Notable Diversity in Peptide Composition of Murine H-2K and H-2D Alloantigens†

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ABSTRACT: NP-40-solubilized radioactive arginine- or lysine-labeled glycoproteins determined by the *H-2K* (*K*) and *H-2D* (*D*) genes of the *H-2* major histocompatibility complex were isolated by indirect immune precipitation and further purified by molecular sieve chromatography in sodium dodecyl sulfate. Double-label comparison of peptides of products of alleles of *K* and *D* genes showed considerable dissimilarity. Thus, comparison of Kb with Db revealed similar chromatographic behavior of only 11 peptide peaks of a total of 21 and 24 peaks obtained respectively. Comparison of Kd and Dd products showed only 7 similar peptides of 20 and 25 visualized. Comparisons of products of alleles of the same gene (*e.g.*, Kb *vs.* Kd; Db *vs.* Dd) were striking since only about 8–9 out of 20–26 peptide peaks showed similar positions. Although peptide comparisons tend to greatly exaggerate actual structural differences, such data still suggest that the H-2 gene products are considerably diverse structurally. This variability in peptide profiles among H-2 products is consistent with the polymorphism of the *H-2* genes, and also with the complex and variable antigenic profiles associated with serological analysis of the H-2 products. An unusual genetic mechanism may be involved to maintain such a polymorphic system.

The major histocompatibility gene complex of mice (*H-2*) is comprised of four regions (Snell and Stimpfling, 1966; Ivanyi, 1970; Klein and Shreffler, 1971) defined by recombination which map in the following order from the centromere: *K, I, S* and *D* (Klein *et al.*, 1974). The outer *K* and *D* regions are separated by about a 0.5% recombination frequency and contain the *H-2K* and *H-2D* structural genes controlling serologically detected cell-membrane antigens which are present in high density on lymphoid cells and play a role in graft rejection. The *S* region contains a gene (Ss-Slp) controlling the quantity of a serum protein (Passmore and Shreffler, 1968) thought to be involved in the complement system (Hinzova *et al.*, 1972) and the *I* region contains genes involved in the immune response to certain polypeptide antigens and immunoglobulin allotypes (Benaceraff and McDevitt, 1972), in lympho proliferative responses (*cf.* review by Demant, 1973), and in susceptibility to mouse leukemogenesis (Lilly, 1973).

The products of the *H-2K* and *H-2D* genes are glycoproteins of about 45,000 molecular weight consisting of approximately 90% protein and 10% carbohydrate (Schwartz *et al.*, 1973). Although they clearly are implicated in tissue graft rejection, a true physiological function of these membrane integrated macromolecules has not yet been established. However, one property, certainly related to their antigenicity and possibly related to their function, is their extraordinary polymorphism. The extent of this polymorphism is attested to by the fact that 41 distinct H-2 haplotypes[1] have been described in laboratory and wild mice (Klein, 1972) and this number clearly is a minimum estimate.

Previous studies have suggested that the antigenic specificity of an H-2 glycoprotein is determined by its polypeptide structure (Kandutsch and Reinert-Wench, 1957; Nathenson and Muramatsu, 1971). It is reasonable to assume, thus, that structural studies on the peptide portion of these products are likely to give information on the basis of their immunochemical properties as well as information on the nature of the polymorphism of their structural genes.

An analysis of the peptides produced by trypsin digestion can provide a qualitative approach to the investigation of protein primary structure. In earlier studies we compared the peptide composition of two papain-solubilized, purified alloantigen fragments using two-dimensional thin-layer cellulose chromatography (Shimada *et al.*, 1970) or ion-exchange chromatography of radiolabeled peptides (Yamane *et al.*, 1972). By these techniques, at least 15–20% of the resolved peptides appeared unique to the haplotypes examined, but this degree of difference was a minimum estimate due to the insensitivity of the methods for detecting differences.

This paper reports our recent studies on the peptide structure of H-2 glycoproteins utilizing more sensitive comparative procedures and carried out on native NP-40-solubilized molecules rather than papain-cleaved H-2 glycoprotein fragments.

