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EXTRACTION OF HL-A TRANSPLANTATION ANTIGEN IN HIGH MOLECULAR WEIGHT SOLUBLE FORM

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SUMMARY

A crude membrane extract was made from a typed human spleen and half of the product was solubilized by proteolytic methods previously described. The remaining half was treated with starch stearate and sodium dodecylsulphate and the soluble product compared with the first for its HL-A specificity and behaviour on Sephadex G-200. The product of the starch stearate method had a molecular weight in excess of 200,000 and might be a membrane subunit.

INTRODUCTION

In the mouse, recent studies of *H*-2 transplantation antigens have shown that, by gentle proteolysis, soluble preparations are obtained that can be separated from many other alloantigens determined by non-*H*-2 genetic loci (Davies, 1968). Further fractionation can be made to separate many of the *H*-2 specificities of a given genotype from each other (Davies, 1969). The *H*-2 molecules analyse as glycoproteins and most of these have molecular weights close to 50,000 (Summerell & Davies, 1969, 1970) but some are smaller (Shimada & Nathenson, 1967; Davies, 1967, 1970).

Human HL-A transplantation antigens are determined by a complex genetic locus that has many features in common with the mouse *H-2* locus; also, the antigens can be extracted by the same sequence of methods (Davies, 1968) and again the different specificities can be separated from each other (Colombani *et al.*, 1970) and have molecular weights in the 50,000 region.

We have recently described a method of solubilization of leucocyte membrane derived material using sodium dodecylsulphate (SDS) and starch stearate (SST). Mouse *H*-2 antigen can then be obtained in soluble form at a much higher molecular weight (the region of several million as judged by Sepharose gel filtration) and where many specificities appear to be present on one molecule (Hämmerling, Davies & Manstone, 1970). Further studies revealed that in the mouse the non-*H*-2 antigens LY-A, LY-B (lymphocyte specific antigens

described by Boyse *et al.*, 1968), TL (thymus-leukaemia) antigen (Boyse, Stockert & Old, 1968) and θ-brain-thymus-lymphocyte antigen (Reif & Allen, 1964) were also solubilized (Davies, Hämmerling & Alkins, 1970).

In the light of thymocyte cell surface mapping data (Boyse, Old & Stockert, 1968) and ferritin label studies (Aoki *et al.*, 1969) it is an attractive possibility that these molecules are membrane units of structural significance. The data given below show that for HL-A a similar large molecule can be obtained, whose further properties may to some extent be predicted from the likelihood that *H-2* and HL-A are genetic homologues and that other alloantigens probably also have homologues generally among mammalian species.

MATERIALS AND METHODS

A human spleen weighing 110 g (wet wt) typed as follows: first segregant series, HL-A1+, HL-A3+, HL-A2 -ve, HL-A9 -ve, Da15 -ve; second segregant series, HL-A12+, HL-A5 -ve, HL-A7 -ve, HL-A8 -ve; Da14+, Da6 -ve. A crude membrane derived extract was made by hypotonic elution (Davies, 1966) and half of this (1·8 g dry wt) was solubilized autolytically (Davies *et al.*, 1968) giving 730 mg of soluble product of the kind previously described (A/T antigen) of molecular weight approx. 50,000.

The other half of the crude membrane material (1·8 g), in 122 ml water suspension was made up to 0·3% sodium dodecylsulphate (SDS) and 0·6 mg/ml starch stearate (SST); the synthesis of SST was described by Hämmerling & Westphal (1967). The suspension was centrifuged at 105,000 *g* for 2 hr and the supernatant dialysed against 0·02 M Tris buffer, pH 8·0, the soluble product being 1130 mg (excluding the content of SST).

One third of the A/T antigen (240 mg) and a quarter of the SST antigen (280 mg) were examined on Sephadex G-200 as previously described (Davies *et al.*, 1968) and the column fractions scanned for HL-A specificities HL-A1, HL-A3, and Da14. These antigens were detected by inhibition of platelet complement fixation (Colombani *et al.*, 1969). The alloantisera selected for inhibition tests were used at a dilution corresponding to 1 antibody unit in 50 μl when tested against platelets of the spleen donor. Such a dilution was equivalent to 0·5 μl of serum BRIG (anti-HL-A1), 1·25 μl of serum FISC (anti-HL-A3) and 6·25 μl of serum KERE (anti-Da14). The doses of column fractions, selected from a preliminary assessment of potency, were 100 μl and 200 μl respectively of A/T column fractions and SST column fractions.

