Serologic and Physicochemical Characterization of Donath-Landsteiner Antibodies from Six Patients

By Carl F. Hinz, Jr.

The hemolysin of the Donath-Landsteiner reaction, responsible for paroxysmal cold hemoglobinuria, has not been characterized physico-chemically. Other erythrocyte agglutinins and hemolysins have been identified in both the 7S and 19S fractions of γ-globulin, and certain other of the cold antibodies have been identified with the 19S globulins. Further, the Donath-Landsteiner hemolysin is often associated with the Wassermann reagent, responsible for serologic tests for syphilis, which is at least in part macromolecular, though the two have been demonstrated to be distinct.

Previous studies from this laboratory, performed during the course of purification of the Donath-Landsteiner hemolysin for studies on the mechanism of action of complement have indicated that it is associated with low molecular weight γ-globulins. The present studies offer further evidence that the electrophoretic, chromatographic, immunologic and ultracentrifugal behaviors of the Donath-Landsteiner hemolysin are consistent with its identification with the 7S γ-globulins.

Materials and Methods

Serum was obtained from six patients with paroxysmal cold hemoglobinuria through the kindness of Dr. R. C. Griggs (serum 1), G. O. Clifford (serum 2), W. S. Jordan, Jr. (serum 3), F. H. Gardner (serum 4), H. Chaplin, Jr. (serum 5), and T. F. Newcomb (serum 6). Several of these (sera 1, 2, 3) were described briefly in an earlier report. Donath-Landsteiner antibody activity of serum or fractions of serum and cold agglutinin activity were tested as described previously utilizing erythrocytes from patients with paroxysmal nocturnal hemoglobinuria which are particularly sensitive to hemolytic antibodies. Zone electrophoresis was performed on starch or on Pevikon according to the method of Müller-Eberhard, column chromatography on DEAE-cellulose according to the method of Fahey, McCoy, and Coulom, ultracentrifugation in a sucrose gradient according to the method of Edelman, Kunkel, and Franklin, and immunoelectrophoresis according to the method of Scheidegger.

Results

Serologic Properties

The Donath-Landsteiner reaction has several unique characteristics including a requirement for the presence of complement and low temperatures at the time of erythrocyte-antibody interaction. All six antibodies fulfilled the criteria for typical Donath-Landsteiner hemolysins (table 1.) They had no
DONATH-LANDSTEINER ANTIBODIES

Table 1.—Serologic Characteristics of Donath-Landsteiner Antibodies from Six Patients

<table>
<thead>
<tr>
<th>Serum</th>
<th>Whole serum</th>
<th>Fractions containing D-L antibody</th>
<th>Hemolysin activity at 37 C.</th>
<th>Cold agglutinin titer</th>
<th>Requirement for complement in cold for optimum lysis</th>
<th>D-L hemolysin titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>positive</td>
<td>negative</td>
<td>0</td>
<td>4</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>negative</td>
<td>negative</td>
<td>0</td>
<td>4</td>
<td>+</td>
<td>128</td>
</tr>
<tr>
<td>3</td>
<td>positive</td>
<td>weakly +</td>
<td>0</td>
<td>4</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>positive</td>
<td>positive</td>
<td>0</td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>negative</td>
<td>negative</td>
<td>0</td>
<td>2</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>positive</td>
<td>negative</td>
<td>0</td>
<td>32</td>
<td>+</td>
<td>32</td>
</tr>
</tbody>
</table>

hemolytic activity at 37 C. in the presence of complement. However, hemolysis occurred after incubation of erythrocytes, antibody and complement, first in the cold and then at 37 C. All showed much greater hemolytic activity when complement was present during the cold phase of incubation and some were inactive unless complement was present in the cold. Titers of Donath-Landsteiner hemolysin varied from 6 to 128 when paroxysmal nocturnal hemoglobinuria erythrocytes were used. Cold agglutinin titers were usually lower than hemolysin titers, and in no instance did they exceed the hemolysin titer. In four of the six sera the serologic tests for syphilis were positive, but in only one instance did the Donath-Landsteiner antibody purified by column chromatography show strong seropositivity.

Physicochemical Characterization

Sera 1 to 5. Zone electrophoresis of sera 1 to 5 revealed all Donath-Landsteiner hemolysin activity in the γ-globulin peak. Figure 1A shows a representative pattern from serum 5. Chromatographic separation of the sera on DEAE-cellulose revealed all the antibody activity in the initial fractions which have been shown previously to contain only 7S γ-globulins (fig. 2A). The fractions containing antibody were found to contain only γ-2 globulins by paper electrophoresis and immunoelectrophoresis. Recovery of antibody activity from zone electrophoresis and column chromatography was from 60 to 90 per cent.