Materials and Methods

Mouse Strains. Inbred mouse strains utilized were C57BL/10 (*H-2*^{b}), B10.D2 (*H-2*^{d}), B10.A (*H-2*^{a}), B10.BR (*H-2*^{k}), HTG (*H-2*^{g}), and HTI (*H-2*^{i}). The mice were either purchased

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[1] The term haplotype refers to a particular form of the *H-2* gene complex. Thus, the *H-2*^{b} haplotype consists of the chromosome segment bearing the two *H-2* genes *H-2K*^{b} (*K*^{b}) and *H-2D*^{b} (*D*^{b}). The numbered specificities which are equivalent to antigenic sites recognized by alloantibodies are carried by the products of each haplotype and include private specificities which uniquely identify each gene product. For example, the *H-2K*^{b} gene product (or the Kb molecule) has private specificity H-2.33, while the *H-2D*^{b} gene product (or the Db molecule) has H-2.2. Other specificities mapping with the determinants of the private specificities, but not associated uniquely with a particular private specificity, are called public specificities. Such determinants may occur in products of several H-2 genes, *e.g.*, H-2.5 occurs in *H-2K*^{b}, *H-2K*^{k}, *H-2K*^{s}, *H-2D*^{k}, etc. Present evidence suggests that each particular H-2 gene product carries one private specificity but also may carry multiple public specificities, *e.g.*, *H-2D*^{b}, which has H-2.2 as its private specificity or antigenic site, also has H-2 public specificities 6, 27, 28, and 29 (*cf.* Nathenson and Cullen, 1974, and Demant, 1973, for reviews).

from Jackson Laboratory or obtained from the breeding colonies of Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, N. Y.

Antisera. The following antisera were used: (1) to detect specificity 2, (HTI × BALB/c)F_1 anti-EL-4; ($H\text{-}2^i \times H\text{-}2^d$)$F_1$ anti-$H\text{-}2^b$; (2) to detect specificity 33, HTG anti-EL-4; $H\text{-}2^g$ anti-$H\text{-}2^b$; (3) to detect specificity 4, (C3H × C57BL/6)F_1 anti-A/Jax; ($H\text{-}2^k \times H\text{-}2^b$)$F_1$ anti-$H\text{-}2^a$; (4) for specificity 31, A/Jax anti-Meth-A; ($H\text{-}2^a$ anti-$H\text{-}2^d$) or (C57BL/6 × A/Jax)F_1 anti-B10.D2; ($H\text{-}2^b \times H\text{-}2^a$)$F_1$ anti-$H\text{-}2^d$; (5) to detect specificity 32 (B10.A(2R) × C3H.SW)F_1 anti-C3H; ($H\text{-}2^h \times H\text{-}2^s$)$F_1$ anti-$H\text{-}2^k$. (6) to detect specificity 11,25 (B10.D2 × PL/J)F_1 anti-B10.A; ($H\text{-}2^d \times H\text{-}2^u$) anti-$H\text{-}2^a$. Sera 1, 2, 3, and 4 were produced according to Snell (1968). Sera 5 and 6 were generously provided by Drs. M. Cherry and G. D. Snell, The Jackson Laboratory, Bar Harbor, Maine.

Radiolabeling and Solubilization of Antigen. Short-term cell cultures were used to label spleen cells. Freshly teased mouse spleen cells (2×10^7 cells/ml) were incubated with 150 μCi/ml of [^{3}H]arginine (30 Ci/mmol, New England Nuclear Corp., Boston, Mass.) or [^{3}H]lysine (30 Ci/mmol) or 50 μCi/ml of [^{14}C]arginine (270 Ci/mol) or [^{14}C]lysine (260 Ci/mol) in Joklik modified MEM spinner medium with freshly added glutamine (Grand Island Biologicals, Grand Island, N. Y.) lacking the amino acid to be added in radioactive form. At the end of incubation for 4 hr at 37° the cells were pelleted and washed once with 0.15 M sodium chloride–0.01 M Tris–0.0015 M magnesium chloride buffer (pH 7.4).

