Soluble HL-A7 Antigen: Localization in the β-Lipoprotein Fraction of Human Serum

Abstract. *The HL-A7 antigen of human leukocytes occurs as a soluble, low-density lipoprotein in the serum of HL-A7-positive individuals. Its presence in high concentration may inhibit direct leukocyte grouping, leading to erroneous results. This finding affords an easy method for the preparation of monospecific cytotoxic antiserums by absorption with serum fractions rather than leukocytes.*

Evidence presented by Zmijewski *et al.* indicates that there is a substance in human serum which is variable in quantity and may either inhibit or augment the detection of HL-A antigens by the defibrinated leukocyte agglutination technique (*1*). The amount of material present appears to vary according to certain environmental stresses to which the test subject is exposed, especially heat, cold, fasting, overeating, exercise, and alcoholic overindulgence, and the manner in which direct serologic testing is influenced strongly suggests its identity to be soluble antigenic material. In an effort to determine the chemical nature of this serum factor, we were guided by the knowledge that stresses on the body can alter the lipid and lipoprotein content of human serum and the significant relationship between the survival of skin grafts and lipoprotein allotypes described by Berg *et al.* (*2*).

Our studies have shown that there is, in the low-density β-lipoprotein fraction of human serum obtained from donors with HL-A7-positive lymphocytes, a substance that can absorb cytotoxic anti-HL-A7 activity from an antiserum containing the antibody. The HL-A7 antiserum selected for this study was produced as a result of isoimmunization of pregnancy. Although other serums containing anti-HL-A7 were also used for comparative purposes, only the results with the main reagent are described, since it was tested in several other laboratories and shown to be monospecific. The absorption is specific insofar as β-lipoproteins from HL-A7-negative individuals have no effect on antibody activity.

The β-lipoprotein from HL-A7-positive individuals was isolated according to the method of Cramer and Brattsten (*3*), and the final product was concentrated to one-half the starting volume.

Serial doubling dilutions of the lipoprotein were added to equal volumes of an HL-A7 antiserum, incubated for 1 hour at 37°C, and then allowed to stand overnight at 4°C. The absorbed antiserum was clarified by filtration and tested against known positive and negative cells by the lymphocyte cytotoxicity test (*4*). There was selective absorption by the isolated lipoprotein, but its low solubility and rapid aggregability caused us to look for another way to perform the absorptions. A system was devised in which the β-lipoprotein bound to the hydroxylapatite bed could be used as an immunoabsorbent. Thus, after washing out free protein, instead of eluting the β-lipoprotein from the column, we left it in situ, and the antiserum to be absorbed was percolated through the bed.

Specifically, 10 ml of fresh human serum from an HL-A7-positive donor was put on hydroxylapatite, and the protein was eluted with 100 ml of 0.25*M* phosphate buffer at *p*H 6.8. After the elution was complete, the excess buffer was removed from the surface of the bed and 1 ml of a 1 : 2 saline dilution of an HL-A7 antiserum was allowed to settle into the β-lipoprotein layer which was visible near the top as an orange-yellow band. This mixture was incubated and the antibody protein was eluted at 4°C with 50 ml of 0.25*M* potassium phosphate buffer. The eluate was dialyzed against 0.9 percent saline for 24 hours and then concentrated to 0.5 ml. The absorbed antiserum was tested by the cytotoxic method against known HL-A7-positive and HL-A7-negative cells. In each series of absorptions, serums obtained from HL-A7-positive as well as HL-A7-negative donors were used in parallel as a source of antigen.

The results obtained in determining the appropriate conditions for absorbing this antibody specificity are shown in Table 1. The optimal time and temperature are 4 hours at 37°C. Absorptions at room temperature for 8 hours or 24 hours resulted in reduction, but not complete removal, of antibody activity against the HL-A7-positive test cells R.C. and J. B-J. Absorption at 4°C for 24 hours did not remove antibody for any of the cells. This is not at all surprising, since the antibody used is a human isoantibody which was developed as a result of isoimmunization of pregnancy and, as is true for most antibodies of this type, it is most reactive at 37°C. In all cases, the HL-A7-negative control cell, F.C., did not react with the absorbed antiserum.

To establish the specificity of the re-

Table 1. Absorption of HL-A7 antiserum at variable conditions with HL-A7-positive and HL-A7-negative serum. R.T., room temperature; N.T., not tested; −, 0 to 20 percent dead cells; ++, 20 to 50 percent dead cells; +++, 50 to 80 percent dead cells, ++++, 80 to 100 percent dead cells.

Absorption conditions	Serum type	HL-A7-positive cells			HL-A7-negative cell (control)
		R.C.	J.B.	J.B-J.	F.C.
37°C, 4 hours	HL-A7+	−	−	−	−
37°C, 4 hours	HL-A7−	++++	+++	++++	−
R.T., 24 hours	HL-A7+	++	−	++	−
R.T., 24 hours	HL-A7−	++++	++++	++++	−
R.T., 8 hours	HL-A7+	++	−	++	−
R.T., 8 hours	HL-A7−	++++	++++	++++	−
4°C, 24 hours	HL-A7+	+++	++++	N.T.	N.T.
4°C, 24 hours	HL-A7−	++++	++++	++++	−

Table 2. HL-A7 antiserum absorbed for 4 hours at 37°C, with β-lipoprotein. See Table 1 for explanation of symbols.

