HUMAN HL-A TRANSPLANTATION ANTIGENS: SEPARATION OF MOLECULES CARRYING DIFFERENT IMMUNOLOGICAL SPECIFICITIES DETERMINED BY A SINGLE GENOTYPE

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The HL-A genetic locus in man determines leucocyte antigens that play a primary role in tissue graft rejection (Dausset, Hamburger and Mathé, 1968). In this paper we show that HL-A antigens from a single individual can be separated by DEAE-Sephadex into different fractions carrying different HL-A immunological determinants.

This complex locus and its phenotypic products are remarkably similar to the mouse H-2 locus and the H-2 transplantation antigens (Davies, 1968a). For H-2 antigens each genotype determines a selection (from 5 to 14 in homozygous animals) of about 28 known specificities. H-2 antigens can be solubilized either autolytically or by the addition of the enzymes ficin, bromelin or papain (Nathenson and Davies, 1966; Davies, 1967). The soluble preparations, run through G200 Sephadex, give H-2 inactive excluded and included fractions and an H-2 active retarded fraction. Further purification can be achieved by conventional methods but yields are very small and insufficient pure material has been obtained for detailed analysis. The best preparations are glycoproteins but it is not yet known in what part of the molecules the immunological determinants reside.

Human HL-A antigens have been found to behave in the same way as mouse

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H-2 antigens, the degree of similarity suggesting that they are likely to be genetic homolgoues (Davies, Viza, Colombani and Dausset, 1967; Davies, Manstone, Viza, Colombani and Dausset, 1968). Mouse studies have revealed that some H-2 specificities can be separated from one another by gel filtration (Shimada and Nathenson, 1967) and also by DEAE ion exchange chromatography (Davies, 1968b). By the latter method nearly all of the eleven H-2 antigens of one mouse H-2 genotype can be separated (Summerell and Davies, 1968).

MATERIALS AND METHODS

Fresh normal human spleens were used and antigens were extracted and solubilized as already described (Davies, 1966; Davies et al. 1968). The results below were obtained with autolytic preparations. Crude soluble material was passed through G200 Sephadex and the retarded fraction pooled, concentrated in the cold under reduced pressure, dialysed and freeze dried. For examination on DEAE columns, material was dissolved in and dialysed against starting buffer (0.05 M Tris, pH8) and in the case quoted (spleen number 18) 59 mg in 10 ml was placed on a column 125 cm x 1.5 cm. A linear gradient was run to 0.05 M Tris, pH9 + 0.35 M NaCl over a volume of 1000 ml. Two hundred 5 ml fractions were collected, each dialysed against 0.01 M NH_4HCO_3 and 4.5 ml of each freeze dried. These were reconstituted in 0.9 ml of buffered physiological salt solution and stored at -70°.

Fractions were tested for HL-A immunological specificities either by inhibition of cytotoxicity induced by isoantisera and measured by the trypan blue exclusion method, or by inhibition of platelet complement fixation (Davies et al. 1968). For the latter, serial dilutions of each column fraction were tested; for inhibition of the cytotoxic reaction a fixed dose of each fraction was used and the extent of combination with antibody assessed as percentage of surviving target cells (peripheral lymphocytes).

The spleen donor was typed for HL-A specificities as follows:

* 1	3	4	5	6	7	8	10	11	12	14	16
-	+	-	+	+	+	+	-	-	-	-	+

RESULTS

The results are shown in Fig. 1, separated into parts (a) and (b) for clarity. For economy, regions of the columns were checked where mouse H-2 antigens would have been expected if H-2 material had been run. This revealed the positions of the HL-A antigens in the predicted places and all other fractions were then tested to confirm negative reactions elsewhere. Fig. 1a shows the clear separation of two antigens, HL-A.16 and one unknown (detected with serum "Mo"), the two peaks being centred on tubes 131 and 104 respectively.

These positions are reflected in a more complex fashion in Fig. 1b. Specificity 5 (an allele of the HL-A second sublocus) appears mainly in a peak coincident with HL-A.16 (an allele of the HL-A first sublocus) but with a smaller peak at tube 104; there is also a possible smaller peak at 113 (but one tube only) and a shoulder at tubes 117-121. Another serum of unknown HL-A specificity, ("Jui"), also shows a major component coincident with antigen 16 but in addition, may have lesser components corresponding to the minor components shown by the specificity 5 reaction. Reaction with antiserum to HL-A.6, tested qualitatively, is shown by the shaded area and again covers the area of both main antigen positions, being negative from tubes 1 to 95 and 141 to 200 as for the other specificities tested. Tubes were tested for reactivity with a serum anti HL-A.1, a specificity the donor did not possess, and no reactions were found.

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* Nomenclature is that of Dausset, Ivanyi, Colombani, Feingold and Legrand (1967). Two of the antisera for which there are alternative terms are:
HL-A.5 = 4^c (Payne) = To.5 (Ceppellini) = 6 (Terasaki).
HL-A.16 = Lc.11 (Walford).

