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Tissue-specific expression of an unusual *H-2* (class I)-related gene

(transplantation antigen/graft rejection/tolerance)

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ABSTRACT **Sequence analysis of mouse *H-2* cDNA clones has suggested the existence of an unusual class of H-2 (class I)-related antigens that, unlike the classical membrane-associated molecules, retains only the extracellular portion and is likely to be secreted. The expression of this class of *H-2*-related mRNA is tissue restricted; it is detectable in liver but not in brain, kidney, testis, thymus, or spleen. In the liver, its accumulation represents about one-fourth of all the H-2 (class I)-specific transcripts. This class of transcripts is present in mice of different inbred strains, but the level of expression differs markedly among them. A model is presented in which such a soluble form of the H-2 antigen would play the role of a blocking factor in maintaining peripheral inhibition of H-2 recognition. This would ensure tolerance of the H-2 molecule as a self antigen while permitting it to act as a guidance molecule for the associative recognition of viral and tumor antigens by cytotoxic T cells.**

The classical transplantation antigens of the mouse are membrane glycoproteins composed of a single polypeptide chain about 346 amino acids long noncovalently associated with a molecule of β_2-microglobulin (1, 2). These antigens (class I antigens) are encoded by a family of genes, including the *H-2K*, *H-2D*, and *H-2L* loci, that have been mapped within the major histocompatibility complex (MHC) on chromosome 17 (3). Recent molecular cloning of H-2 sequences has identified other regions both from within the MHC (like the *Qa2,3* and *T1a* loci) and from outside the MHC (but yet to be mapped) that code for related membrane antigens (4–6).

These class I antigens, the H-2K and H-2D products in particular, are classical cell-surface antigens. The cell-surface presentation of these antigens is required for their role in allograft rejection (3), as well as in the associative recognition of viral and tumor antigens by cytotoxic T cells (7). Biochemical analyses have shown that the NH_2-terminal ≈280 amino acids are external to the plasma membrane and contain the alloantigenic determinants (1, 2). This extracellular portion of the molecule is followed by a stretch of about 25 hydrophobic residues that penetrates the lipid bilayer of the plasma membrane and an adjoining 4 or 5 basic amino acids that serve to anchor it to the membrane. The remaining COOH-terminal 30–40 residues are intracellular and presumably function to transmit external signals to the inside of the cell.

In a recent analysis of *H-2* cDNA clones, we have described an unusual class of *H-2* molecules whose nucleotide sequence is closely related to those of the classical H-2 antigens except for the transmembrane domain (8). Within this region of the molecule, extensive nucleotide substitutions and a short deletion not only result in the replacement of hydrophobic residues by charged and polar amino acids but also introduce a translational termination codon toward the end of this structurally important region of the molecule. Such a mRNA would encode a shorter polypeptide containing the biologically active portion of the H-2 antigen located NH_2 terminal to the transmembrane region but lacking the intracellular domain. Because of the nonconservative replacement of hydrophobic amino acids by charged and polar residues in the transmembrane region, the predicted protein product would not be expected to insert into the plasma membrane but most likely would be secreted.

The prospect of having a secretory form of the H-2 antigen is conceptually intriguing with respect to its possible role in immunological regulatory mechanisms. The present study was directed at comparison of the expression of this class of RNA molecules with the expression of those encoding the classical H-2 antigens.

MATERIALS AND METHODS

Isolation of RNA. Total RNA was extracted from tissues of SWR/J, BALB/cJ, C57BL/10J, C3H/HeJ, AKR/J, RF/J, and NZB/BINJ mice (obtained from Jackson Laboratory) by the guanidine·hydrochloride method (9). Polyadenylylated RNA was selected by using oligo(dT)-cellulose as described (10).

Electrophoresis of RNA. Aliquots of RNA were adjusted to 50% formamide/20 mM 4-morpholinepropanesulfonic acid (Mops), pH 7.0/5 mM sodium acetate/1 mM EDTA/2.2 M formaldehyde, heated at 60°C for 10 min, and subjected to electrophoresis on 1% agarose/2.2 M formaldehyde slab gels. The running buffer was 20 mM Mops, pH 7.0/5 mM sodium acetate/1 mM EDTA, and electrophoresis was performed at 35–40 mA for 4 to 5 hr at 4°C. The RNA was transferred to nitrocellulose filters as described by Thomas (11).

