Human Transplantation Antigens: Isolation and Radioimmunoassay[1] (35390)

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The current status of the isolation of transplantation antigens has been reviewed by Kahan and Reisfeld (1). In the case of HL-A antigens, known major transplantation antigens of the human, soluble antigens have been obtained by diverse methods and some of the antigenic specificities have been reported to be found on molecular fragments showing different properties on gel-filtration (2) or on ion exchange chromatography (3, 4).

We have now been able to isolate HL-A1 antigen and HL-A2 antigen from cells of a single hematopoietic cell line which has both antigens, giving additional direct evidence that the specificities of HL-A antigens, which are under control of the first sublocus (5) and are expressed in the same cells, are present on molecular fragments with properties sufficiently different to permit separation.

The antigen fragments appear to be of protein nature. They can be labeled with radioiodine by direct iodination without significant loss of antigenic activity. The incorporation of the radiolabel permits the use of radioimmune methods for the detection and quantitation of HL-A antibodies and HL-A antigens both in the soluble and insoluble forms and on intact cells.

Materials and Methods. Particulate HL-A antigens, starting materials for solubilization, were obtained by gently shaking a 10% cell suspension in isotonic buffered saline (pH 7.3) for 3 days at 4°. This procedure separated virtually all the HL-A2 antigenic activity of cells in a particulate form. The particulates obtained from 10 ml of packed cells were concentrated by centrifugation and digested with papain (230 units from Worthington Biochemical Corp., Freehold, N.J.) in the presence of cysteine (0.01 *M*) for 30 min (pH 7.0 at 37°), at which time the maximal HL-A2 antigenic activity was present in the fluid phase. The solubilized HL-A antigens were then applied to a column of DEAE-Sephadex A-25 (1.5 g) equilibrated with 0.02 *M* Tris–HCl buffer containing 0.38 *M* NaCl (pH 8.0 at room temperature). The material which passed through the column with the above buffer was collected, concentrated to 0.5 ml and then applied on a column of Sephadex G-150 superfine (bed volume 50 ml) equilibrated with Tris–HCl buffer (pH 8.6 at 4°, Γ/2 0.05). Both HL-A1 and HL-A2 activities were eluted as a single peak at the position corresponding to a molecular size of 48,000. The active fractions were pooled, concentrated to 1.0 ml and finally subjected to electrophoresis on a column of Bio-gel P-2 (bed volume 82 ml) equilibrated with Tris–HCl buffer (pH 8.6 at 4°, Γ/2 0.05). After electrophoresis at 3 mA/cm^2 for 24 hr in the cold, 0.5-ml fractions were collected by elution with the same buffer.

Results. When the cells of a cell line, RPMI 5287, which has both HL-A1 and HL-A2 antigens as shown by cytotoxicity tests with monospecific antisera to HL-A1 and HL-A2, were treated as described, a single protein peak was obtained on column electrophoresis. The peak activity of HL-A1 antigen appeared at the ascending part of the pro-

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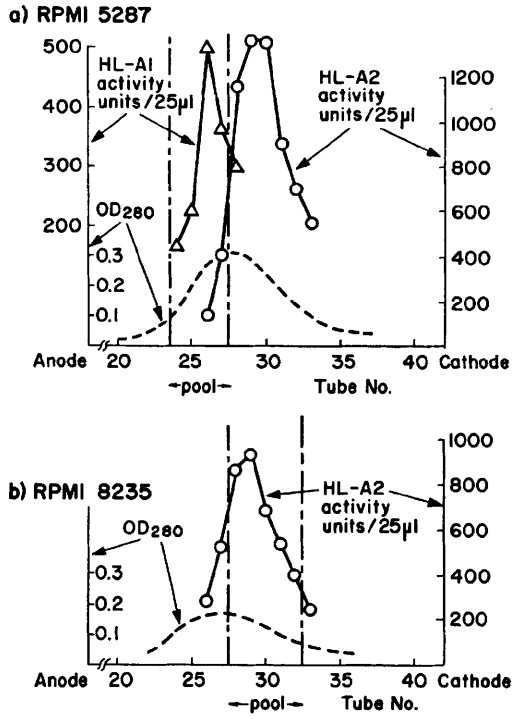

