SOLUBILIZATION OF MEMBRANE H-2 ISOANTIGENS: CHROMATOGRAPHIC SEPARATION OF SPECIFICITIES DETERMINED BY A SINGLE H-2 GENOTYPE

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Membrane located isoantigens determined by the Histocompatibility-2 (H-2) locus in the mouse carry an array of antigenic specificities involved in immunological rejection of tissue transplants. The H-2 locus is complex, and at least five ordered regions controlling these specificities have been detected by recombination studies (cf. Snell, 1967 and Shreffler, 1967).

Digestion of cell membranes by papain releases the H-2 isoantigens in a soluble form, and when further purified they are found to have the general composition of glycoproteins with molecular weights in the range of 50,000 to 70,000 (Nathenson and Shimada, 1967). We now present evidence that one of the specificities (H-2.31) determined by the $H\text{-}2^d$ allele differs in solubilization properties from several other specificities determined by $H\text{-}2^d$; moreover, that the H-2.31 antigenic specificity can be physically separated from the others by chromatography on Sephadex G-150. Products carrying two antigenic specificities determined by the $H\text{-}2^b$ locus can also be separated by the latter technique.

MATERIALS AND METHODS. Mice: The following inbred mice, purchased from Jackson Labs, Bar Harbor, Maine, or obtained from the breeding colonies of Dr. Frank Lilly, were used: B.10.A ($H\text{-}2^a$); C57BL/10, C57BL/6, ($H\text{-}2^b$); DBA/2, BALB/C, ($H\text{-}2^d$); H-2H, ($H\text{-}2^h$); AKR, B10.BR ($H\text{-}2^k$). Meth A ($H\text{-}2^d$) ascites tumor cells were maintained in BALB/C mice and E.L. 4 ($H\text{-}2^b$) tumor cells in C57BL/6 mice.

Isoantisera and test system: Isoantigenic activity was detected as described previously (Nathenson and Davies, 1966) by the method of inhibition of immune cytolysis (Sanderson, 1964, Wigzell, 1965). The specificities were determined by use of the following isoantisera and test cells.

H-2.31: B10.A anti-Meth A serum tested on B10.D2 target cells;

H-2.3,8: C57BL/10 anti-Meth A serum tested on B10.BR target cells;

H-2.4,10,13: (AKR x C57BL/6)F_1 anti-Meth A serum tested on B10.A target cells;

H-2.5: B10.D2 anti-E.L.4 serum tested on B10.BR target cells;

H-2.2: (BALB/C x C3H)F_1 anti-E.L.4 serum tested on H-2H target cells.

H-2.6,14,27,28,29: B10.BR anti-C57BL/10 serum tested on B10.D2 target cells.

Membrane fractions were prepared as described (Nathenson, 1967).

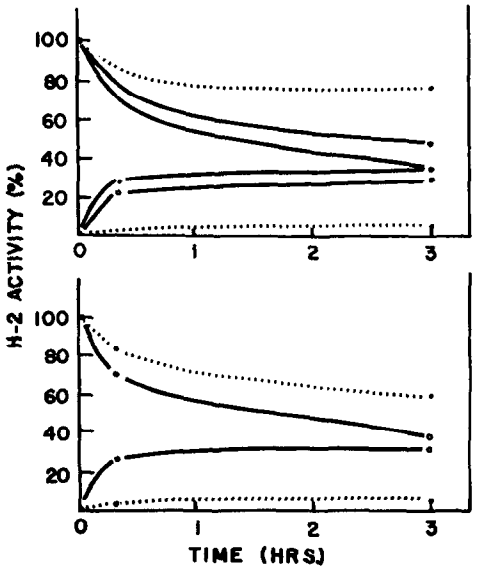

Figure 1. Time course of papain solubilization of H-2^d specificities. Crude membranes from DBA/2 spleen cells (upper fig.) and Meth A tumor cells (lower fig.) were suspended at 10 mg/ml in Tris-Cl 0.05M, pH 8.4, with papain at 0.5 mg/ml and cysteine 0.06 mg/ml and incubated at 37° for times indicated. The reaction was stopped with neutralized iodoacetic acid (final concentration 0.01M) and membranes and supernatant fluids separated by centrifugation at 144,000 x g for one hour and then assayed. For both DBA/2 and Meth A preparations, membrane isoantigen activity is plotted in the set of curves starting at 100% and supernatant activity is plotted in the set of curves starting at 0%. Specificities H-2.3,8 o——o ; H-2.4,10,13 •——• ; and H-2.31 •-----• were measured. The results are average determinations from two different membrane preparations of each source and three separate time course studies.

RESULTS. Figure 1 presents the time course of release of different antigenic specificities from DBA/2 spleen membranes (upper part of Fig. 1) and Meth A tumor membranes (lower half of Fig. 1). The data show that from membranes of either type H-2.31 is solubilized very slowly and in low amount, whereas the sets of specificities, H-2.3,8 and H-2.4,10,13, are released rapidly and in considerably higher yield.