Hybridization of RNA Blots. Nitrocellulose filters were baked at 80°C for 3 to 4 hr and prehybridized at 45°C for 4–6 hr in 50% formamide/750 mM NaCl/75 mM Na citrate/5-fold Denhardt's solution/0.1 M sodium phosphate, pH 7.5, with denatured herring sperm DNA at 250 μg/ml. Hybridization was carried out in the same buffer for 16–48 hr at 45°C. In some experiments, 10% dextran sulfate was also included. After hybridization, filters were washed with 450 mM NaCl/45 mM Na citrate at 68°C with several changes of buffer and then with 75 mM NaCl/7.5 mM Na citrate at 68°C. Blots were dried and exposed to Kodak XAR-5 films at −70°C.

Preparation of Hybridization Probes. *Pst* I fragments derived from clones pH2 and pH8 (8) were subcloned into pBR322 at the *Pst* I site. Plasmid DNA was nick-translated to a specific activity of 1–5 × 10^8 cpm/μg (12).

RESULTS

Detection of Subclasses of *H-2*-Related mRNA. Analysis of liver *H-2* cDNA clones derived from SWR/J mice has shown three classes of molecules that share extensive sequence homology throughout their expected coding regions and part of

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Abbreviations: MHC, major histocompatibility complex; Mops, 4-morpholinepropanesulfonic acid.

their 3′-noncoding regions but differ completely in their 3′-terminal 140–170 nucleotides (8). In each class of cDNA clones, a small *Pst* I fragment (170–185 bases) defines the nonhomologous region; this fragment has been used as a specific hybridization probe for that class of cDNA molecules.

The availability of such specific 3′-noncoding probes has allowed us to compare expression at the RNA level of the various classes of *H-2*-related genes. The DNA probes used in these studies (Fig. 1) are the following. (*i*) Probe A is a common 250-base-pair fragment from the coding region that spans amino acids 151–236. This probe, derived from clone pH2, includes the coding sequence for much of the third external domain, the region of the molecule that is suspected to be highly conserved among all class I (H-2) antigens and is responsible for the binding of β_2-microglobulin (1, 6). (*ii*) Probe B is a unique 185-base-pair noncoding fragment derived from clone pH8, a representative of the class of cDNA clones that codes for an H-2 antigen that has a "classical" transmembrane domain composed of entirely hydrophobic residues. (*iii*) Probe C is a unique 170-base-pair noncoding fragment derived from clone pH2. This clone is a representative of the class of cDNA clones that codes for an H-2-related molecule that has a "nonclassical" transmembrane region in which extensive nonconservative substitutions of hydrophobic residues have been detected and that lacks the cytoplasmic domain.

These cDNA probes were hybridized to blots of total poly(A)$^+$RNA obtained from livers of SWR/J mice (Fig. 2). Probe A which spans the coding region for the third external domain and is expected to detect transcripts for all class I (H-2)-related antigens, hybridizes to a heterogeneous population of RNA molecules with a size range of 1,700–1,800 bases (Fig. 2*A*). The heterogeneity in this RNA population is further confirmed by densitometric scanning of the autoradiogram (Fig. 2 *Right*), which indicates a main peak (component I) having a distinct shoulder on the leading edge (component II). A minor component about 4,000 bases long probably represents the unspliced nuclear RNA precursors.

Probe B, derived from the 3′-noncoding region of a classical *H-2* molecule, hybridizes to a more discrete RNA component about 1,800 bases long (Fig. 2*B*), which by densitometric scanning overlaps the major RNA species (component I) detected with probe A. The absence of component II clearly indicates that only a subset of all class I-related transcripts detected with the coding probe is in fact hybridizing to the noncoding probe derived from a gene having a classical transmembrane region. Considering the known specific activities of the two DNA probes and their differences in size, it can be estimated that

