DR-Bearing T Lymphocytes in Thoracic Duct Lymph

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T cells having DR antigens were shown to be present in high numbers in the thoracic duct lymph of patients undergoing long-term drainage. As drainage progresses the proportion of T DR cells in the lymph increases to levels as high as 70% at 6 weeks. These cells were demonstrated by showing that T cells isolated by sheep red cell rosetting were killed by the action of rabbit anti-B-cell sera and of HLA-DR antisera. The HLA-DR specificities found on the T cells corresponded with those on the patients' B lymphocytes.

INTRODUCTION

Thoracic duct drainage has been used as a method of lymphoid depletion for kidney transplantation (1-5). Recently, essentially through the work of Starzl and his colleagues (4, 5), it has become apparent that the most effective method of this form of immunosuppression is to initiate the drainage well before transplantation. We have been testing methods to demonstrate in vitro the optimum time for transplantation.

During this attempt we noted that the characteristics of lymphoid cells in the thoracic duct changed markedly after a few weeks of drainage. Others observed that the counts dropped dramatically (6, 7) and immature cells appeared (2). As reported in our preliminary studies (8), in many patients thoracic duct cells with DR markers increased markedly upon drainage. We present here evidence for the abundant existence of Tla-positive cells following prolonged drainage.

MATERIALS AND METHODS

Thoracic duct lymph was obtained from patients undergoing continuous thoracic duct drainage as preparative therapy for kidney or liver transplantation. Details of this procedure have been reported previously (4, 5, 9).

Lymphocytes were prepared from thoracic duct lymph by centrifuge spinning the lymph at 1500g for 10 min. The pelleted cells were suspended in McCoy's media (0.5% fetal calf serum) at a concentration of 10-20 x 10^6 cells/ml. This suspension was layered over Ficoll and centrifuged for 10 min at 1500g. The interface yielded a homogeneous mononuclear lymphocyte preparation.

B cells were prepared from TD lymphocytes by nylon wool adherence (10). T cells were prepared from nonadherent TD cells using neuraminidase-treated sheep erythrocyte rosette formation (11). Rosetted cells were isolated by layering and spinning over Ficoll and then by lysing with isotonic NH_4Cl (12). The rosetted

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cells were checked for purity by either rerosetting after NH₄Cl treatment or by determining the percentage of rosetted cells in the Ficoll pellet. Lymphocytes which had three or more sheep red cells bound to their surface were counted as positive rosettes.

Cytotoxicity was performed by the complement-mediated microcytotoxicity test (11). Heterologous rabbit anti-DR sera which have been extensively characterized (13) and recognize common determinants of the alpha and beta polypeptides of DR antigens were used to determine the presence of DR. Alloantisera from parous women were used to determine the specific HLA-DR groups (DR-1,2,3,4,5,7, and MT1). These DR alloantisera have been characterized previously (11, 14). The T-cell antiserum was prepared by intravenous immunization of rabbits with human thymus cells. Data demonstrating the antiserum's specificity has been published (15).

Surface membrane immunoglobulin was detected (6). Lymphocyte populations were incubated with FITC-conjugated rabbit anti-human immunoglobulins (IgG, IgM, IgA heavy and light chains), and then washed. Fluorescent cells were counted using fluorescence microscopy.

RESULTS

T cells from thoracic duct lymph produced by rosetting with neuraminidase-treated sheep red blood cells did not react, as expected, with the rabbit anti-B-cell sera. Negative reactions were found with the cells of patients tested during the first and second week of drainage (Fig. 1). As drainage continued, the T cells became more and more susceptible to lysis by the anti-B sera. After more than 6 weeks, most of the T cells were lysed by anti-B sera, showing that such T cells had the DR antigen structures recognized by the antisera. The antisera do not react served against peripheral blood T cells (13), nor did they react against T cells from patients who had just started on drainage.

Further studies were performed to obtain more quantitative information on the percentage of T cells which may have the DR antigen. The anti-B serum was

![Graph](image-url)

**Fig. 1.** Percentage of la-positive T cells (E rosetting) in thoracic duct following drainage. Each (●) represents thoracic duct cells from a different patient. *Score: 1 = 0–10% ; 2 = 11–20% ; 4 = 21–40% ; 6 = 41–80% ; 8 = 81–100%.*
tested in titrations and, as a control, heterologous anti-T serum (15) was also used (Table 1). To be certain that the isolated T cells were indeed pure T cells, all cells were rerosetted to determine the percentage of E cells. The preparations were 89 to 97% E-rosetting cells. In the first patient (AC), the anti-T serum killed all cells to a dilution of 1:16 whereas the anti-B serum was not cytotoxic. The 10% killing was within the background level found in the control negative serum. In all other samples taken from patients after further periods of drainage, the percentage of cells reactive with the anti-B serum increased sharply. The anti-T serum continued to kill all preparations.

In the last patient (CW) in Table 1 as many as 80% of the T cells were killed by anti-B serum at a dilution of 1:64. Since rerosetting has shown 93% to be T cells, we concluded that at least 60% of the T cells were dual marked with DR antigens.

Since the heterologous antiserum against DR could be suspected to be reacting against other antigens, the T cells from the thoracic duct were tested against HLA-DR antisera. Four to six antisera of each of the DR specificities were reacted against the T cells. These antisera had been absorbed thoroughly with platelets and did not react with peripheral blood T cells. Certain DR antisera were cytotoxic to T cells of the thoracic duct (Table 2). Sera which were negative are not shown. All positive reactions were those which were positive on the peripheral blood B cells of each individual patient. For example, the first patient whose B cells reacted with DR2 and MT1 antisera also had T cells which reacted only with those antisera. The HLA-DR antigens which appear on T cells are therefore the genetically determined DR of the individual.