FIG. 1. Migration pattern of HL-A1 and HL-A2 antigens on column electrophoresis (a) RPMI 5287 cells, (b) RPMI 8235 cells. HL-A1 and HL-A2 activities were determined by the ^{51}Cr cytotoxicity test and plotted as units per 25 µl. Fractions were pooled as indicated and concentrated and used as HL-A1 and HL-A2 antigen sources, respectively.

tein peak, whereas that of HL-A2 antigen appeared at the descending part (Fig. 1a). The fractions with high HL-A1 activity were pooled, concentrated, and used for radioiodination. The fractions with high HL-A2 activity were apparently contaminated with HL-A1 antigen as judged by the distribution pattern of the activities determined by inhibition of the ^{51}Cr cytotoxicity reaction (6).

HL-A2 antigen, free of HL-A1 antigen, was obtained by the same procedure from another cell line, RPMI 8235, which has HL-A2 antigen but no HL-A1 antigen. HL-A2 antigenic activity from RPMI 8235 was found to appear at the same position as that from RPMI 5287 both on gel filtration and on electrophoresis (Fig. 1b). The final yield of these pooled fractions was approximately 430 µg for HL-A1 antigen derived from cell line RPMI 5287 and 270 µg for HL-A2 antigen derived from cell line RPMI 8235 assuming that OD_{280} of 1 mg/ml of solution is 1.0 unit.

Soluble HL-A1 and HL-A2 antigens thus isolated were radioiodinated with ^{125}I by the chloramine-T method (7) to have a level of 0.6 iodine atoms/mole of 48,000 Daltons and a specific radioactivity of approximately 3 mCi/mg. The iodination process did not significantly diminish the HL-A antigenic activities as determined by inhibiton of the ^{51}Cr cytotoxicity reaction.

These HL-A antigens (not necessarily the specific regions) appear to be of protein nature since prolonged digestion with papain, chymotrypsin, trypsin, and pronase destroys HL-A2 antigenic activity. Moreover, appreciable amounts of radioiodine are attached to the antigens, further indicating their protein nature.

The binding activities of the radioactive HL-A1 and HL-A2 antigens with alloantisera to each antigen were determined by coprecipitation (8). Seven ng of the radioactive preparations (as estimated by OD_{280}) was incubated with various amounts of alloantisera for 2 hr at room temperature and overnight in the cold. Goat antihuman IgG serum was then added to precipitate the human antibody globulin and the radioactive antigen bound to it. After incubation, the resultant precipitate was washed and the radioactivity was determined.

As shown in Fig. 2, 34% of the radioactivity in 7 ng of radioactive HL-A1 preparation derived from cell line RPMI 5287 was bound by 16 µl of alloantiserum to HL-A1, D-66-17058V, while 3% reacted with 16 µl of alloantiserum to HL-A2, Jochum, and 1.4% with 10 µl of alloantiserum to HL-A2, Piquard I.[4] The nonspecific binding with 16 µl of a normal human serum was 1%. This high binding activity of the HL-A1 preparation with anti-HL-A1 alloantiserum and low binding with anti-HL-A2 alloantisera clearly in-

[4] Alloantisera, D-66-17058V and Piquard I were obtained from the National Institutes of Health, alloantiserum, Jochum, from Dr. Elias Cohen of the Department of Clinical Laboratories, Roswell Park Memorial Institute, and the original cell cultures were obtained from Dr. George E. Moore.