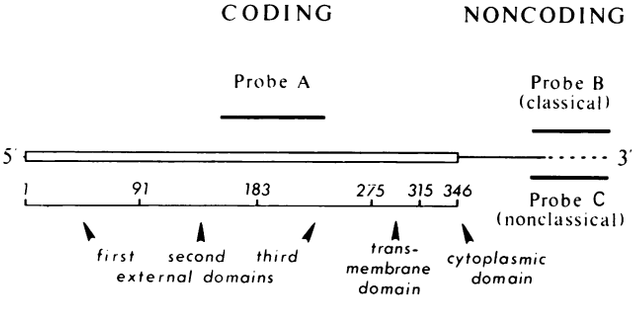

FIG. 1. Schematic representation of the structure of an *H-2* cDNA molecule and location of the three hybridization probes used in this study. The open box represents coding sequences, the solid line represents 3′-noncoding sequences that are common to different cDNA clones, and the broken line represents 3′-noncoding sequences that are nonhomologous among different classes of cDNA clones. Numbers indicate amino acid residues encoded within the various domains.

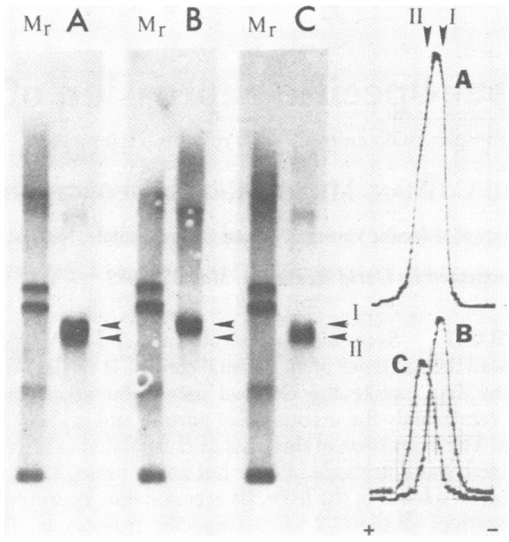

FIG. 2. Detection of classes of *H-2*-related RNA. Total polyadenylated RNA (3 μg) from SWR/J mouse liver was fractionated by electrophoresis in a formaldehyde-containing agarose gel, transferred to a nitrocellulose membrane, and hybridized with probe A, probe B, or probe C. (*Left*) Autoradiograms of the hybridized blots. (*Right*) Densitometric scans of the autoradiograms. The scans of *B* and *C* are superimposed to demonstrate the different mobilities of the two hybridizing components, designated I and II, respectively. M_r markers were ^{32}P-labeled DNA fragments of 4,400, 2,300, 1,950, 1,100, and 600 base pairs.

30–40% of all the H-2 transcripts detected with probe A are also detected with probe B.

In contrast, probe C, derived from the 3′-noncoding region of a nonclassical *H-2* molecule (with extensive substitutions in the transmembrane region), hybridizes almost exclusively to the faster migrating RNA species (component II) that is about 1,700 bases long (Fig. 2*C*). A conservative estimate indicates that this DNA probe detects 20–30% of all H-2 transcripts identified by the coding probe. This suggests that the class of cDNA clones represented by pH2 is derived from a pool of stable mRNA that represents a significant fraction of all class I-related transcripts present in liver RNA.

The remaining fraction of the class I-related transcripts detected by the coding probe, which cannot be assigned to either one of the two noncoding probes shown here, most likely belongs to a third cDNA class that we have described and that also contains an unrelated 3′-noncoding sequence (8). Unfortunately, the unique fragment derived from this third class of clones (represented by pH12) contains a repetitive sequence, detectable in both DNA and RNA studies (data not shown), that limits its usefulness as a hybridization probe. A similar repetitive sequence has also been detected by others in analyses of *H-2* cDNA clones (13, 14).

Tissue-Specific Expression of the Nonclassical *H-2*-Related mRNA. The unusually high level of accumulation of the nonclassical H-2 transcripts in the liver prompted us to analyze other tissues for its expression. Total RNA extracted from kidney, brain, testis, thymus, and spleen was compared with that obtained from liver. With probe A, which detects the coding region representing the third external domain, a component of similar size (about 1,800 bases) is detected in all of the tissues (Fig. 3*A*). Since an equivalent amount of total RNA from each tissue was analyzed, it can be concluded that liver contains relatively more class I (H-2) transcripts than any other tissue. Lym-