Antibody titers were sufficient to permit successful ultracentrifugation in a sucrose gradient in three of the sera (fig. 3A). In each instance the antibody activity was contained in fractions corresponding to known 7S γ-globulins. Figure 3B demonstrates comparison with anti-A agglutinins in serum from a hyperimmunized group O individual which contained two peaks of activity corresponding to 7S antibody in intermediate fractions and 19S antibody at the bottom of the tube.

Serum 6. In the serum of patient 6, the antibody activity was in electrophoretic fractions intermediate between γ- and β-globulins (fig. 1B). On column chromatography with DEAE cellulose, all the antibody activity eluted in a narrow band at slightly higher ionic strength than did the other antibodies. On stepwise elution from DEAE cellulose, most of the antibody was eluted in .03 M phosphate buffer at pH 8. The fractions containing antibody...
activity showed precipitation on immunoelectrophoresis with anti-\(\gamma\)-2 globulin serum but not with anti-B2A antiserum (kindly provided by Dr. J. L. Fahey). Ultracentrifugation in a sucrose gradient showed all antibody activity to be in intermediate fractions at the same level as the other five antibodies. In summary, antibody 6 appeared to be a 7S \(\gamma\)-globulin with slightly faster electrophoretic mobility than the other five antibodies studied.

**Discussion**

These observations offer several lines of evidence in support of the thesis that the Donath-Landsteiner hemolysins are associated with the 7S \(\gamma\)-globulins. Evidence of some heterogeneity is offered in the observation that one antibody exhibited slightly different electrophoretic and chromatographic behavior than the others. Heterogeneity of this sort has been reported for a number of blood group antibodies.\(^9\)

The Donath-Landsteiner hemolysin therefore has different physicochemical characteristics than the cold hemagglutinin responsible for high titer cold hemagglutinin disease, which has been demonstrated to be macromolecular by several investigators.\(^5,10\) As has been reported previously and confirmed again in the present study, the Donath-Landsteiner hemolysin bears no constant relationship to the Wassermann antibody which is in both the heavy and light fractions of serum.\(^3\)

Though a number of cold acting antibodies are 19S in nature, the present observations emphasize that macromolecular size is not prerequisite for cold
DONATH-LANDSTEINER ANTIBODIES

Fig. 2.—Chromatographic separation of Donath-Landsteiner antibodies on DEAE cellulose. (A) Serum 5—representative of sera 1 to 5. (B) Serum 6. The cross-hatched area represents the area of antibody recovery.

activity. Nor does molecular size seem to determine the relative agglutinating and hemolytic activities of red cell antibodies. It is true that the 7S Donath-Landsteiner antibody is predominantly a hemolysin and the 19S cold antibody is predominantly an agglutinin, but the studies of Abelson and Rawson have shown both agglutinating and hemolytic activity in many chromatographic fractions of the isoantibodies anti-A and anti-B.9

Of particular interest is the recent demonstration by Levine et al.11 that all Donath-Landsteiner antibodies tested, including sera 5 and 6 of the present series, have specificity resembling anti-P + P_1 (anti Tj^*). Their observations were based on the failure of Donath-Landsteiner antisera to react with erythrocytes from the extremely rare donors of genotype pp. Thus, another of the
autoimmune antibodies is demonstrated to have antigenic specificity, further evidence in support of their classification as true antibodies. In this manner the Donath-Landsteiner antibodies are comparable to cold agglutinins which have been demonstrated to have anti-I specificity, and to a variety of warm autoantibodies which have various specificities in the Rh system.

**SUMMARY**

Donath-Landsteiner antibodies from six patients were studied serologically and physicochemically. All had similar serologic properties including a requirement for complement in the cold phase. Five of the antibodies were associated with the 7S γ2-globulins, while the sixth was associated with 7S γ-globulins of slightly faster electrophoretic mobility and slightly different chromatographic behavior.

**SUMMARIO IN INTERLINGUA**

Anticorpores Donath-Landsteiner ab sex patientes esseva studiate serologicamente e physicochimicamente. Omnes habeva simile proprietates serologic, incluse
un requirimento pro complemento in le phase figide. Cinque del anticorpores eseva associate con le globulinas γ² 7S, durante que le sexte eseva associate con globulinas γ 7S de levemente accelerate mobilitate electrophoretic e de levemente differente comportamento chromatographic.

ACKNOWLEDGMENTS

Valuable technical assistance was contributed by Mrs. Mary Picken and Miss Anna Marie Mollner. Dr. Russell Weisman has kindly supplied erythrocytes from a patient with paroxysmal nocturnal hemoglobinuria.

REFERENCES


Carl F. Hinz, Jr., M.D., John and Mary R. Markle Scholar in Academic Medicine; Assistant Professor of Medicine, School of Medicine, Western Reserve University, Cleveland, Ohio.
SeroLogic and Physicochemical Characterization of Donath-Landsteiner Antibodies from Six Patients

CARL F. HINZ, JR.