1983), glucagon receptor (Lafuse and Edidin 1980). In some cases, the relationship derives from the fact that the receptor for the ligand demonstrates an affinity for class I molecules. We suspect that it is in this arena that the monomorphic function of class I molecules will be found. If that be the case, then Syrian hamsters would be an excellent experimental model for further study of this physiologic and presumably nonimmune function of MHC molecules.

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