One consistent finding has been that the cytotoxicity produced by the heterologous anti-DR sera is stronger than that produced by the allogeneic antisera. The heterologous sera may be more efficient in producing cytotoxicity or in detecting low levels of DR antigens. Alternatively, the "true" DR is detected by the allogeneic antisera and the rabbit sera may contain other impurities. We are not able to distinguish between the two possibilities at the present time.

DISCUSSION

Dual-marked lymphocytes, that is, T cells with DR antigens have been found on T cells stimulated by mitogens or allogeneic stimuli (17, 21), on some leukemia cells (15, 18, 21), and on erythroid and myeloid precursor cells (18). Moreover, recently they have been found at low levels (about 2% of cells) in the peripheral blood of healthy persons (18, 21, 22). The high levels of dual-marked cells upon prolonged thoracic duct drainage reported here have not been encountered in prior studies. As many as 80% of T cells produced by sheep red cell rosetting were lysed by anti-DR sera. The DR sera were from both heterologous and allogeneic sources. We reported earlier that the T cells also reacted with zymosan, indicating that as many as 50% of the T cells had C3 receptors (8). These unprecedented high levels of dual-marked cells in patients with prolonged drainage may have some biologic significance.

We postulate that the unusual state of having mostly TDR-positive cells produced in patients on prolonged thoracic duct drainage may be associated with the ability to induce tolerance in these patients. That is, it is possible that thoracic...
TABLE 1
COMPLEMENT-DEPENDENT CYTOTOXICITY OF RABBIT ANTI-B-CELL (DR) AND ANTI-T-CELL ANTISERA AGAINST E-ROSETTING MONONUCLEAR CELLS FROM THE THORACIC DUCT OF PATIENTS DRAINED FOR VARIOUS LENGTHS OF TIME

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percentage E</th>
<th>Weeks on drainage</th>
<th>Control</th>
<th>Anti-B (DR) titrations</th>
<th>Anti-T titrations</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>AC</td>
<td>89</td>
<td>1</td>
<td>10</td>
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<td>100</td>
</tr>
<tr>
<td>CW</td>
<td>95</td>
<td>3</td>
<td>5</td>
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<td>40</td>
</tr>
<tr>
<td>AB</td>
<td>92</td>
<td>3</td>
<td>10</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
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<td>4</td>
<td>10</td>
<td>100</td>
<td>90</td>
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<tr>
<td>CW</td>
<td>93</td>
<td>6</td>
<td>5</td>
<td>100</td>
<td>95</td>
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</tbody>
</table>
## TABLE 2

**Reactivity of Thoracic Duct T Cells to DRW Alloantisera at Various Times during Thoracic Duct Drainage**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percentage E rosettes</th>
<th>DR types</th>
<th>Weeks on drainage</th>
<th>Rabbit antisera lot No.</th>
<th>Percentage of killing</th>
<th>DR2 Sera</th>
<th>MT1 Sera</th>
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<tr>
<td>RL</td>
<td>88</td>
<td>2, MT1</td>
<td>4</td>
<td>1 2 3</td>
<td>1 2 3 4 5 6</td>
<td>25 20</td>
<td>35 10</td>
</tr>
<tr>
<td>CH</td>
<td>90</td>
<td>1, MT1</td>
<td>4</td>
<td>0 90 95 95</td>
<td>30 50 10 40 40</td>
<td>30 10</td>
<td>75 80</td>
</tr>
<tr>
<td>CW</td>
<td>ND</td>
<td>4, 7</td>
<td>6</td>
<td>ND 30 20 20</td>
<td>10 10 10 10 10</td>
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<td>6</td>
<td>ND 30 60 30 30 50 50 50</td>
<td>30 30 50 50 50 20</td>
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<tr>
<td>CW</td>
<td>93</td>
<td>4, 7</td>
<td>7</td>
<td>95 95 95 90 40 25 70 80 50 60</td>
<td>60 80 80 40 20 100</td>
<td>60 80 80</td>
<td>40 20 100</td>
</tr>
</tbody>
</table>

*Note.* Reactivity of cells against DR alloantisera, scored in percentage of killing. Negative control = normal serum, less than 10% killing on all trays. ND, not done.

* Counted after spun through Ficoll.
duct drainage removes adult immunocompetent cells and that, upon prolonged drainage, only immature cells remain. Thus, a state of immunologic immaturity is produced. The condition would then be the same as that shown by Medawar and his associates in classic immunologic tolerance (23). These immature cells may be identifiable by their dual markings. If this is verified, the cell which is susceptible to tolerance would be the TDR-positive cell.

At this point, we can only offer the following as evidence for the hypothesis. First, there is a rough correspondence of effectiveness of thoracic duct drainage before transplantation (4, 5) and the increase in TDR-positive cells. Whether this association indicates a direct relationship, of course, remains to be determined. Second, immature cells apparently are often dual marked (15, 18, 21) and with maturity, the E+ rosette and DR markers become unique to the T and B cells, respectively. Stimulation of T cells may possibly involve a dedifferentiation process.

The current findings also suggest that finding TDR-positive cells in a small proportion of normal circulating blood (18, 21, 22) may not be unusual. Moreover, these dual-marked cells may be more common in various immunologic disorders in which immature cells spill into the peripheral blood. We have preliminary data which show that spleen cells from healthy persons have relatively high levels of the TDR-positive cells. Such cells are therefore a normal component of the body's cells but apparently do not normally occur in high percentages in the blood.

ACKNOWLEDGMENTS

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REFERENCES