INVESTIGATIONS ON HUMAN LEUCOCYTE ANTIGENS (HLA) FROM URINE

I. BERNIER, A. DAUTIGNY, J. COLOMBANI and P. JOLLÈS

Laboratory of Proteins, University of Paris V, 45 rue des Saints-Pères, 75270 Paris Cedex 06 France, and Groupe de Recherche sur l'Immunogénétique de la Transplantation humaine, Hôpital Saint-Louis, 75010 Paris, France

Received 28 January 1976

1. Introduction

The poor yields and the high costs which characterize the purification of HLA antigens from cells (essentially from lymphoid cells in culture) led us to investigate another source for the transplantation antigens: the urine [1,2]. The present paper is dealing with the purification of urinary HLA antigens in order to obtain highly purified active molecules.

2. Materials and methods

The urine was provided by a single donor (a patient suffering from cystinosis), whose HLA specificities had been phenotyped (HLA-A9, AW 33, B 12, B 14). The antigenic activity was monitored, at the different purification steps of the urine, by the complement fixation inhibition test [3]. 5 litre samples of urine (concentrated on Amicon DC-2 to 30–50 ml) were purified by (a) chromatography on DEAE-Sephadex; (b) preparative polyacrylamide gel electrophoresis (a and b as described in [1]); (c) electrofocusing (LKB column, 110 ml capacity, carrier ampholines pH 4–6, 180–500 V, 45 h) and (d) gel filtration on Sephadex G-25 (elimination of the ampholines). Analytical polyacrylamide gel electrophoresis of the purified active fractions was carried out in SDS containing systems [4]. Proteins were determined by the Folin procedure of Lowry et al. [5].

3. Results and discussion

Table 1 points out the increase of specific activity (HLA-A9) during the different purification steps. A total 470-fold increase was obtained. Fig.1 represents the elution profil after electrofocusing and indicates the isoelectric points of HLA-A9 and B 12 antigens (5.1 and 4.7, respectively). But in spite of the high resolution power of this latter technique, the purified active fractions always contained 2 or 3 contaminants

Table 1
Proteins and activity (HLA-A9) during the purification of urinary HLA antigens

Crude urine and purification steps	Protein[a] (mg)	Specific activity (I.U./mg protein)
10 litre urine	4000	150
DEAE-Sephadex A-50	660	1200
Preparative polyacrylamide gel electrophoresis (Canalco apparatus)	60	3750
Electrofocusing (LKB column; ampholines pH 4–6)	0.700	69 000

[a] The protein content was determined on samples dialyzed on Diaflomembranes UM-20.

North-Holland Publishing Company – Amsterdam

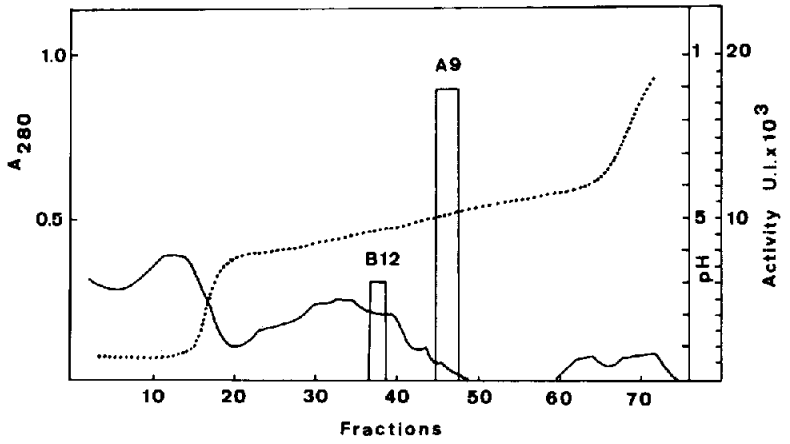

Fig. 1. Electrofocusing of a 5 litre sample of urine (after ion exchange chromatography and preparative polyacrylamide gel electrophoresis). 1.5 ml fractions. (———) Absorbance at 280 nm; (- - - - - - -) pH. Inhibition unit (I.U.): 1 I.U. = ID_{50} = 50% inhibition dose.