Ammonium sulfate fractionation of the solubilized H-2 isoantigens from DBA/2 membrane preparations showed that 95% of the activity for each of the three sets of specificities was soluble at 50% saturation, but precipitated from solution at 75% saturation. Chromatography on a Sephadex G-150 column (Fig. 2) of the redissolved, dialyzed 50 to 75% ammonium sulfate precipitate

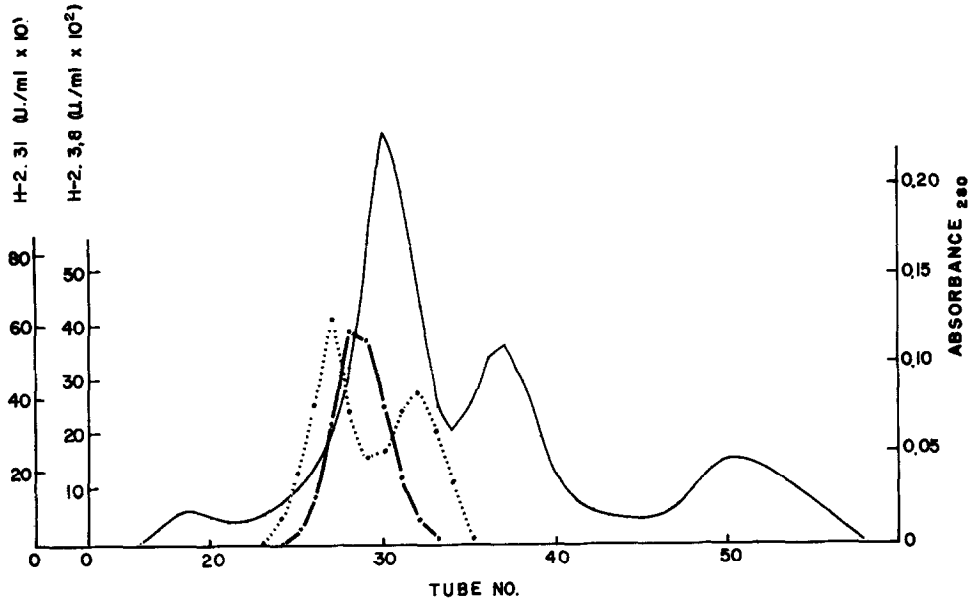

Figure 2. Chromatographic separation of specificities of H-2^d isoantigens. A sample of H-2^d isoantigen was solubilized by incubating DBA/2 cell membranes from 1000 spleens at 20 mg/ml, with crude papain (Sigma Chemical Co.) 6 mg/ml, and cysteine, 0.3 mg/ml in Tris buffer 0.05M, pH 8.4 at 37° for 2 hours. Iodoacetic acid at a final concentration 0.01M was added to stop the reaction, and supernatant recovered by centrifugation at 144,000 x g for one hour. The 50-75% saturated ammonium sulfate fraction, after dialysis against 0.01M Tris-Cl, pH 8.4, was placed on a Sephadex G-150 column (95 cm x 4 cm) equilibrated and eluted with 0.01M Tris-Cl, 0.15M NaCl buffer pH 8.4 at 4°C, and 22.5 ml samples collected. V_o was 382 ml (tube 17). Absorbance at 280 millimicrons ——— ; and the quantity of H-2 specificities H-2.3,8 ●——● and H-2.31 ●-----● were determined on each tube.

showed that the isoantigen profiles for the sets of specificities H-2.3,8 and H-2.4,10,13 (not plotted) were almost identical to each other with a peak at tubes 28 and 29.[1] The elution pattern for the molecular species carrying H-2.31, however, revealed two peaks, one at tube 27 and the other at tube 32. Recovery of units of activity applied to the column were 95% for H-2.3.8; 103% for H-2.4,10,13 and 93% for H-2.31. Identical chromatographic findings have been obtained with preparations from Meth A tumor cell membranes.

Papain solubilized, ammonium sulfate fractionated isoantigenic material from cell membranes of C57/BL6 spleen cells (H-2^b) was chromatographed on the same Sephadex G-150 column (Fig. 3). The activity profile for the specificity H-2.5 showed a single sharp peak at tube 28, whereas specificity H-2.2