The cells were suspended in the same buffer and 10% NP-40 (Shell Chemical Co. Ltd., London) was added to give a final concentration of 0.5%. After 1 hr at 0° the mixture was centrifuged for 1 hr at 30,000 rpm. The pellet was discarded and the supernatant was treated to remove radiolabeled IgG formed during the incubation. Goat anti-mouse γ-globulin serum was added (in an amount of $\frac{1}{5}$th the volume of NP-40 extract) and after 30 min at 4°, $\frac{1}{20}$th the volume of normal mouse serum (BALB/c, Jackson Laboratory, Bar Harbor, Maine) was added as carrier and the mixture was allowed to incubate for 2 hr more. The precipitate that formed was removed by centrifugation and the supernatant was frozen at −70° in small aliquots.

Immunoprecipitation. A typical experiment demonstrating the isolation of H-2.31 (H-2Kd) glycoprotein from an NP-40 extract of B10.D2 ($H\text{-}2^d$) spleen cells was carried out as follows. A 300-μl portion of the anti-H-2.31 specific antiserum was first incubated with 600 μl of unlabeled NP-40 extract from spleens of $H\text{-}2^a$, ($H\text{-}2K^k$, $H\text{-}2D^d$) mice for 10 min at room temperature. This amount of unlabeled antigen "blocker" (prepared from spleen cells at 5×10^8 cells/ml) was used so that on a cell basis, there was a 2.5- to fivefold excess over labeled antigens. Then 1200 μl of the radiolabeled antigen was added and the mixture was incubated for 30 min at 4°. Goat anti-mouse immunoglobulin serum (four times the volume of alloantiserum) was then added and the incubation was continued at 4° for 3 hr. The precipitate formed was removed by centrifugation and washed four times with 0.01 M Tris–0.15 M NaCl (pH 7.4). The immune precipitate was then suspended in 0.576 M Tris–9% sodium dodecyl sulfate (pH 8.5) (2 ml/300 μl of mouse antiserum originally used). This was mixed vigorously, boiled for 5 min, and then dithiothreitol (final concentration 0.11 M) was added as a solid. The solution was boiled for 2 min and saturated with nitrogen, capped, and incubated for 3 hr at 37°. Iodoacetamide to the concentration of 0.25 M was then added followed by a small amount of 1 N NaOH to keep the pH at 8.5 and the alkylation was allowed to proceed for 30 min. A two- to fourfold excess of mercaptoethanol was then added to stop the reaction.

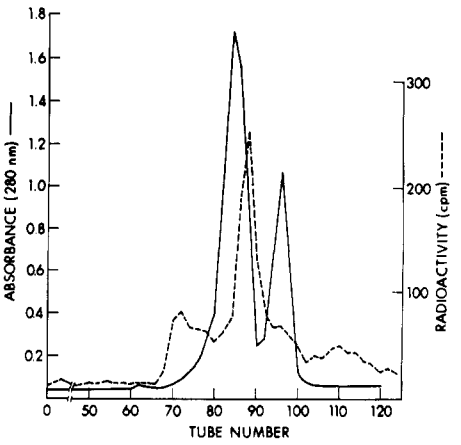

FIGURE 1: Purification of radiolabeled, NP-40-solubilized antigen by column chromatography on Bio-Gel A-0.5 m. The specific immune precipitate of [^{3}H]arginine-labeled, NP-40-solubilized H-2.4 was prepared as described in Materials and Methods. This was reduced with dithiothreitol and alkylated with iodoacetamide in 9% sodium dodecyl sulfate–0.576 M Tris (pH 8.6) and loaded directly on a 1.5 × 110 cm Bio-Gel A-0.5 m column. The sample was eluted with 0.5% sodium dodecyl sulfate–0.05 M Tris (pH 7.5) and the resulting fractions were sampled to determine the radioactive profile and the optical density at 280 nm. The optical density tracing shows the heavy and light chains of the combined mouse and goat immunoglobulin. The peak of radioactivity between fractions 86 and 92 was pooled. Analytical electrophoresis of the pooled material on 7.5% acrylamide–0.1% sodium dodecyl sulfate gels showed a single sharp peak of radioactivity with about mol wt 45,000.