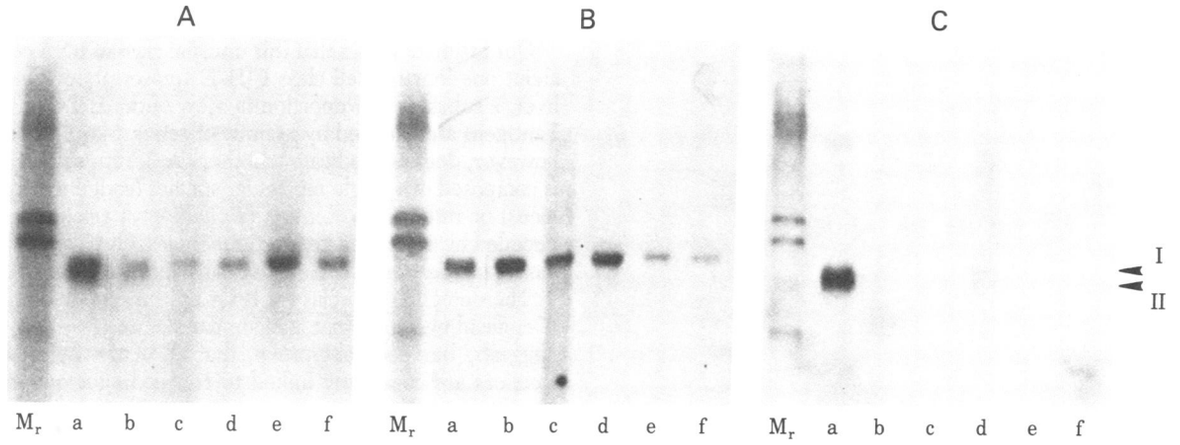

FIG. 3. Detection of *H-2*-related RNA in different tissues. Total RNA (5 μg) extracted from different tissues of SWR/J mice was subjected to electrophoresis, blotted, and hybridized to ^{32}P-labeled cDNA probes. Autoradiograms of the blots after hybridization with probe A (*A*), probe B (*B*), and probe C (*C*) are shown. Tissues tested were liver (lane a), kidney (lane b), brain (lane c), testis (lane d), thymus (lane e), and spleen (lane f). M_r markers were as in Fig. 2.

phoid organs, like thymus and spleen, contain relatively high amounts when compared with brain and testis, which have the lowest level of H-2-related transcripts of the tissues tested. While this coding probe detects a similar RNA component in all tissues, the heterogeneity observed in liver is much greater than that seen with most tissues, which have H-2-related transcripts migrating as a rather discrete component.

The extent of hybridization with probe B, the noncoding probe specific for a class of H-2 molecules having a classical transmembrane region, parallels that of the coding probe in that all tissues tested are positive (Fig. 3*B*). The relative amount of RNA in the various tissues detected with probe B, however, varies somewhat compared with that seen with probe A, suggesting that subclasses of H-2-like molecules have different tissue distributions.

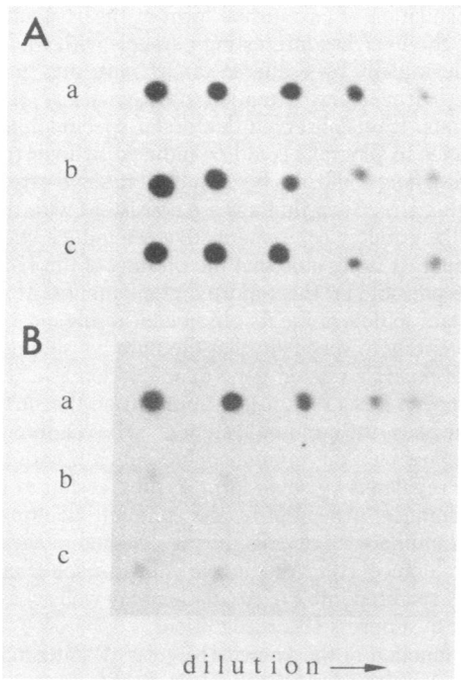

FIG. 4. Quantitation of amounts of *H-2*-related RNA in different tissues. Total RNA from liver (row a), thymus (row b), and spleen (row c) was diluted twofold serially in water and spotted onto nitrocellulose filters. (*A*) Hybridization with probe A. (*B*) Hybridization with probe C.

Analysis with probe C, the noncoding probe specific for a class of H-2 molecules having a nonclassical transmembrane region, produced significantly different results (Fig. 3*C*). Hybridization with this probe detected a RNA species present only in liver; none was found in RNA from kidney, brain, testis, thymus, or spleen. The faint bands seen with kidney and testis RNA are irreproducible and may result from the approximately 30 base pairs of homology between probes B and C (unpublished results).