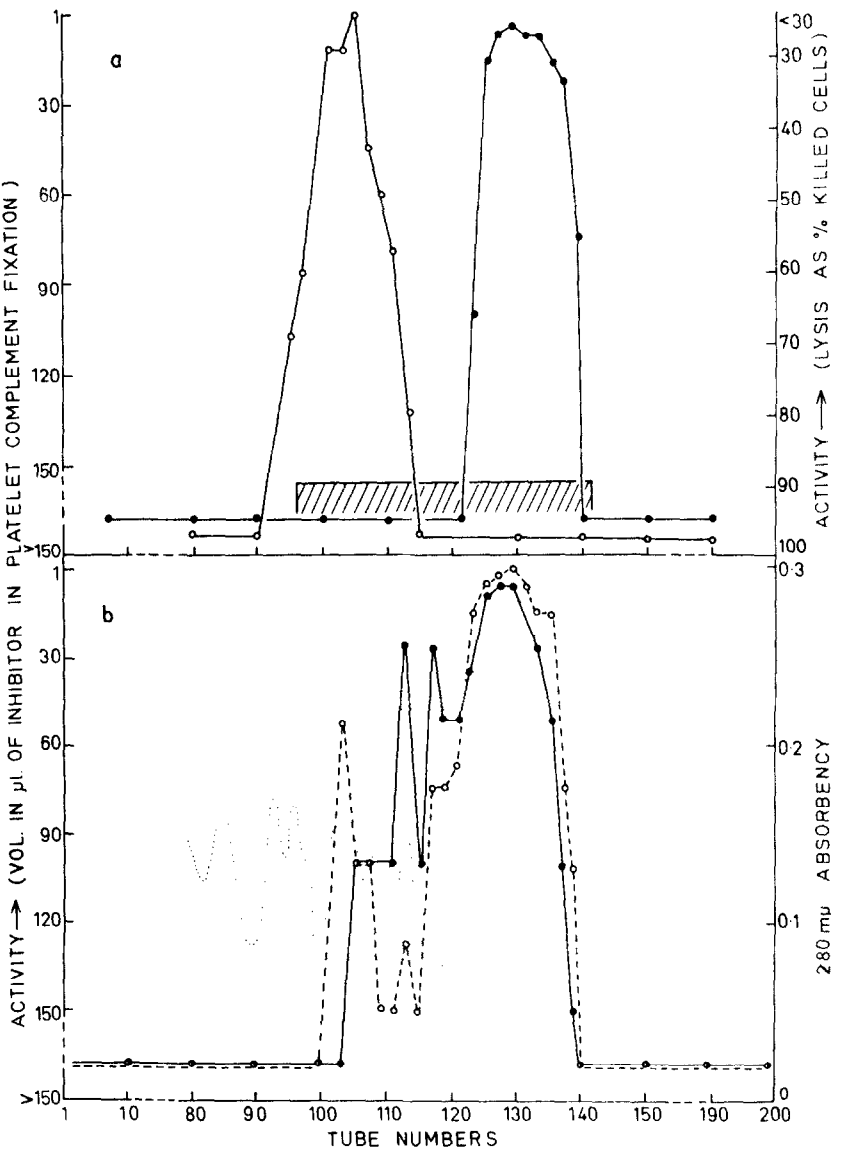

Fig. 1. Human soluble HL-A transplantation antigen examined on DEAE-Sephadex A50 (linear salt gradient), center section shown. Tube numbers are for 5 ml fractions. Tests for different HL-A specificities of the original spleen donor have been plotted on arbitrary scales. (a) Reaction with serum "Mo", O—O, (cytotoxicity inhibition). Reaction with serum anti-HL-A.16, ●—● (platelet complement fixation inhibition). Shaded area is the extent of qualitative reaction with serum anti-HL-A.6, (inhibition of cytotoxicity). (b) Reaction with serum anti-HL-A.5, O—O, and reaction with HL-A antiserum "Jui", ●—●, (both by platelet complement fixation inhibition). Protein profile (280 mµ absorbence), dotted line.

The protein profile (by 280 mµ absorption) is shown by the dotted line in Fig. 1b from tubes 80 to 155. There are protein peaks coinciding with each of the antigen positions in the region of serological reactivity.

DISCUSSION AND CONCLUSIONS

The separation of different HL-A specificities into separate positions on DEAE columns enhances the idea of genetic homology with the mouse H-2 system. Two main positions are indicated so far, e.g. by the two components detected with serum anti HL-A.5. The other peaks appear to coincide with one of these positions but exact coincidence is not claimed. In the mouse almost all specificities of a given H-2 genotype are separable (Summerell and Davies, 1968). The interpretation of multiple peaks revealed by a reaction with a single antiserum is not yet clear. In the mouse this usually means that the serum is not monospecific. The probability is that few of the available HL-A antisera are monospecific and the HL-A.5 antiserum used may recognise more than one HL-A antigen. Antiserum HL-A.6 reacts over the extent of both positions defined in Fig. 1a and might have resolved into two (or more) peaks if it had been possible to test dilutions of each fraction in that system.

Correspondence of protein and antigen peaks is suggestive that the antigens themselves may be revealed in the protein profile but it would be premature to attach significance to this until more data have accumulated.

Failure of any fractions to react with the antibody HL -A.1 (M ac) serves as a specificity control; antigen 1 was not present in the original spleen donor, but only other alleles of the HL-A first sublocus (e.g. HL-A.16). There is no suggestion thus far that the two main peaks represent separations of antigens determined by the two main HL-A subloci that have recently been defined (Dausset, Walford, Colombani, Legrand, Feingold and Rapaport, 1968).

The importance of establishing the similarity of human HL-A and mouse H-2 systems is that experimental work can be carried out in the mouse where human studies are impossible and the importance of human tissue transplantation requires the information that mice may possibly provide. Thus antigens that are separable may have different biological properties e.g. of immunogenicity, cell stimulation for matching (Viza, Degani, Dausset, and Davies, 1968) or tolerance induction.

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