essentially albumin and α-globulins characterized by immunoelectrophoresis [6]. Analytical polyacrylamide gel electrophoresis of the purified fractions showed 2–3 proteins and glycoproteins with mol. wts. of about 60 000–80 000 and a glycoprotein with a mol. wt. of about 33 000. It was not possible to separate these molecules by gel filtration on Sephadex G-150 in a 0.05 M Tris-HCl, 0.15 M NaCl buffer pH 7.4 because of the presence of relatively high amounts of albumin known for its strong interaction with other molecules. The separation could only been obtained in denaturing-conditions (6 M guanidine chloride) (fig.2). The characterization of the purified active HLA molecules was ascertained by the following two experiments: the active fractions provided by the purification of three 5 litre samples of urine were submitted to a second electrofocusing. Analytical polyacrylamide gel electrophoresis, in the presence of SDS, indicated an important increase of the 33 000 dalton band, corresponding to the concomitant increase of the serological activity. The second indication that the HLA antigens in urine correspond to 33 000 dalton molecules was obtained by ultrafiltration (Amicon) on XM-50 membranes: the increase of the amount of 33 000 dalton molecules in the ultrafiltrate went in parallel with the increase of HLA activity.

No β2-microglobulin was present in the highly purified fractions. As already reported [2], most of the β2-microglobulin was lost during preparative polyacrylamide gel electrophoresis.

It should be emphasized that HLA antigens purified from urine resemble those isolated from serum [7]. In both cases the transplantation antigens showed mol. wts. similar to the molecules obtained by papain solubilization of cellular material [8], suggesting the rapid cleavage of a fragile peptide bond or/and a partial loss of carbohydrates.

It is difficult to determine the yield of the HLA antigens purified from urine on account of the high

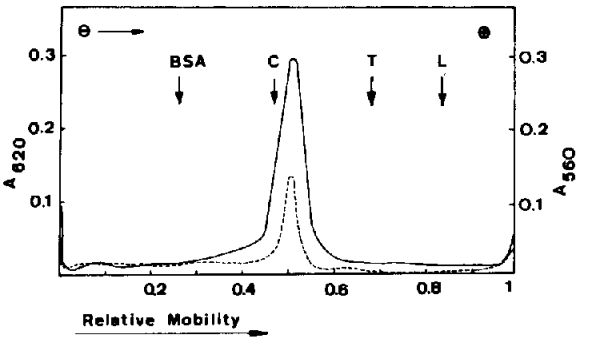

Fig. 2. Sodium dodecylsulfate-acrylamide gel scan analysis of about 50 μg of the 33 000 dalton band obtained after gel filtration on Sephadex G-150, in 6 M guanidine-chloride. Amido black (———) and periodate-Schiff (- - - - - - -) stained protein pattern. The abcissa gives the relative mobility, the ordinate the absorption for Amido black (620 nm) and periodate-Schiff reagent (560 nm). The arrows indicate the positions of the following marker proteins: bovine serum albumin (BSA), carboxypeptidase B (C), trypsine (T) and lysozyme (L).

number of purification steps. Thus our efforts are presently directed toward a simplification of the preparation procedure of the active primary material. Nevertheless the present results indicate that it was possible to obtain from pathological urines a highly purified HLA antigen.

Acknowledgements

This research was supported in part by the I.N.S.E.R.M. (Research group U-93 and U-116), the C.N.R.S. (ER. 102), the D.G.R.S.T. (Action concertée 'Immunologie de la Transplantation') and the Fondation pour la Recherche Médicale Française.

References

[1] Robert, M., Vincent, C. and Revillard, J. P. (1974) Transplantation 18, 89–91.
[2] Bernier, I., Dautigny, A., Colombani, J. and Jollès, P. (1974) FEBS Lett. 45, 308–311.
[3] Colombani, J., d'Amaro, J., Gabb, B., Smith, G. and Svejgaard, A. (1971) Transplant. Proc. 3, 121–126.
[4] Laemmli, U. K. (1970) Nature 277, 680–685.
[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[6] Hirschfeld, P. (1960) Science Tools, 7, 18–25.
[7] Oh, S. K., Pellegrino, M. A., Ferrone, S., Sevier, E. D. and Reisfeld, R. A. (1975) Eur. J. Immunol. 5, 161–166.
[8] Parham, P., Terhorst, C., Herrmann, H., Humphreys, R. E. Waterfield, M. D. and Strominger, J. L. (1975) Proc. Nat. Acad. Sci. USA 72, 1594–1598.