

## Preliminary Evidence of Dual-Marked Lymphocytes in Thoracic Duct Lymph Fluid

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**T**HORACIC duct drainage (TDD) has been an effective immunosuppressive procedure, although the precise mode of action has not been determined.<sup>1-5</sup> Changes in the lymph percentages of B and T lymphocytes and other immunologic alterations after drainage have been noted.<sup>6,7</sup> In the present study we examined thoracic duct lymphocytes of patients on prolonged drainage for B and T surface markers and Ia-like antigens. We present preliminary evidence for the occurrence of Ia and complement receptor (CR) bearing T cells after TDD.

### MATERIALS AND METHODS

TDD cannulation was accomplished as previously reported.<sup>4,5,8</sup> Lymphocytes were isolated from TDD lymph according to the method of Terasaki et al.<sup>9</sup> Briefly, TDD lymph was centrifuged, the cells were resuspended in McCoy's media, and layered over Ficoll. Lymphocytes were recovered from the interface.

Sheep erythrocyte-rosetting cells (T cells) were prepared as previously reported.<sup>9</sup> The percentage of T cells in the preparations was determined by counting 100 viable rosetted and nonrosetted cells, as indicated by fluorescein diacetate.<sup>10</sup> Rosetted cells were isolated by centrifugation over Ficoll.<sup>9</sup> Rosettes in the pellet were

lysed with  $\text{NH}_4\text{Cl}$ .<sup>11</sup> Greater than 90% of these cells rosetted, indicating minimal contamination of nonrosetted cells. The percentage of CR-positive cells in the E-rosette and non-E-rosette populations was determined using yeast rosetting, which detected CR by zymosan fixing of Cr.<sup>12</sup> Yeasts were boiled for 10 min, washed 3 times, and adjusted to  $1.5 \times 10^9$  yeast/ml in 0.1%  $\text{NaN}_3$ . One milliliter of yeast suspension was incubated with 0.5 ml of fresh human serum for 1 hr at 37°C and then washed 3 times. A  $10^9$ /ml complement-coated yeast suspension was incubated with an equal volume of lymphocytes at  $2 \times 10^6$ /ml at 5°C for 30 min. A yeast rosette was considered positive when more than 2 yeasts were bound per visible lymphocyte.

Cytotoxicity tests were carried out as previously described.<sup>9</sup> Production and documentation of the specificity of the rabbit anti-B-cell antisera was reported earlier.<sup>13</sup>

### RESULTS

The least square line ( $R=0.5$ ,  $B=-0.56$ ) in Fig. 1 demonstrates a slight downward trend in the percentage of T cells of thoracic duct lymphocyte preparations as a function of drainage time. Individual patients reflect this same trend but also show great fluctuations in the percentage of T cells. These trends are in agreement with previously reported work.<sup>6,7</sup>

Rosette-positive and rosette-negative cells were examined for the presence of CR. Figure 2 shows two representative samples and indicates that as many as 60% of the T cells also had complement receptors. Moreover, when we examined TD lymphocytes with heterologous anti-Ia serum, we found cytotoxicity that indicated the appearance during drainage of Ia-like antigens on TDD cells. The percentages of killing indicate that some cytotoxicity must be due to killing of T cells. Calculating from the cytotoxicity score, on the 34th drainage day, between 38% and 100% of patient E's T cells had Ia antigen; at 27 days, patient K had 18%–78%  $\text{Ia}^+$  T cells.

These data indicate that there are large populations of T cells that bear CR or Ia-like

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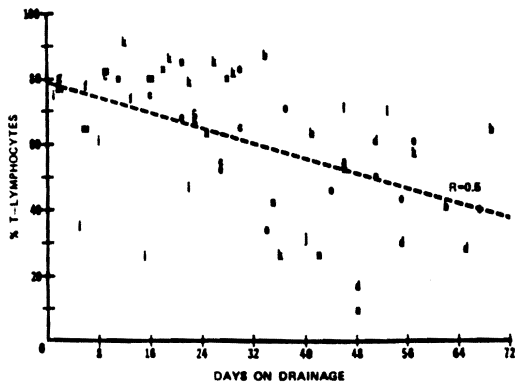


Fig. 1. Percentage of T lymphocytes in the lymph of 14 patients at various drainage times. Letters "a-n" designate individual patients.

antigens in thoracic duct drainage patients. It is possible that these two populations overlap and some of the cells in TDD lymph bear Ia-like antigens, CR, and sheep erythrocyte receptors.