To obtain a more quantitative assessment of the levels of RNA homologous to the various probes, dot blot analysis was carried out using total RNA from the same tissues. Fig. 4 presents a comparison of liver, thymus, and spleen, in which the input RNA was adjusted such that the amounts of class I (H-2) transcripts detected with probe A, the coding probe, were equivalent (Fig. 4*A*). Under these conditions, probe C (the nonclassical/noncoding probe) still does not hybridize significantly to either thymus or spleen RNA at levels that react strongly with liver RNA (Fig. 4*B*). Based on quantitation of this dot blot, we conclude that, if RNA transcripts related to the nonclassical probe are indeed present in the various tissues tested, they account for less than 5% of the level of this RNA species present in liver.

Comparison among Different Mouse Strains. The experiments described above were carried out with RNA and cDNA probes derived from SWR/J (*H-2*^{q}) mice. We also wished to examine the livers of other strains of mice for expression of this unusual class of *H-2*-related RNA. The additional mouse strains tested included C57BL/10 (*H-2*^{b}), BALB/c (*H-2*^{d}), RF (*H-2*^{k}), NZB (*H-2*^{d}), C3H (*H-2*^{k}), and AKR (*H-2*^{k}). In all cases, both the coding probe, probe A (Fig. 5*A*), and the nonclassical/noncoding probe, probe C (Fig. 5*B*), hybridized to a component of the expected size, showing that the expression of this *H-2*-related molecule is not unique to SWR/J mice.

Although all mouse strains tested expressed the nonclassical H-2 transcripts in liver tissue, the level of expression varied 5- to 10-fold among the strains. It should be pointed out that the fluctuation in expression of this class of H-2-related transcripts correlates well with the level of expression of class I (H-2) transcripts detected by the coding probe. Thus, there may be coordinate expression in the liver of the unusual *H-2*-related molecule and other *H-2* transcripts in the strains we have tested.

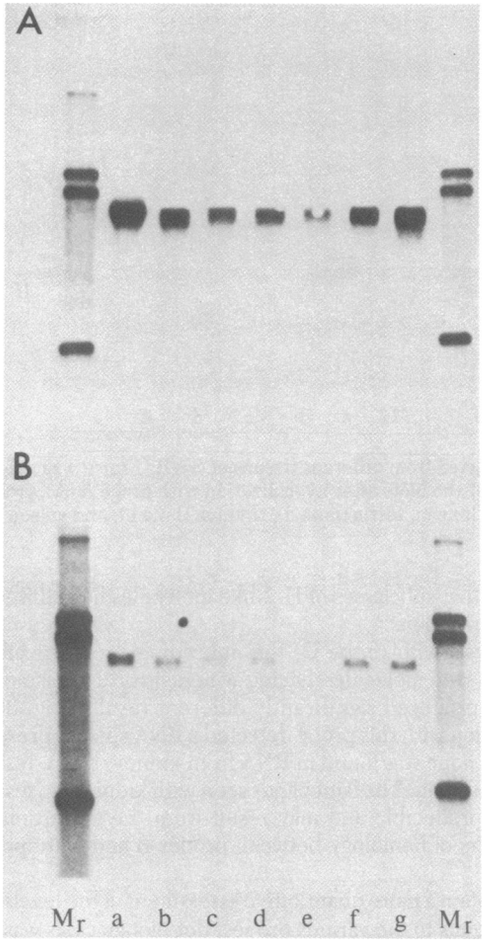

FIG. 5. Detection of *H-2*-related RNA in livers from different strains of mice. Total RNA was extracted from livers of mice of the following strains: SWR (lane a), C57BL/10 (lane b), BALB/c (lane c), RF (lane d), NZB (lane e), C3H (lane f), and AKR (lane g). Each lane contained 5 μg of total RNA except lane a, which contained 10 μg. (*A*) Autoradiogram of the blot after hybridization with probe A. (*B*) Autoradiogram of the blot after hybridization with probe C. M_r markers were as in Fig. 2.

