The Use of Homogenized Human Leukocytes for the Preparation of Anti-Lymphocyte Serum

By ULRICH W. JEHN AND HELMUT H. RENNER

Mitogenically active antilymphocyte sera (ALS) are generally prepared by intravenous immunization with intact cells. Immunization procedures utilizing spleen cells that had been disrupted by repeated freezing and thawing have yielded ALS with insignificant lymphocyte-stimulating activity. The purpose of this paper is to present a method that utilizes immunization with mechanically homogenized cells to prepare antileukocyte sera with high mitogenic activity.

Human leukocytes were prepared from peripheral blood as described by Skoog et al. The cells used for immunization consisted, on the average, of 83 per cent granulocytes, 14 per cent lymphocytes and 3 per cent monocytes. A total of 3.5 × 10⁸ cells were suspended in 4 ml of 0.15 M NaCl, and homogenized for one minute at 0°C and 15,000 RPM with a 7-109 AF Virtis 23 homogenizer. The disrupted cell suspension was emulsified with 4 ml of complete Freund's adjuvant and used to immunize a rabbit in the footpads and other subcutaneous sites; four weeks later, on three consecutive days, each rabbit received a subcutaneous injection: on the first day 0.5 ml., and on the next two days 1 ml. of a leukocyte homogenate containing 5 × 10⁸ cells in 4 ml of 0.15 M NaCl; adjuvant was omitted. This last series of three daily injections was repeated three times at five day intervals. Three days after the last injection the animals were bled. The pooled sera were absorbed overnight.

Table 1.—Antibody Activity of RAHLS

<table>
<thead>
<tr>
<th>Tested Against</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphotoxicity</td>
<td>1:256</td>
</tr>
<tr>
<td>Leukagglutination</td>
<td>1:256</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>1:16,000</td>
</tr>
<tr>
<td>Soluble leukocyte extract</td>
<td>7 precip. lines</td>
</tr>
<tr>
<td>LDH, catalase, peroxidase, carboxypeptidase A, TAME-ase, amylase</td>
<td>antibodies detected against each enzyme</td>
</tr>
<tr>
<td>SRBC coated with: human-γ-globulin</td>
<td>1:32</td>
</tr>
<tr>
<td>human albumin</td>
<td>neg.</td>
</tr>
</tbody>
</table>

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Table 2.—Morphological Changes of Lymphocytes after Stimulation In Vitro

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Blast-Transformation</th>
<th>Cells in Mitosis</th>
<th>Cells Containing Two Nuclei</th>
<th>Cells Containing Deformed Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>RAHLS</td>
<td>6.3%</td>
<td>56.6%</td>
<td>0.1-0.2%</td>
<td>absent</td>
</tr>
<tr>
<td>PHA</td>
<td>74.8%</td>
<td>75.7%</td>
<td>0.5-1.5%</td>
<td>1%</td>
</tr>
<tr>
<td>HAHLG</td>
<td>76.8%</td>
<td>80.0%</td>
<td>0.5-1.0%</td>
<td>0.5-1.5%</td>
</tr>
<tr>
<td>Without Stimul.</td>
<td>1.5%</td>
<td>3.9%</td>
<td>absent</td>
<td>absent</td>
</tr>
</tbody>
</table>

a) Culture containing complement (1.5 × 10^6 cells/1.5 ml. medium—Behring Co, 60% Eagles MEM, 40% Hamacel; 12% plasma; pH 7.2 cultured for five days at 37° C)
b) Culture without complement
RAHLS = Rabbit-Anti-Human-Leukocyte-Serum (0.02 ml/ml.)
PHA-M = Phytohemagglutinin (1/100 U/ml.—Difco Co.)
HAHLG = Horse-Anti-Human-Lymphocyte-Globulin (0.02 ml/ml.)
with 10 mg. lyophilized normal human serum per ml. RAHLS (rabbit-anti-
human-leukocyte serum), and clarified by centrifugation at 10,000 RPM for
20 minutes.

The RAHLS was characterized by a number of in vitro assays including
lymphocytotoxicity,\textsuperscript{11} leukoagglutinin titer,\textsuperscript{12} antinuclear antibody titer,\textsuperscript{13}
passive hemagglutination of tanned sheep red cells coated with serum albumin
or globulin, antibodies against leukocyte enzymes and double diffusion in gel
against various antigens.

The lymphocyte-stimulating activity of RAHLS was tested by adding it to
culture of human lymphocytes.\textsuperscript{14} As controls, other cultures were incubated
with PHA or an antilymphocyte serum produced by immunizing horses with
intact lymphocytes.

The antibody activities of RAHLS are shown in Table 1. Very high anti-
nuclear antibody activity was found, and this may have produced the large
number of deformed nuclei seen in lymphocytes cultured with RAHLS (Table
2). Since these cells with abnormal nuclei are absent in the presence of com-
plement, it suggests that they are peculiarly fragile and readily lysed.

It is noteworthy that RAHLS has marked in vitro lymphocyte-transforming
activity, as shown in Table 2. This compares favorably with the activity of both
PHA and antilymphocyte serum prepared with intact cells. These results indi-
cate that, with respect to the in vitro assays described here, potent antileuko-
cyte serum can readily be prepared by immunizing rabbits with homogenized
cells, rather than intact lymphocytes. Therefore it is important to stress two
major advantages of the method described above: first, viable cells are not
required, and secondly, complicated lymphocyte purification procedures are
avoided.

\textbf{SUMMARY}

This is the first report of the preparation of a potent antileukocyte serum
produced by immunization with homogenates of human leukocytes. High titers
of antibody activity were found against nuclei and a number of other intra-
cellular materials, including several leukocyte enzymes. This antiserum, pre-
pared in rabbits, also had marked in vitro lymphocyte stimulating activity that
compared favorable with the activity of antilymphocyte serum prepared with
intact cells. Cytotoxicity was enhanced in the presence of complement.

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