Serum donor	Serum type	HL-A7-positive cells			HL-A7-negative cell (control)
		R.C.	J.B.	J.B-J.	F.C.
R.C.	HL-A7+	−	−	−	−
J.B.	HL-A7+	−	++	−	−
F.C.	HL-A7−	+++	++++	++++	−
N.D.	HL-A7−	++++	+++	++++	−

action we absorbed the HL-A7 antiserum with β-lipoprotein from different HL-A7-positive and HL-A7-negative individuals under the previously determined optimal conditions. The absorbed serums were tested and some of the results obtained with two HL-A7-positive as well as two HL-A7-negative donors are shown as an illustrative example in Table 2. Absorption with the β-lipoprotein fraction of serum from donor R.C., who is HL-A7-positive, removed the cytotoxic antibody reactive with HL-A7-positive cells R.C., J.B., and J. B-J. The β-lipoprotein from HL-A7-positive donor J.B. completely absorbed the antibody activity for test cells R.C. and J. B-J. and reduced the antibody activity for cell J.B., indicating that there is some quantitative relationship between the amount of antigen present on the cells and in the plasma of a particular donor, which may vary from one individual to another. Absorption with the β-lipoprotein from HL-A7-negative donors, F.C. and N.D., failed to remove antibody activity. As expected, the HL-A7-negative cell, F.C., did not react with any of the absorbed serums.

These data furnish convincing evidence that there is a soluble HL-A7 antigen associated with the β-lipoprotein fraction of serum from HL-A7-positive donors. This is equatable with the human red cell isoantigens which have soluble counterparts. However, two distinct categories of soluble antigens exist in the erythrocyte systems.

In the ABO blood-group system the soluble antigens are secreted independently of the antigenic structures on the erythrocyte surface. In the Lewis system, on the other hand, the determinants are primarily soluble plasma polysaccharides and are secondarily adsorbed onto the red cell surface, thereby imparting the observed cellular polymorphisms (*5*).

The latter mechanism is intriguing and may gain further support from the findings of Morton *et al.*, who claim to have found the HL-A7 antigen on red cells (*6*). This was shown by means of a highly sensitive hemagglutination method which used the autoanalyzer and could be interpreted as meaning that the soluble HL-A7 lipoprotein was adsorbed onto the erythrocyte surface. Such a possibility is especially likely, since in their experiments washed, packed erythrocytes failed to absorb the antibody.

Although lipoproteins can be polymorphic (*7*), at our present state of understanding we do not know whether the β-lipoprotein antigen we are detecting is primarily soluble material which is adsorbed onto the surface of leukocytes or a degradation product resulting from normal cellular destruction.

The method of absorption devised for this study is well suited to antiserum purification. Large quantities of antiserums can be absorbed by enlarging the size of the column and increasing the amounts of antigen-containing serum and thus the amount of β-lipoprotein on the column. Batch procedures may also be employed.

Since the initiation of this work, van Rood *et al.* have reported that the antibody activity of HL-A2 and 7^b antiserums can be neutralized with whole serum from donors carrying these HL-A antigens (*8, 9*). Van Rood has also been able to sensitize skin graft recipients by injecting plasma from donors positive for the 7^b antigen (*8*). The use of purified β-lipoprotein fractions rather than lymphocytes could conceivably facilitate production of HL-A antibody.

These findings have additional implications. It is known that the transfusion of whole blood can stimulate the production of leukocyte antibodies. These can in turn cause hyperacute rejection of renal transplants, as pointed out by Kissmeyer-Nielson and his associates (*10*). Therefore, in order to avoid immunization of potential transplant recipients who are on chronic hemodialysis a method of rendering blood buffy-coat-free should be selected which also removes a large proportion of plasma.

RONALD K. CHARLTON*
CHESTER M. ZMIJEWSKI†
Division of Immunology,
Duke University Medical Center,
Durham, North Carolina 27706

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* Present address: Dental Research Center, University of North Carolina, Chapel Hill.
† Present address: Division of Transplantation Immunology, Ortho Research Foundation, Raritan, N.J. 08869.

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Tuberculin-Active Carbohydrate That Induces Inhibition of Macrophage Migration but not Lymphocyte Transformation

Abstract. *A tuberculin carbohydrate fraction, GAE, in sensitized animals induced a delayed type of skin reactivity and inhibited the migration of macrophages but failed to stimulate lymphocyte transformation in vitro. Tuberculin protein-containing fractions were active in each test. These results show that in vitro lymphocyte transformation is not necessarily a corollary of delayed type hypersensitivity.*

The ability of polysaccharides to elicit a delayed type of hypersensitivity has been a subject of controversy for a number of years. We have shown previously that GAE, a carbohydrate preparation from culture filtrates of *Mycobacterium bovis* (strain BCG) containing less than 0.4 percent nitrogen (in the form of peptide or protein) is skin reactive in tuberculin-sensitive guinea pigs and causes in vitro inhibition of migration of macrophages from sensitized guinea pigs (*1–4*). The present study shows that this carbohydrate had little or no lymphocyte-transforming activity even though it caused macrophage inhibition and had dermal reactivity in sensitized animals, whereas protein-containing fractions from the same culture filtrates were active in each test.

The antigens used were GA, GAE, GB, and GX (*1, 3–5*). These substances were prepared from the acid-soluble (*p*H 4.0) portion (G) of exhaustively dialyzed 3-month-old culture filtrates