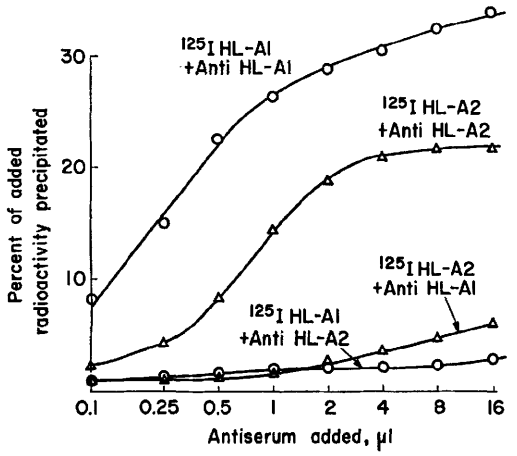

FIG. 2. Binding of ^{125}I HL-A1 and ^{125}I HL-A2 antigens by specific alloantisera. Percentage binding of the radioactive antigens at various levels of antiserum against HL-A1, D-66-17058V and antiserum against HL-A2, Jochum, was determined by the coprecipitation technique.

dicates that the HL-A1 antigen was almost completely separated from HL-A2 antigen originally present on the same cells and that these anti-HL-A2 sera did not contain significant amounts of anti-HL-A1 antibody. When 7 ng of the radioactive HL-A2 preparation derived from cell line RPMI 8235 was used, 21.5 and 20.4% of the radioactivity was bound by 16 µl of each anti-HL-A2 alloantiserum, Jochum and Piquard I. A fairly high binding (6%) observed with an alloantiserum to HL-A1, D-66-17058V, suggests that this HL-A2 preparation contains some antigens other than HL-A2 and HL-A1 since no HL-A1 antigen has been detected on the cells or solubilized fractions of this line by cytotoxicity methods using still different anti-HL-A1 alloantisera. Moreover the alloantiserum to HL-A1, D-66-17058V, which was used here is not completely monospecific to HL-A1 and contains antibodies against other antigens in accord with the description of this serum (9).

HL-A antigens in the insoluble form and on intact cells as well as in the soluble form can also be determined by radioimmunoassay methods, *i.e.*, by inhibition of binding of radioactive antigens. Thus 50% inhibition of the binding in the HL-A1 system, utilizing 0.5 µl of D-66-17058V and 7 ng of ^{125}I-HL-A1, was observed with 9.5 ng of the purified HL-A1 antigen or 1.5×10^4 cells of RPMI 5287 cell line. In the HL-A2 system, binding of 7 ng of ^{125}I-HL-A2 by 2 µl of Jochum serum was inhibited 50% by 12 ng of the purified HL-A2 antigen or 3.2×10^4 cells of RPMI 8235 cell line. Cells do not have to be removed from the system prior to assay for binding of radioactive antigen and the incubation time can be shortened considerably for routine purposes. The method should be amenable to detection and estimation of HL-A antigens on peripheral leukocytes and should require extremely small amounts of specimens (probably less than 10 µl of peripheral blood). Prior fractionation of lymphocytes would not be necessary as required for cytotoxicity methods. On the other hand, reasonably purified antigens of known specificity and high-titer antisera are required for the successful execution of the radioimmunoassay method. A radioimmune method depending on the binding of a radiolabeled microsomal lipoprotein fraction of mouse cells to mouse alloantiserum adsorbed on plastic tubes has been reported by Foschi and Manson (10). However they found that their fraction showed a very high nonspecific binding.

Work is in progress in isolation of HL-A antigens of other specificities and on the further application of the radioimmunoassay method to the determination of other transplantation antigens.

Summary. Human transplanation antigens, HL-A1 and HL-A2, have been obtained from cells of a single hematopoietic line which has both antigens. HL-A1 has been obtained essentially free of HL-A2. Both have a molecular weight of about 48,000. They appear to be of protein nature and can be labeled with radioiodine. The radioactive antigens permit the use of sensitive radioimmunoassay methods for identifying and quantitating HL-A antigens.

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