Bio-Gel A Chromatography. The reduced and alkylated precipitates were loaded directly on Bio-Gel A 0.5 M (Pharmacia Fine Chemicals) columns (either 1.5 × 115 cm or 2.5 × 115 cm), which had been equilibrated with 0.5% sodium dodecyl sulfate–0.05 M Tris (pH 7.5), and were eluted with the same buffer. Figure 1 shows the profile of such a column run in which the immune precipitate of H-2.4 reactive material (H-2Dd) was fractionated before trypsin digestion and peptide chromatography. The pooled material migrated as a single peak of about 45,000 mol wt upon electrophoresis on 7.5% polyacrylamide–0.1% sodium dodecyl sulfate gels (Shapiro *et al.*, 1967) and on 10% polyacrylamide–0.1% sodium dodecyl sulfate discontinuous gels (Maizel, 1971).

Trypsin Digestion. The material from polled fractions of the specific H-2 peak of the Bio-Gel column (*cf.* Figure 1) was treated with 15% CCl_3COOH after addition of sufficient unlabeled human IgG (Schwarz/Mann, Orangeburg, N. Y.) to form a visible precipitate. This precipitate was washed successively with 5% CCl_3COOH, a mixture of 95% ethanol and anhydrous ether (1:1), and finally with anhydrous ether. The glycoprotein was then dissolved in 0.05 M ammonium bicarbonate (NH_4HCO_3), pH 8.0, to give a concentration of 10 mg/ml. Tos-PheCH_2Cl-trypsin was added in 0.001 N HCl at a ratio of 1:20 the amount of protein. After incubation for 1 hr at 37°, one-half as much trypsin as the first time was added again and the incubation was continued for 3 more hr. The pH was then lowered to 2.0 with glacial acetic acid and the "core" was removed by centrifugation at 5000 rpm.

Chromatography of Trypsin Peptides. Spherix resin (type XX8-60-0, from the Phoenix Co., Long Island, N. Y.), equilibrated with 0.05 M pyridine–acetic acid buffer (pH 3.13), was used in a column (0.9 × 23 cm) enclosed in a water jacket. The soluble peptides from the digest (usually 2–3 ml) were loaded and washed onto the column with 0.05 M pyridine–acetic acid

