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# SPECIFICITY OF ANTI-LYMPHOCYTIC ANTIBODY AND ANTIGENICITY OF LYMPHOID CELLS

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## (1) Specificity of anti-lymphocytic antibody

Recent progress of immunohematology has revealed that two or more clones of socalled lymphocytes are necessary for initiation of antibody formation. They are postulated to be thymus-derived lymphocytes and bone marrow-derived lymphocytes. The possibility of employing fluorescent antibody techniques in the identification of those two kinds of lymphocytes has been investigated.

Antibodies against inbred rat thymus cells, thoracic duct lymphocytes and bone marrow cells were obtained by immunizing rabbits with the corresponding living cells intravenously. The antibodies were conjugated with fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate. All conjugates used were absorbed twice with kidney homogenate from inbred rat before staining. Furthermore, specific absorption of conjugates with lymphoid cells or with rat gamma globulin was done.

### **RESULTS**

- 1. Small thymus cell has its specific antigen in its cell membrane and cytoplasm which may be possessed by some of the small lymphocytes in the thoracic duct lymph in their cytoplasm and/or cell membrane scantly. Another possibility is that such antigenic substance may be contained in greater amount in a small thymus cell than in a small lymphocyte of thoracic duct lymph.
- 2. Small thymus cells and small lymphocytes may have a common lymphocyte specific antigen which reacts either with anti-thymus cell antisera (ATS) or anti-thoracic duct lymphocyte antisera absorbed with  $\tau$ -globulin. Large or some medium-sized lymphocytes in thoracic duct lymph and plasma cells lack such common lymphocyte specific antigen on their cell surfaces.
- 3. Rhodamine-labeled anti-bone marrow cell antibody (ABMS) stained almost all of the cells in the thymus, thoracic duct lymph, or lymph nodes and about 40 percent mononuclear round cells in the bone marrow. The specific binding capacity to lymphoid cells of ABMS was diminished by absorption with thymus cells or thoracic duct lymphocytes. Oh the other hand, ATS remainded its specific binding capacity to small thymus cells and thoracic duct lymphocytes after absorption with bone marrow cells. So, it may

be concluded that ABMS reacts with basic common antigen in lymphoid organs besides lymphocyte specific antigen and thymus antigen.

4. The percentage of cells which have lymphocyte specific antigen in rat lymphoid organs was also investigated in cytocentrifuge slides by fluorescent antibody technique. It has been revealed that 82.4% of cells of the thymus, 74.0% in the lymph node, 57.5% in the spleen and 4% in the bone marrow were fluorescent positive in the case of normal rat by staining with fluorescein labeled ATS. The percentage of fluorescent positive cells was diminished by either intraperitoneal administration of ATS into rat or chronic thoracic duct drainage.

# (2) The effect of thoracic duct drainage on the population of the lymphocytes from thoracic duct fistula.

- 1. Rapid and drastic diminution in number of small lymphocyte was observed. Such rapid diminution occurred within first three days. ATS-treated rat would not show such rapid output of small lymphocyte from the fistula. It was not observed that the more diminution in number of lymphocyte of the adult thymectomized rats than in non-treated rats.
  - 2. The output of larger lymphocyte did not change remarkably.
- 3. Per cent fluorescent cells with FITC-ATS was diminished in thoracic duct lymph by the chronic drainage of thoracic duct.
- 4. Chronic thoracic duct drainage caused the diminution of per cent fluorescent cell with FITC-ATS in lymphoid organs.