Identification of a Cytotoxic and Agglutinating Anti-4b Leucocyte Antibody

H. J. DOWNING, Natal Institute of Immunology, Durban

SUMMARY

A cytotoxic and agglutinating serum for the detection of the 4b antigen on human leucocytes is described.


The 4a/4b leucocyte antigens were first reported by Van Rood et al. who used a leuco-agglutination antibody. So far there has been no report of a cytotoxic antibody giving a high level of agreement with Van Rood's agglutinating anti-4b sera. Of the cytotoxic sera listed as anti-4b in the NIH Catalogue, the sera Brewer and Burke (NIH 2-51-4-11-22-01 and 2-51-4-04-28-01 respectively) are now believed to be heterospecific (NIH Catalogue (1969) pp. 262 and 263). The serum Oshita (NIH 2-55-8-12-10-04), when used in a population study gave only 33% positive results as opposed to Van Rood's figure of 91%. Similarly the only other two cytotoxic sera alleged to contain anti-4b antibodies, TO/29/01 and TO/29/02 (NIH 1-02-8-05-29-28 and 1-02-8-05-29-29) which were obtained at different times from the same donor, give only 75% positive results in population studies.

We report here an agglutinating and cytotoxic serum (Melb P 1500) that gives a high level of agreement with Van Rood's agglutinating sera 1057 and 110.

METHODS AND MATERIALS

The serum (Melb P 1500) was obtained from a multiparous woman attending the Maternity Clinic at Queen Victoria Hospital, Melbourne. The serum was tested against leucocyte suspensions from 10 random donors at the Red Cross Blood Transfusion Service by the agglutination test of Van Rood et al. and by the cytotoxicity test of Walford et al. When positive results were obtained by both of these methods, the serum was tested against leucocyte suspensions from 143 donors whose leucocyte antigens had been previously determined.

RESULTS

The serum was found to contain cytotoxic and agglutinating antibodies which gave positive results with 85% of the panel of donors. Furthermore, there was almost complete agreement between the results obtained with this serum and those obtained with Van Rood's sera 1057 (De Goede) and 110 (Smits). These findings are summarized in Table I.

<table>
<thead>
<tr>
<th>TABLE I. COMPARISON BETWEEN VAN ROOD'S ANTI-4b SERA (1057 AND 110) AND SERUM MELB P 1500 WHEN TESTED BY AGGLUTINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1057/Melb P 1500</td>
</tr>
<tr>
<td>110/Melb P 1500</td>
</tr>
</tbody>
</table>

There is a highly significant agreement between the results obtained with Melb P 1500 and each of Van Rood's sera (1057 and 110) when tested by agglutination. When the results obtained by the agglutination test for serum Melb P 1500 were compared with the results obtained by the cytotoxicity test there was complete agreement. These results are shown in Table II.

<table>
<thead>
<tr>
<th>TABLE II. COMPARISON OF THE RESULTS OF AGGLUTINATION TESTS WITH RESULTS OF CYTOTOXICITY FOR SERUM MELB P 1500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Cytotoxicity</td>
</tr>
</tbody>
</table>

DISCUSSION

This is the first report of an antiserum that, while giving almost complete agreement with Van Rood's anti-4b sera by agglutination was also cytotoxic. The complete agreement between the agglutination and cytotoxicity tests meant that the 4b antigen, as originally defined by Van Rood for polymorphonuclear leucocytes, is present on lymphocytes. The presence of this antigen in human lymphocytes had been presumed from its importance as a transplantation antigen, but the direct demonstration of the presence of an antigen on lymphocyte requires the appropriate cytotoxic antibody. Therefore the discovery of a cytotoxic 4b antibody confirmed the presence of the 4b antigen on lymphocytes.

The discovery of a cytotoxic 4b antibody almost 7 years after the discovery of the agglutinating 4b antibody suggests that the same could occur with other specificities, such as 5a and 5b, which so far have been demonstrated only by agglutination. This suggestion is supported by the demonstration of the 5a and 5b antigens on kidney and spleen cells.

Therefore, when investigating the specificity of the leucocyte antibody or antibodies in a serum, it is desirable to use leucocyte suspensions that have been typed for as wide a range of specificities as possible. This in turn requires the use of agglutination as well as cytotoxicity tests.
A Case of Transfusion Reaction due to Cytotoxic Anti-4b Leucocyte Antibody

H. J. DOWNING, Natal Institute of Immunology, Durban

SUMMARY

Two transfusion reactions are reported, one a febrile and the other an allergic reaction, in a patient with erythrocyte and leucocyte antibodies of known specificity.


It has been shown that leucocyte antibodies can cause febrile reactions in patients transfused with blood possessing the appropriate leucocyte antigens. The 4a/4b leucocyte antigens were first reported by Van Rood in 1961; the antibodies concerned were agglutinating only and it is only recently that a cytotoxic antibody was discovered that gave a high correlation with Van Rood's agglutinating sera.

We report here a transfusion reaction caused by a cytotoxic anti-4b antibody.

CASE REPORT

A 63-year-old woman blood group O, Rh-positive (Dce/dce) had her first blood transfusion of 2,25 litres of O Rh-positive blood in May 1966 for a gastro-intestinal haemorrhage. In May 1967 she received 1,75 litres of O Rh-positive blood for the same reason. She was re-admitted to hospital on 21 September 1968 with a further episode of melaena and haematemesis. Her haemoglobin fell to 8,5 g/100 ml. Transfusion was commenced with O Rh-positive blood (third transfusion), but after 200 ml the patient developed marked pain in her back, associated with rigors. Her pulse rate fell to 80/min and blood pressure rose to 180/100 mmHg.

Subsequent investigation showed that the patient had anti-E antibodies, but the transfusion she had received did not in fact have E antigen and the red-cell crossmatch had been negative by saline, Coombs' and papain techniques. The patient was then given CDe/CDe blood (fourth transfusion). After 2 units she developed a widespread eruption of itchy red weals which were controlled by antihistamines. The patient had no history of allergic phenomena such as asthma, rhinitis, eczema, or drug or food allergy.

Investigations

The patient's serum was tested for leucocyte antibodies by cytotoxic tests and by leuco-agglutination. The serum was also tested for antibodies against serum by gel immunodiffusion using 2 mobile phases. The donors of the various units of blood used in the transfusions were called to the Blood Bank and the leucocyte antigens were determined by cytotoxicity tests. Similar tests were performed on the patient's husband, son and daughter.

Results

The gel immunodiffusion tests did not demonstrate any antibodies against human serum. The agglutination tests were also negative but the cytotoxicity tests showed that the patient's serum contained a cytotoxic leucocyte antibody to a titre of 4.

The specificity of the antibody was determined by performing further cytotoxicity tests with lymphocyte suspensions from 145 donors and comparing these results with those obtained with sera of known specificity. The unknown antibody and Van Rood's anti-4b serum 110 (Smits) gave identical results in a panel of 145 donors. The leucocyte antigens of the patient, her husband, son and daughter and of the various units of blood that the patient received are shown in Table 1.

*Date received: 26 July 1971.