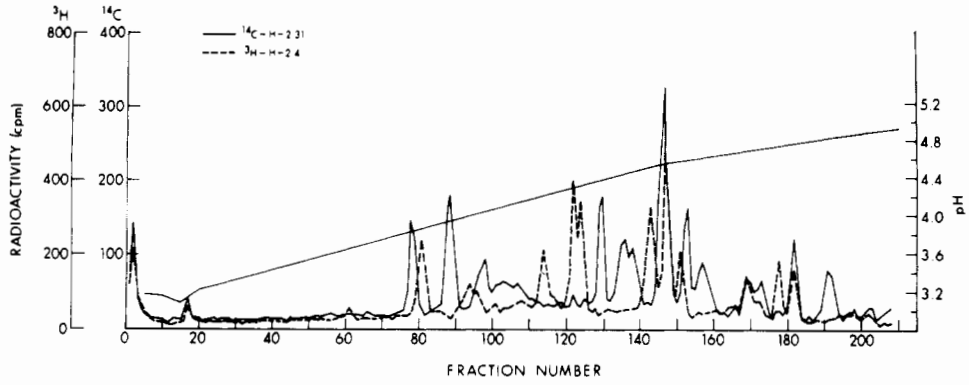

FIGURE 2: Comparison of the tryptic peptides of a K gene product ([^{14}C]arginine-labeled H-2.31) and a D gene product ([^{3}H]arginine-labeled H-2.4). [^{3}H]Arginine-labeled H-2.4 and [^{14}C]arginine-labeled H-2.31, purified on Bio-Gel A-0.5 m as in Figure 1, were mixed to give a total of 20,000 cpm of ^{3}H and 15,000 cpm of ^{14}C; precipitated with 15% CCl_3COOH; washed; and digested with Tos-PheCH_2Cl-trypsin. The supernatant containing the soluble peptides (recovery from the digest was 79% for ^{3}H and 71% for ^{14}C) was separated on a column of Spherix XX8-60-0 resin as described in Materials and Methods. (----, ^{3}H—H-2.4; —, ^{14}C— H-2.31) H-2.4 is the D gene product of B10.D2 (*H-2^d*) and H-2.31 is the K gene product of B10.D2.

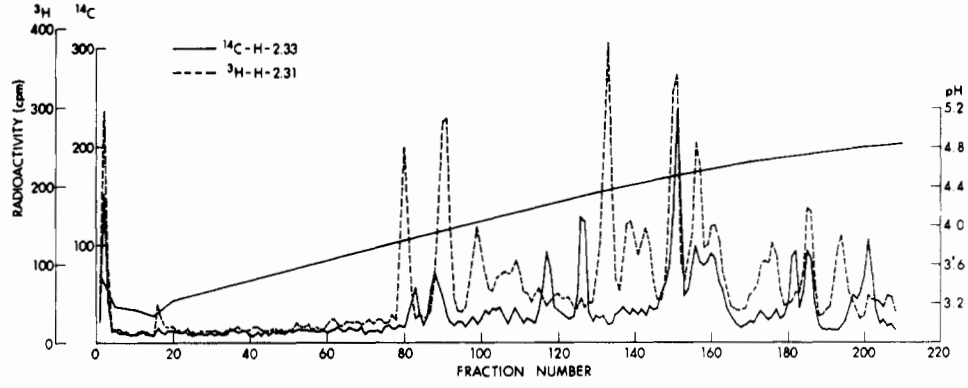

FIGURE 3: Comparison of the tryptic peptides of two K gene products: [^{14}C]arginine-labeled H-2.33 and [^{3}H]arginine-labeled H-2.31. Preparation of the sample for the peptide column and its elution was the same as in Figure 2. (----, ^{3}H—H-2.31; —, ^{14}C—H-2.33) H-2.31 is the K gene product of B10.D2 (*H-2^d*) and H-2.33 is the K gene product of B10 (*H-2^b*).

buffer (1 ml). A pH gradient was then applied using a Varigrad gradient making chamber (Model No. VG-1200, Virtis Research Equipment, Gardiner, N. Y.) and 120 ml of each of the following pyridine–acetic acid buffers used (0.05 M, pH 3.13; 0.10 M, pH 3.54; 0.20 M, pH 4.02; 0.5 M, pH 4.5; and 2.0 M, pH 5.0). Elution was carried out at 51° and 200 fractions of 60 drops per tube were collected. The samples were collected in glass minivials, the pH's recorded and, after evaporation to dryness, dissolved in 0.1 ml of H_2O, and counted with Aquasol (New England Nuclear Corp., Boston, Mass.) in a Beckman scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.). The counts in the ^{3}H channel were corrected for spill from the ^{14}C channel.

Results

In order to gain information on the properties of products of alleles of different H-2 genes as well as of the same gene, we compared the tryptic peptide patterns of products of alleles of genes of one haplotype, *e.g.*, *K^d vs. D^d*, and products of alleles of the same gene but different haplotype, *e.g.*, *K^b vs. K^d*. In all cases, arginine as well as lysine labeled peptides were studied.

