The American Journal of
PATHOLOGY

JANUARY 1977 • VOLUME 86, NUMBER 1

Reactivity of Anti-Human Brain Serum With Human Lymphocytes

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Specific anti-human T-cell serum was prepared in rabbits by multiple subcutaneous injections of human brain homogenates in incomplete Freund's adjuvant. The serum was exhaustively absorbed with human RBCs, lyophilized human liver, lyophilized normal human serum, and peripheral blood lymphocytes from patients with chronic lymphocytic leukemia (CLL). Specificity of the antiserum for human T lymphocytes was tested by indirect immunofluorescence. It stained 70 to 80% of lymphocytes in circulation, 95% of thymus, 27 to 35% of spleen, 5 to 10% of tonsil lymphocytes, and over 90% of phytohemagglutinin-stimulated lymphocytes *in vitro*. Only T-dependent areas of cryostat-sectioned human lymph nodes stained with the antiserum. It did not stain circulating lymphocytes which formed HEAC rosettes, plasma cells in marrows of multiple myeloma patients or macrophages. After removal of HEAC rosettes by centrifugation in Ficoll-Hypaque, 75% of interface cells formed E rosettes and 65 to 75% stained with the antiserum. The antiserum was used in studies of lymphocytes in chronic and acute lymphocytic leukemias, lymphomas, and other lymphoproliferative diseases. Numbers and distribution in the circulation, spleen, and nodes of lymphocytes bearing the T marker were significantly altered in patients with these disorders. (Am J Pathol 86:1–16, 1977)

THE T-LYMPHOCYTE POPULATION is functionally heterogenous, composed of antigen-reactive cells,[1,2] T-helper cells,[3,4] T-effector or killer cells,[5,6] and T-suppressor cells.[7-9] These different populations of T cells may express surface markers that vary in quality and/or quantity from one subpopulation to another.[10,11] The expression of surface markers may be affected by the degree of activation, stage of differentiation, or mitotic activity of lymphocytes.[12,13] It is reasonable to expect that disease processes, particularly those involving the lymphorecticular system, may either affect or alter the expression of such surface markers.

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Presented in part at the Seventy-third Annual Meeting of the American Association of Pathologists and Bacteriologist, Boston, Mass., March 22, 1976.

Accepted for publication August 24, 1976.

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Two markers commonly used for identifying circulating T cells are their ability to form spontaneous rosettes with SRBCs [14,15] and their reactivity with anti-T cell serum.[16] The T-cell populations identified by these two assays have been reported to either overlap [16,17] or not to overlap.[18] This suggests that some circulating T cells bear only one kind of T receptor. Experiments of Williams *et al.*[19] and Bentwich *et al.*[20] suggested that thymocytes possess two distinct T markers, one of which is expressed on CLL lymphocytes. If human T cells indeed express distinct T antigens at each stage of development, differentiation, or dedifferentiation, then the ability to identify these antigens on lymphoid cells is of importance in clinical medicine.

This communication describes the preparation and properties of an anti-human T-cell serum and its use in studying changes in distribution and numbers of T lymphocytes in circulation and lymphoid organs in patients with various lymphoproliferative diseases.

Materials and Methods

Immunization

White albino rabbits weighing 5 to 6 lbs were injected subcutaneously into multiple sites at weekly intervals. Normal human cerebrum was obtained at autopsy, homogenized in a Sorvall Omni-mixer [2 mg/ml phosphate-buffered saline (PBS), pH 7.4] dispensed into tubes and stored frozen at −70 C. Tubes were thawed when needed, and their contents emulsified with an equal volume of complete (4 mg *Mycobacterium tuberculosis* H37 RA/ml) (CFA) or incomplete (IFA) Freund's adjuvant (Difco). Each rabbit received 300 mg (wet weight) of brain per injection. Rabbits immunized with brain and IFA received a total of seven injections. Most rabbits were test bled from the ear after four injections and bled by cardiac puncture after immunization was terminated. Rabbits injected with brain and CFA all developed acute experimental encephalomyelitis and some had to be exsanguinated after the third injection. Sera were heat inactivated at 56 C for 30 minutes and stored at −20 C.

Preparation of Lymphoid Cell Suspensions

Human peripheral blood lymphocytes (PBL) were isolated on Ficoll-Hypaque as detailed elsewhere.[21] Thymocytes were obtained from fresh thymus glands surgically removed from young children during open-heart surgery. Thymic tissue was pressed through a fine stainless steel mesh into a small volume of PBS, pH 7.2, containing 0.1% disodium salt of ethylenediamine tetracetate (EDTA-Na_2). The suspension of thymocytes was centrifuged at 250*g* briefly to remove tissue clumps, and its concentration was adjusted as needed.

