Comparison of the ability of monoclonal and polyclonal anti-D antibodies to promote the binding of erythrocytes to lymphocytes, granulocytes and monocytes

ANTHONY H. MERRY,* EVA BROJER,J BARBARA ZUPANSKA,J ANDREW G. HADLEY,* BELINDA M. KUMPEL,J and NEVIN C. HUGHES-JONES§

*The Blood Group Reference Laboratory, Radcliffe Infirmary, Oxford OX2 6HE, U.K., †Department of Serology, Institute of Haematology, Warsaw, Poland, §U.K. Transplant Centre, Southmead Road, Bristol, U.K. and ¶Institute for Animal Physiology, Babraham, Cambridge, U.K.

Human monoclonal antibodies to the D antigen of the Rh blood group system have been produced by long-term culture of Epstein Barr virus (EBV)-transformed lymphocytes. In future, it may be possible to use these antibodies for prophylaxis against haemolytic disease of the newborn, if a convenient way of assessing the interaction between sensitized erythrocytes and Fc-receptor bearing leucocytes (Zupanska et al., 1986). Such assay may also provide information on Fc-receptor characteristics on different types of leucocyte.

Ten culture supernatants were tested, six containing IgG1 anti-D antibodies and four containing IgG3 antibodies. Seven supernatants were produced by culture of EBV-transformed lymphoblastoid cell lines 1A3(IgG1), FC3(IgG1), CB6(IgG1), CB6-1(IgG1), CB6-3(IgG3), 6D10(IgG3) and (UCHD4(IgG1)). The latter was a gift from Dr D. H. Crawford, (University College Hospital, London) and two supernatants were produced by culture of mouse–human heterokaryoma cell lines namely Fog-B(IgG1) and Fog-3(IgG3). Rosette assays were repeated several times for each antibody as shown in parentheses.

Abbreviation used: EBV, Epstein Barr virus.

1To whom correspondence should be addressed.

Table 1. Minimum levels of erythrocyte sensitization for rosette formation with leucocytes

<table>
<thead>
<tr>
<th>IgG subclass</th>
<th>Leucocyte</th>
<th>Monoclonal</th>
<th>Polyclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>Monocytes</td>
<td>2000–3000</td>
<td>500–1300</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>Not found</td>
<td>3400–14200</td>
</tr>
<tr>
<td></td>
<td>Granulocytes</td>
<td>Not found</td>
<td>Not tested</td>
</tr>
<tr>
<td>IgG3</td>
<td>Monocytes</td>
<td>300–600</td>
<td>180–500</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>5000–15000</td>
<td>500–1300</td>
</tr>
<tr>
<td></td>
<td>Granulocytes</td>
<td>8000–10000</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

These data suggest that IgG3 anti-D is more effective in promoting rosette formation than IgG1 anti-D and that interaction with monocye FcRI occurs at lower levels of erythrocyte IgG sensitization than with lymphocyte or granulocyte FcRII and FcRIII. These results in vivo may, however, not be directly applicable to effects of these antibodies in vitro.
Isoelectric focusing spectrotypes of anti-thyroglobulin autoantibodies in autoimmune thyroiditis: an AUG rat model

DAVID I. STOTT,* ROSEMARY HASSMAN,† LYNNE NEILSON* and ALAN M. McGREGOR†
*Department of Bacteriology and Immunology, University of Glasgow, Western Infirmary, Glasgow G11 6NT, U.K. and †Department of Medicine, King's College, London SE5 8RX, U.K.

Hashimoto's thyroiditis is an organ-specific autoimmune disease involving infiltration of the thyroid by B- and T-lymphocytes, destruction of thyroid follicles and production of autoantibodies against thyroglobulin and other thyroid antigens. Immunization of AUG strain rats with autologous thyroglobulin induces an autoimmune response with similar lesions (Penhale et al., 1975), but the mechanism whereby tolerance is abrogated is still unknown. Proposed mechanisms can be divided into polyclonal activation models and those predicting a restricted response. Previous studies of autoantibodies produced by Hashimoto's patients showed that the majority were polyclonal with approximately 3% of patients having monoclonal antibodies in their sera (Stott et al., 1986). We have therefore investigated the isoelectric focusing (IEF) spectrotypes of autoantibodies produced by AUG rats to determine their clonality.

Six groups of 6–8-week-old female AUG rats were injected with rat thyroglobulin in Freund's complete adjuvant followed by a boost on day 7. The animals were bled, killed and the thyroid assessed for lymphocyte infiltration at various times from 0 to 8 weeks after immunization. A seventh (control) group was not immunized. Rats from a separate group were not killed, but bled sequentially at the same time intervals.

Anti-thyroglobulin levels were measured by enzyme-linked immunosorbent assay (ELISA) (Rennie et al., 1983) and thyroid stimulating hormone (TSH) by radioimmunoassay. Lymphocyte infiltration of the thyroid was determined histologically and scored on a scale of 0–4 (McGregor et al., 1983).

Spectrotypes of serum anti-thyroglobulin antibodies were analysed by IEF, electrophoretic blotting on to a nitrocellulose membrane, overlay with 125I-labelled rat thyroglobulin and fluorography (Stott & McLearie, 1986).

Anti-thyroglobulin titres rose progressively, peaking at weeks 4–6 and beginning to decline 8 weeks after immunization. Serum TSH also rose to a peak at 3–6 weeks and maximum thyroid infiltration occurred at 6 weeks. A strong correlation existed between antibody titre and grade of thyroiditis ($r = 0.86$, $P < 0.001$), and less strongly between antibody titre and TSH ($r = 0.64$, $P < 0.001$).

IEF bands appeared in a similar sequence to the ELISA titres, reaching maximum intensity at 4–6 weeks, each animal displaying a unique spectrotype. All sera gave polyclonal or oligoclonal banding patterns except for one rat which had only one clonotype. Dominant clonotypes were evident in other tracks.

Fig. 1. Sequential changes in the anti-thyroglobulin IEF spectrotypes of immunized AUG rats

Each rat (A, B, C and D) was bled at the times indicated and the sera analysed by IEF and reverse immunoblotting. Arrowheads indicate presumed clonotypes. N: serum from an untreated rat.

1988