Comparison of Peptides from Proteins Determined by Alleles of H-2K Genes and H-2D Genes of the Same Haplotype. Figure 2 shows the peptide profile of the glycoprotein bearing H-2.31 (Kd) compared with that bearing H-2.4 (Dd). This double-label peptide comparison was carried out with arginine-labeled peptides produced by trypsin digestion of the glycoproteins isolated by indirect immune precipitation (*cf.* Materials and Methods) and purified by Bio-Gel sodium dodecyl sulfate chromatography as shown in Figure 1. About 12 well-separated radioactive peaks were seen for each gene product.[2] Clearly, only three of the peaks from the two products coincide in elution position under these chromatographic conditions. Comparison of the lysine-labeled Kd and Dd products showed four peaks of the same elution position of 13 Kd and 9 Dd peaks obtained (data not shown). Accounting for both arginine and lysine peptides, therefore, there appears to be at most only a similarity of about 1/3 between these products.

Comparison of the alleles of the *K* and *D* genes of the *H-2^b* haplotype showed for the total arginine plus lysine profiles, a sharing of 11 out of 21 peaks visualized for H-2.33 (Kb) and 24 peaks visualized for the H-2.2 (Db) product. This finding of similarity of about one half of the peaks was somewhat higher than was found in the comparison of Kd *vs.* Dd.

Comparison of the Peptides of Proteins Determined by Alleles of the Same Gene. An example of comparison of products of alleles of the same gene is given in Figure 3 which shows the double-label comparison of the arginine peptides of H-2.33 (Kb) and H-2.31 (Kd). Clearly, of the 10 Kb and 15 Kd peaks, about 4 are coincident in elution position. Lysine label comparison (data not shown) also showed 4 coincident peaks out of 10 Kb and 12 Kd total visualized peaks.

Comparison of the H-2.2 (Db) and H-2.4 (Dd) arginine- and lysine-labeled allelic products showed about 9 coincident

[2] The peak appearing at tubes 5–10 contains the glycopeptides since it is radiolabeled with fucose or glucosamine. It has not been considered in tabulation of the profiles.

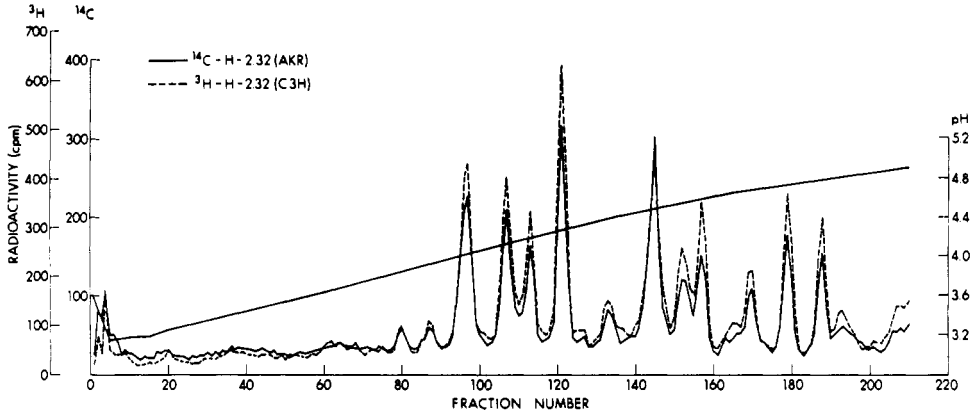

FIGURE 4: Comparison of tryptic peptides of [^{14}C]arginine-labeled H-2.32 and [^{3}H]arginine-labeled H-2.32 from two different mouse strains of the same genotype (*H-2^k*). Preparation of the sample for the peptide column and its elution were the same as in Figure 2. (----, ^{3}H—H-2.32 from C3H; —, ^{14}C—H-2.32 from AKR). This is a comparison of the K gene product carrying the same private and public specificities in two different strains of mice of the *same* genotype.

peaks of 23 and 26 peaks visualized respectively (data not shown).

Comparison of the Peptides of Gene Products of Serologically Identical Haplotype but from Mice of Different Backgrounds. In view of the striking differences in peptide composition of the products of alleles of the H-2 gene products, we examined the serological representatives of the *H-2^k* haplotype present in mice of quite divergent origin, the AKR (*H-2^k*) and C3H (*H-2^k*) strains. These strains were not derived from a common stock, and the H-2 genes presumably arose independently.