Lymph nodes and spleens from patients with leukemias or lymphomas were processed immediately after surgical removal by pressing the chopped lymphoid tissue through a mesh screen and Ficoll-Hypaque centrifugation of the resulting lymphocyte suspensions.

Viability of lymphocytes in suspensions was determined by trypan blue dye exclusion and was generally $\geq$ 90%.

Immunofluorescent Staining

Aggregate-free monospecific fluorescein-labeled antihuman IgG, IgA, IgM, κ, and λ antisera (Meloy Laboratories, Springfield, Va.) were used to detect surface immunoglobu-

lins on lymphocytes. The antisera were tested for monospecificity as described elsewhere.[22] The direct method used for staining of lymphocytes was detailed elsewhere.[21]

The indirect method was employed to detect the presence of T antigen. Lymphocytes in suspension (2×10^6 cells/ml) were incubated with appropriately diluted (usually 1:16, depending on the batch of antiserum), anti-human brain serum for 30 minutes at 4 C. The cells were washed twice with PBS and then reacted with fluorescein-conjugated goat antirabbit immunoglobulin serum (7S, Meloy Laboratories) for 30 minutes at room temperature. As control, lymphocytes were reacted directly with labeled goat antirabbit globulin without the antibrain sera. To test for nonspecific staining, controls with fluorescein-labeled human albumin were run simultaneously with specimens. Controls were always negative.

Cryostat sections of lymphoid tissues (4 μ thick) were fixed in acetone for 2 minutes, air dried, washed in PBS, and reacted with fluorescein-labeled antiimmunoglobulin sera or with antibrain serum followed by labeled goat antirabbit globulin. All sections were stained for 30 minutes at room temperature and washed three times with PBS, each wash lasting 10 minutes. The sections were mounted in PBS-glycerol and examined in a Zeiss Photoscope II.

Rosette Formation

T rosettes with sheep red blood cells (SRBC) were formed by the method of Jondal, Holm, and Wigzell [14] modified as follows. Washed lymphocytes were resuspended in GVB buffer (Cordis Laboratories) to a final concentration of 4×10^6 cells/ml. Equal volumes (0.25 ml) of the lymphocyte suspension and of 1% (v/v) suspension of SRBC in PBS were combined in a small tube. The mixture was centrifuged at 200*g* for 5 minutes and then placed at 4 C overnight. Next morning, the pellet was resuspended by gentle tapping of each tube, and the percentage of rosette-forming cells (lymphocytes with three or more SRBC) was determined in a hemacytometer by counting 200 or more lymphocytes in several fields.

B rosettes were determined by a modified technique of Mendes *et al.*[23] Human red blood cells (type A, Rh negative) were coated with heat-inactivated anti-A serum at a subagglutinating dilution. Anti-A serum was made in rabbits by a single subcutaneous injection of human A-type red blood cells in complete Freund's adjuvant followed by bleeding 6 or 7 days later. Serum-coated erythrocytes were then incubated with 0.2 ml of mouse serum as a source of complement. The indicator cells (0.3 ml) were added to an equal volume of the lymphocyte suspension at the concentration of 2×10^6 cells/ml in GVB buffer. The mixture was centrifuged at 200*g* for 5 minutes, the pellet resuspended after addition of a drop of toluidine blue (0.2% in PBS), and the number of rosettes determined by counting at least 200 lymphocytes in consecutive fields.

In some experiments both immunofluorescence and rosetting were performed with the same cell preparation, lymphocytes were stained as described above, washed gently, resuspended at the cell concentration suitable for rosette formation, and reacted with either SRBC or C'-coated indicator cells. Duplicate tubes were set up, one for counting rosettes in a hemocytometer, the other for examining rosettes in the fluorescent microscope.

Rosette Inhibition

Lymphocytes were resuspended in PBS at the concentration of 5×10^6 cells/ml. An aliquot of this suspension was mixed with an equal volume of undiluted antibrain serum and incubated for 30 minutes at room temperature. Normal rabbit serum was used as control. After incubation, lymphocytes were centrifuged at 500*g*, washed twice with PBS, and allowed to form rosettes with SRBC as described. An untreated lymphocyte control was always included.