DISCUSSION

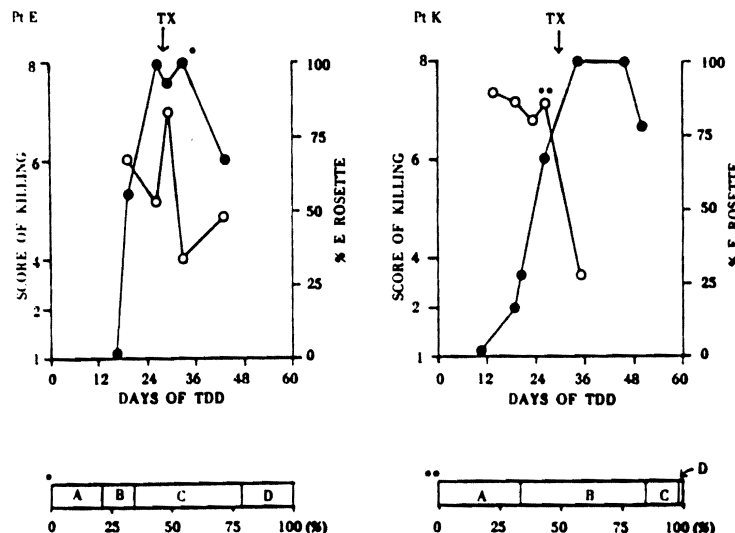
Several authors have reported that Ia<sup>+</sup> T cells appear after stimulation with allogeneic sperm, mitogens, or B cells.<sup>14-18</sup> Of normal peripheral blood T cells, 0%–6% have been reported to have Ia antigens.<sup>15,18,19</sup> Normal peripheral lymph may contain Ia<sup>+</sup> T cells in slightly higher percentages.<sup>20</sup> Increased pro-

portions of Ia<sup>+</sup> T cells have been shown in some leukemias,<sup>15,18</sup> and it is important to note that Ia antigen has been found on precursor lines.<sup>15</sup> Hence, it has been proposed that dual-marked leukemia cells originate from immature populations.<sup>21</sup> The percentages of Ia<sup>+</sup> T cells reported here are relatively high, compared to that found in normal lymph fluid or peripheral blood.

As many as 51% of the whole lymphocytes in our patients had both Ia and CR, as compared to the 0%–8% of normal peripheral blood lymphocytes which bear both markers.<sup>22-27</sup> These cells, called "D" cells, were in higher proportions in some patients with lymphoproliferative disorders.<sup>22,26-29</sup> The data presented here show large percentages of T lymphocytes that have Ia antigens or CR and possibly a subpopulation of T cells that has both.

Whereas the mechanism of immunosuppression by TDD has been associated with the lymphopenia developed by the procedure, this effect cannot explain the continued suppression<sup>1-5</sup> and the tolerance induction<sup>30</sup> that has occurred after TDD is discontinued. Based on the preliminary evidence reported here, we propose that prolonged TDD induces cell surface marker changes in the TDD lymphocyte population similar to those found in

Fig. 2. Monitoring of percent E rosette and reactivity against rabbit antisera to human B lymphocytes: O-O, % E-rosette; ●-●, reactivity to rabbit antisera. Score of killing: 1, 0%–10% dead cells; 2, 11%–20%; 4, 21%–30%; 6, 31%–80%; 8, over 80%. Each point represents an average of the reaction to 3 lots of rabbit antisera. (•, ••) Classification of different cell subpopulations according to E-rosette receptor and complement receptor: A, E-rosette positive and complement receptor negative; B, E-rosette positive and complement receptor positive; C, E-rosette negative and complement receptor positive; D, E-rosette negative and complement receptor negative.



leukemia and precursor cell populations. We suggest that prolonged thoracic duct drainage induces an immature state in the immune system as indicated by the presence of the Ia<sup>+</sup>CR<sup>+</sup> T cells. We propose that this immature state is transient, therefore, antigen must be presented to the patient at the appropriate time for tolerance to occur. Detection of Ia<sup>+</sup>CR<sup>+</sup> T cells may be important clinically to optimize TDD as an immunosuppressive therapy.

Mixed lymphocyte culture experiments and further characterization of Ia<sup>+</sup>CR<sup>+</sup> T cells are underway to confirm the results presented here and to elucidate the function of dual-marked cells in immunosuppression.

#### SUMMARY

Thoracic duct lymphocytes from patients receiving thoracic duct drainage as a pretransplant therapy were examined for cell surface markers. Patients followed over the drainage time period showed a variable but decreasing percentage of E-rosette-positive cells in the lymph fluid. A substantial percentage of these E-rosette-positive cells also had C3 receptors on their cell surface. Reactions of the whole lymphocytes with a heteroantisera to human B-lymphocyte antigens reflected the increasing proportion of B cells in the samples, but also indicated that a fraction of the T cells have Ia-like antigens on their surface. Some cells may have all 3 surface marker characteristics. Significance of these cells with respect to graft survival is discussed.

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