CONCLUSIONS

Analysis of cDNA clones has allowed us to identify an unusual class of *H-2*-related genes that could encode a shorter polypeptide. This protein would contain the entire biologically active portion of the H-2 antigen, located NH_2 terminal to the transmembrane region, but probably would not be able to insert into the plasma membrane or to interact with cytoplasmic components of the cell (8). Such a molecule seems likely to be secreted, although we have not yet unequivocally demonstrated its existence.

The availability of a specific DNA probe derived from the 3′-noncoding region of a representative nonclassical cDNA clone (pH2) has allowed us to study the expression of this unusual *H-2*-related gene. A single size class mRNA about 1,700 nucleotides long can be detected among total liver poly(A)$^+$RNA by using this specific DNA probe and a probe from the common *H-2* coding region. The finding that this RNA transcript is slightly smaller than those that encode the classical H-2 antigens (≈1,800 nucleotides) is consistent with our previous analysis of cDNA clones, which showed that the 3′-noncoding region of the nonclassical molecule is shorter. This suggests that the length of the 5′-noncoding region of this mRNA is not significantly different from that of the classical *H-2* mRNA molecules.

Our estimate shows that this unusual class of RNA comprises about one-fourth of all class I (H-2) transcripts present in the liver, a substantial proportion in view of the fact that the class I antigens are encoded by a family of genes (5, 6). This analysis, however, does not indicate whether the 1,700-nucleotide RNA is composed of a single molecular species (coded for by a single locus) or rather represents a population of related molecules encoded by a family of genes. Sequence analysis of more cDNA clones of this class will hopefully answer this question.

The detection of a class of RNA by the specific nonclassical 3′-noncoding probe that is of similar size to *H-2* RNA strongly suggests, but does not prove, that all of the hybridizing sequences are covalently linked to *H-2* coding sequences. Two observations, however, are consistent with a covalent linkage. First, about 20% of cDNA clones isolated from a liver cDNA library by screening with *H-2* coding probes are of the nonclassical variety (8). This proportion is consistent with our estimate of the abundance of nonclassical H-2 transcripts measured in blot hybridization experiments. Second, hybridization of the 3′-noncoding nonclassical probe to Southern blots of liver DNA detects a small number of bands that appear to be a subset of the 20–30 bands detected with an *H-2* coding probe (unpublished data).

Of particular interest is the apparent tissue-specific expression of this class of *H-2*-related RNA. The liver is the only tissue in which it is detected at significant levels, an observation that has been confirmed in at least three different inbred strains of mice (SWR, NZB, and AKR). In contrast, the *H-2K* and *H-2D* products are expressed on virtually all cells in the body. Other membrane-associated class I antigens, such as Qa2,3 and T1a, do show a more restricted tissue distribution (15) but one that is very different from that of the unusual RNA described here. The possibility that other tissues express altered forms of *H-2*-like genes with dissimilar 3′-nontranslated regions remains to be tested.

The postulation of an unusual form of the H-2 antigen secreted by the liver has interesting parallels with the observations made initially by Calne *et al.* (16) with pigs and subsequently confirmed and extended by Kamada *et al.* (17) by studying rats. It was observed that donor-specific immunological tolerance to allografts could be induced by liver transplantation. This effect could also be mimicked to some extent by the donor serum (18). Such findings are consistent with the secretion by a liver graft of components that can induce tolerance in the recipient. It is possible that the product of the *H-2*-related gene is responsible for this reported phenomenon. If so, it will be important to determine its physiological role.

It is tempting to speculate that the putative soluble protein product of this *H-2*-related gene may function as a "blocking" factor. Since H-2 is a self antigen, present on the surface of all cells in the body, the immune system must be rendered tolerant to it. It is possible that immunocompetent cells with H-2 reactivity are regulated by some form of suppression in the adult (active tolerance) instead of by the complete deletion of H-2-specific immunoreactive cells during prenatal or neonatal life (passive tolerance) (19). A molecule with H-2 specificity that is constantly secreted into the circulation may well act as a blocking factor to suppress H-2 recognition.

A key function of the known H-2 (class I) antigens is to act as guidance molecules in the recognition of foreign antigens by cytotoxic T cells. In this context, there appears to be a need immunologically to recognize nonself against the background of self. Whether a soluble form of H-2, representing a related yet distinct polypeptide, plays a role in the delicate balance of associative recognition remains to be determined.

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