RESULTS

In Fig. 1 column A illustrates the A/T antigen picture and column B the SST antigen result. The antigen doses used, when tested with HL-A1 antibody, gave inhibition shown in building-block style relating to complete or less than complete inhibition indicated by the scale. For HL-A3 and Da14 antibody inhibition only qualitative results could be recorded, as shown.

For HL-A1, complete or partial inhibition was confined to the excluded fraction of the column eluate in the case of SST antigen and to the retarded fraction in the case of A/T antigen; weak reactivity was a little more spread in each case. For SST antigen, HL-A3 and Da14 reactivity was confined to the gel excluded fraction while for A/T antigen activity was retarded but also extended to the excluded fraction.

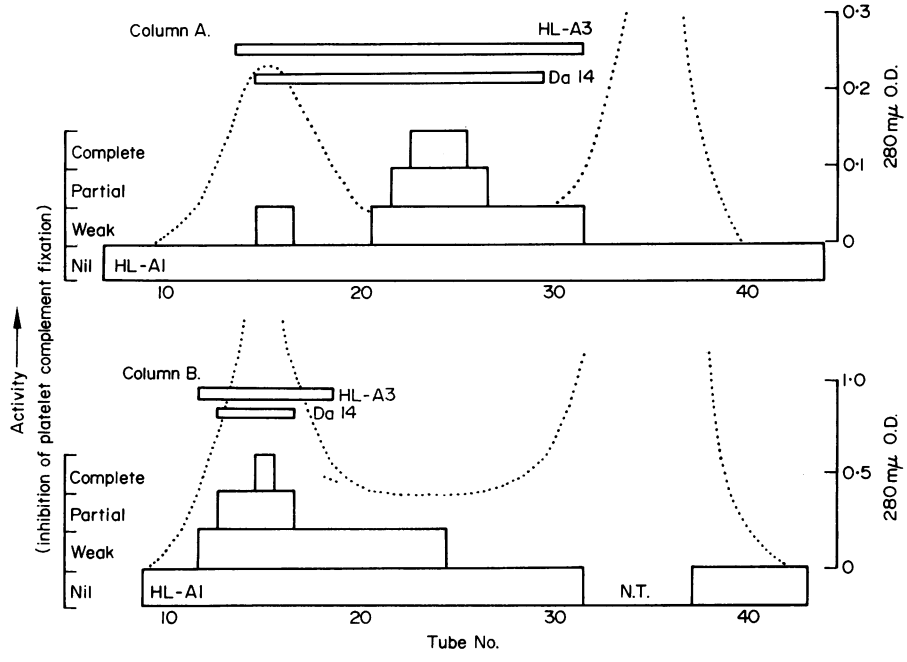

FIG. 1. Sephadex G-200 gel filtration of HL-A antigen solubilized by autolytic methods, column A, and by sodium dodecylsulphate with starch stearate, column B. Absorbance at 280 mμ is shown by the dotted line; markers for the exclusion point on both columns indicated tube 15. Regions of specific HL-A reactivity are shown for HL-A1, HL-A3 and Da14.

DISCUSSION

All the methods developed for extraction of mouse *H*-2 antigens and their solubilization and purification have proved to be equally useful for processing human HL-A active material. This implies a close similarity in chemical properties between *H*-2 and HL-A antigens that is confirmed by analysis of the most purified products. The soluble antigens presently available, however, are artefacts of proteolytic degradation of cell membrane material and consequently little can be deduced about the membrane structure to which they contribute.

Studies of mouse *H*-2 material solubilized by SDS+SST show that many specificities are probably present on the same molecular complex. Moreover, some non-*H*-2 antigens are also present on molecules similar in size, some of which are separable from others. This kind of material may be a subunit of membrane structure (Davies *et al.*, 1970).

The present data show that a large soluble molecule can be prepared in the same way from human lymphoid cell membranes and we can confidently predict further properties from the mouse data. Non-HL-A alloantigens (other than those on red cells) have been very little studied but such extracts may serve as a source of these. In any event, the present data consolidates the view of *H*-2 and HL-A genetic homology (Davies *et al.*, 1968).

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