Figure 4 shows the double-label map of arginine peptides for the H-2.32 (Dk) product from these mice. The complete identity of these profiles is evident and is particularly remarkable in view of the wide diversity of origin of the original mouse stocks. The findings also attest to the reproducibility of the peptide comparison method. Similar patterns of identity were found when H-2 antigens of mice of the same strain were examined by the double-label technique.

Discussion

In the present studies, because of the extremely limited amounts of material, and difficulties of purification, we chose to use immunoprecipitation to isolate radiolabeled H-2 glycoproteins for peptide comparison. By utilizing H-2 glycoproteins from arginine or lysine *in vitro* radiolabeled spleen cells, we could take advantage of the high sensitivity of isotopic methods. In addition, since mice of any strain can be analyzed, examination of rare genotypes, recombinants, and mutants is feasible.

To assure that our results would reflect a precise examination of only H-2K and H-2D products, as defined by mouse alloantisera, we utilized: (1) antisera carefully selected to exclude possible antibodies to other gene products; (2) a blocking procedure; and (3) a final purification step by sodium dodecyl sulfate molecular seive chromatography.

Since the ultimate purity of the H-2 gene products obtained is dependent on the specificity of the alloantisera used in the immunoprecipitation, these alloantisera are raised in donor–recipient combinations which, to the extent of our present knowledge of *H-2* genetics, would permit reaction only with the private or unique antigenic specificity of either the K or D gene product of a given haplotype. In these immunizations, recombinant strains were used so that the recipient could respond only to either the H-2K or H-2D antigen of the donor. For example, to detect the Kb product, *H-2^g* mice (*H-2K^d; H-2D^b*) were immunized with tumor cells of an *H-2K^b; H-2D^b* mouse. Thus, since Db is shared by donor and recipient, the only possible antibody reaction is *H-2K^d* anti-*H-2K^b* or anti-H-2.33.[3]

In addition to the use of antisera prepared by alloimmunization of genetically defined mice, a "blocking" step was used to assure further the detection of only the particular gene product sought (*i.e.*, either the K or D product of the haplotype tested). In this procedure, nonradiolabeled, NP-40-solubilized spleen cell extract containing a five- to tenfold excess of antigenic material from a selected recombinant haplotype was included in the precipitation procedure to block possible inappropriate antibody reactions. If, for example, Kb (H-2.33) was sought, the anti-Kb antiserum was first incubated with an unlabeled spleen extract containing the Db glycoprotein (*e.g., H-2^g*), but lacking the Kb antigen. The radiolabeled extract containing the Kb antigen was then added, and the indirect precipitation carried out as outlined in Materials and Methods.

We noted that even specific immune precipitates contained a variable amount of radioactive material which was not H-2 glycoprotein. Therefore, as outlined in the Materials and Methods section, each precipitate was dissolved in sodium dodecyl sulfate, reduced with dithiotheitol, alkylated with iodoacetamide, and chromatographed on a Bio-Gel A-0.5 m column in 0.5% sodium dodecyl sulfate (Figure 1). The major radiolabeled peak (approximately 45,000 molecular weight) was utilized for peptide comparisons.

Although we have shown only examples of several of our comparisons, we have examined thoroughly the peptide profile of K and D gene products of two haplotypes, *H-2^b* and *H-2^d*, and partially of *H-2^k*.