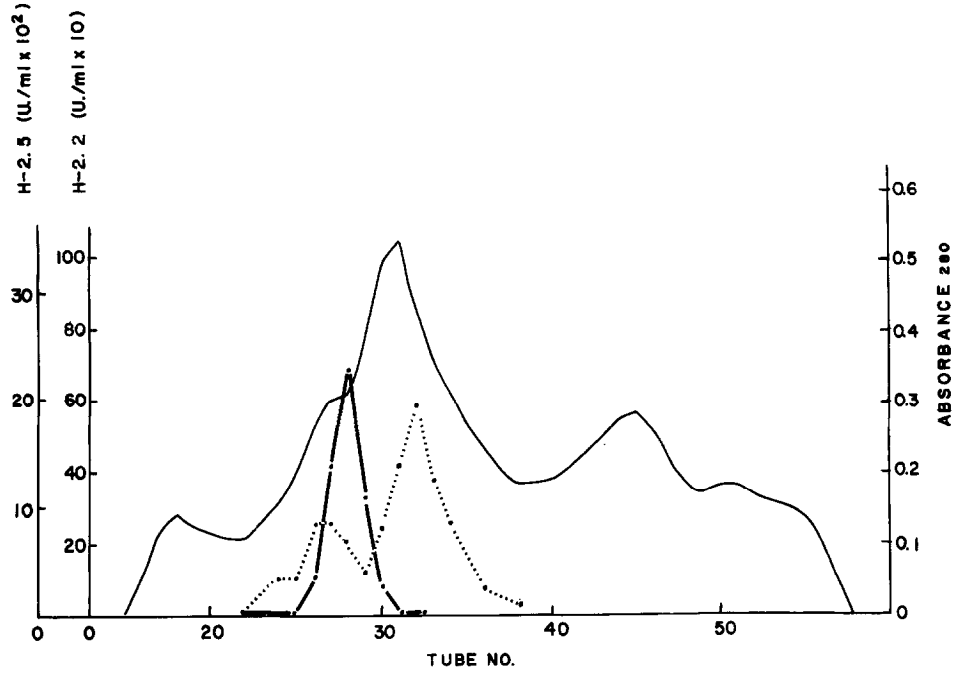

Figure 3. Chromatographic separation of specificities of H-2^b isoantigens. A sample of H-2^b isoantigen prepared and chromatographed as described for the H-2^d preparation of Figure 2, except membranes from 2000 spleens were used. $Absorbance_{280}$ ——— ; H-2.5 ●———● ; H-2.2 ●-----● .

[1] The set of specificities H-2.6,14,27,28,29, as a measure of the remaining specificities determined by H-2d, likewise showed a single peak at tube 28 and 29.

peaked at tube 27 and 32 to give a profile remarkably similar to that of H-2.31. Specificity H-2.33 and the set, H-2.6,14,27,28,29 (neither plotted) were eluted in a pattern similar to H-2.5.

DISCUSSION. Our results with papain solubilized isoantigen preparations from mice homozygous with respect to two different H-2 alleles show that molecules carrying one specificity can be separated from molecules carrying other specificities determined by the same H-2 allele. We have further evidence (Shimada and Nathenson, in preparation) that Sephadex chromatography of papain solubilized isoantigen preparations of an ($H\text{-}2^b$ x $H\text{-}2^d$)F_1 hybrid separates molecules carrying the H-2.5 specificity (from the $H\text{-}2^b$ allele) from molecules carrying H-2.3,8 (from the $H\text{-}2^d$ allele).

Since some of the sets of specificities of an H-2 genotype, e.g., H-2.3,8 and H-2.4,10,13 ($H\text{-}2^d$) chromatographed together on Sephadex (Fig. 2) we cannot conclude without further fractionation whether the molecules carrying these specificities are separate. It is quite possible that some specificities are expressed uniquely on one molecule, while others coexist together on another molecule. This possibility, together with differences in solubilization techniques, may account for apparent disagreement in our findings with those of Davies (1967), who concluded that the soluble H-2 specificities which he tested were found on the same molecule, both for preparations from mice homozygous and heterozygous with respect to the H-2 allele.

The molecules containing the specificities H-2.5 and H-2.3,8 have been further purified and both were found to be glycoproteins composed of about 85% protein, 8% neutral carbohydrate, 5% amino sugar and 2-3% sialic acid, with a molecular weight of 66,000 (Shimada and Nathenson, in preparation). Of course, further chemical and structural studies of the purified products are needed in order to precisely define the chemical nature of the antigenic site of each H-2 specificity on the glycoprotein. The separation of molecules showing a single specificity will make this task easier.

The present findings bear in a general way on the question of the

molecular relationships of the H-2 cellular product as it may exist in situ in the membrane. The solubilized specificities which we found on separate molecules may be derived from separate molecules on the membrane, or in fact, from separate membranes. This would be supported by the finding of different solubilization properties for the specificity H-2.31 (Fig. 1), and would have precedent in the experiments of Ozer and Wallach (1967) who showed that certain specificities are expressed on plasma membrane fragments and others on endoplasmic reticulum. It is possible, however, that the proteolytic solubilization has split a very complex membrane macromolecule into smaller, but still sizeable glypoproteins (perhaps subunits) some of which uniquely contain one specificity. In that case also, it would seem that the antigenic site or sites for such a specificity on the larger membrane macromolecule must be localized to a single region and not intermixed with the sites of other specificities.

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