Comparisons of products of D and K genes of the same haplotype showed slightly different results, since Kd *vs.* Dd showed about ⅓ similarities in peptide profile, whereas Kb *vs.* Db showed about ½. Surprisingly, comparisons of products from one gene series, *e.g.*, Kb *vs.* Kd, and Db *vs.* Dd while more uniform still showed a similarity of only ⅓ of the peptide peaks. Such results suggest that in some cases, products of alleles of

[3] Strictly speaking, such sera could also detect Ia antigens recently described (David *et al.*, 1973; Cullen *et al.*, 1974), in this particular case those products detected by I^d anti-I^b. The antisera used for the present study lacked such antibodies since they were raised against tumor cells which lack the Ia products as detected by the sera of David *et al.* (1973). Since the Ia products have a molecular weight of approximately 30,000 (Cullen *et al.*, 1974) such materials would be separated from the 45,000 molecular weight H-2 glycoproteins during gel filtration chromatography in sodium dodecyl sulfate (*cf.* Figure 1).

the D and K genes may be more similar than of the same gene. Nonetheless, analysis of all the comparisons gives the overall general impression that there is considerable structural uniqueness for the product of each allele.

Of course, such conclusions must be interpreted with extreme caution. We have assumed, for example, that the techniques for isolation of the glycoprotein are valid, and that the products are homogeneous. Such assumptions are supported by the findings of homogeneity of the product by both sodium dodecyl sulfate acrylamide gel electrophoresis, and discontinuous sodium dodecyl sulfate gel electrophoresis (Maizel, 1971), and by the genetic and immunological criteria outlined earlier.

An additional finding which supports the validity of our methods is the complete coincidence of peptide profiles of H-2.32 (Dk) from the C3H and AKR mouse strains. Contaminants due to non-H-2 components would be expected to show up as differences in these profiles since such strains share *H-2* haplotype but are otherwise very different in genetic background.

A crucial factor to be considered in the interpretation of peptide comparison is the fact that the technique tends to overestimate differences in actual protein structure since a single amino acid change could alter the chromatographic behavior of a peptide containing many conserved amino acids. For example, while κ and λ light chains are 30–40% homologous in sequence (Barker *et al.*, 1972), their peptide maps are essentially nonidentical (L. Hood, personal communication). Thus, we cannot predict the actual homology of the H-2 proteins until more structural results are available. A complication of our peptide profiles, further, is that we have not established the actual number of peptides in each peak, and have not determined the identity of putatively similar peptides by criteria other than mobility on ion-exchange chromatography.

In spite of all qualifications, however, the data of our present studies, when considered in a semiquantitative manner, still present the striking suggestion that the products of alleles of the K and D genes show a marked degree of diversity. These results confirm and extend the results of the previous peptide comparisons (Shimada *et al.*, 1970; Yamane *et al.*, 1972).

Possibly the structural differences between the H-2 allelic products suggested by the peptide comparisons are not unexpected in view of the complex serological profile often associated with the products. For example, in the comparison of K^b with K^d (Figure 3), a consideration of the presently defined antigenic specificities (Klein, 1972) shows a considerable number of differences: K^b is positive for its unique specificity 33, as well as for public specificities 5, 25, 35, and 36; the K^d product is positive for unique specificity 31, but also carries specificities 8 and 34. Thus, even though we presently have no direct understanding of the precise chemical basis for an antigenic specificity, and cannot establish that each antigenic site represented by an antibody-defined specificity represents a particular unique amino acid configuration on the polypeptide, the dissimilarity of peptide profile coincides with the diversity of serological profile.

The present studies have examined only a few of the potential 40–50 H-2 gene products. If our present sample reflects the case for other H-2 chromosomal arrangements, the remarkable variability observed between the structural products of H-2 genes is consonant with the extraordinary serologically detected polymorphism of the H-2 system. The mechanism for generating and/or selecting for and maintaining this variability is presently unknown. The variation among the so-called "alleles" is particularly extraordinary. Possibly an unusual mechanism is functioning to maintain such different forms of a gene. One suggestion as yet unsubstantiated is that the H-2 products are actually determined by gene clusters, and that each haplotype contains many H-2D or H-2K genes for a mouse haplotype, but that only one gene is selected for expression by some control mechanism. Evidence in favor of this interesting possibility is still lacking. It is clear that more extensive studies, including sequence analyses, are needed to further explain the genetic implications of the histocompatibility systems, as well as to explain the immunochemical properties of the H-2 products.

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