Cytotoxicity Testing

Peripheral blood lymphocytes, thymocytes, or lymphocytes isolated from spleen and/or lymph nodes were suspended in barbital buffer and a two-step microcytotoxicity assay was performed as described by Amos.[24]

Absorptions of Antisera

Rabbit antisera reactive with PBL in the immunofluorescence and cytotoxic assays were absorbed twice with human RBC type AB, Rh+. Equal volumes of antisera and washed packed RBCs were combined, agitated gently, and incubated at room temperature for 30 minutes. Antisera were then absorbed twice with homogenized and lyophilized normal human liver at the concentration of 50 mg/ml for 1 hour at 4 C and with lyophilized normal human serum (40 mg/ml). To amplify the specificity of the antiserum for T cells, absorptions with lymphocytes from two different patients with chronic lymphocytic leukemia followed. Approximately 2×10^7 lymphocytes from each patient were used, and the absorptions were judged complete if the antisera gave no positive reactions with absorbing CLL lymphocytes in indirect immunofluorescence or cytotoxicity assays. Following each absorption, the antisera were centrifuged at 200,000*g* for 1 hour in the Beckman ultracentrifuge model L2 (rotor 50 Ti). Centrifuged antisera were filtered through a 0.22 nm Millipore filter (Millipore Corp., Bedford, Mass.) and stored frozen in small aliquots.

Results

Properties of Anti-Human Brain Serum

Anti-human brain serum raised in rabbits and absorbed as described in Materials and Methods was tested against human lymphocytes by three different procedures: immunofluorescence, cytotoxicity, and T-rosette inhibition. The potency and ability of the antisera to stain, lyse, or inhibit T rosettes depended on the length of immunization and type of adjuvant used. Rabbits immunized with brain emulsified in complete Freund's ajuvant all developed acute experimental encephalomyelitis and had to be bled out, usually after the third injection. For that reason, antiserum obtained had a low titer and was reactive only in immunofluorescence (at 1:2 dilution after absorption). With the use of IFA, antibrain serum with higher titers was obtained following seven subcutaneous injections into multiple sites (Table 1).

Some of the properties of anti-human brain serum made with IFA are listed in Table 2. A partial inhibition of spontaneous rosettes was observed when lymphocytes or thymocytes were preincubated with the undiluted antiserum. This partial inhibition indicates that the receptors for sheep erythrocyte rosettes are distinct from the receptors defined by the anti-T cell serum. Thus, the observed partial inhibition may have resulted from the steric hindrance by the antibody rather than direct blocking of the rosette receptor site.

The anti-T cell serum lysed PBL and thymocytes in the presence of rabbit serum as a source of complement. The preferential *in vitro* cyto-

Table 1—Properties of Absorbed Anti-Human Brain Serum Made in Rabbits and Tested on Circulating Human Lymphocytes and Thymocytes

	Immunofluorescence titer*	Cytotoxicity titer†	Percent T rosette inhibition
Brain + CFA			
PBL	2	0	0
Thymocytes	2	0	0
Brain + IFA			
PBL	32–64	128–256	50–60
Thymocytes	128	≥512	40

* Immunofluorescence titer is defined as the reciprocal of the highest dilution at which 70 to 75% of peripheral blood lymphocytes and ≥95% of thymocytes were stained.
† Cytotoxicity titer is defined as the reciprocal of the highest dilution at which 50% of cells were lysed in presence of rabbit complement.

xicity toward thymocytes is suggestive of the presence of increased quantities of the T antigen on thymocytes as compared to circulating T cells.

Specificity of the Antiserum for T Cells

Absorbed anti-human brain serum was tested for its specificity to T lymphocytes by immunofluorescence. Freshly prepared cell suspensions from human blood, thymus, spleen, and tonsils were stained with the antiserum previously titrated to a plateau with PBLs (Table 3). In addition, PBLs cultured in the presence of various mitogens were stained. Lymphocytes stimulated with PHA and con A, the putative T-cell mitogens, all stained with the anti-T cell serum,[22] while lymphocytes transformed with PWM did not. As the antiserum was absorbed with human RBCs and CLL cells, it did not stain these cells in suspension. The antiserum failed to stain plasma cells in bone marrow preparations of patients with multiple myeloma. Macrophages, defined as those cells in normal human circulation that stick to glass and/or plastic, were negative.

To exclude the possibility that the antiserum reacts with B lymphocytes, complement-receptor lymphocytes (CRLs), which can be identified by their ability to form HEAC rosettes, were labeled with fluores-

Table 2—Properties of the Absorbed Anti-T Cell Serum

Partially inhibits spontaneous rosettes formed by PBLs and thymocytes (50% inhibition)
In presence of rabbit C′ is cytotoxic to PBL at 1:128 dilution and thymocytes at 1:512 dilution
PBL forming HEAC mouse rosettes (CRLs) were stained by FITC-polyvalent anti-Ig serum but *not* by anti-T cell serum
Cells left at the interface after Ficoll-Hypaque centrifugation of HEAC mouse rosettes formed T rosettes (75%) and stained with anti-T cell serum (65 to 75%)

Table 3—Staining Properties of the Absorbed Anti-T Cell Serum (Indirect Immunofluorescence)

Stained:	
	70-80% of lymphocytes in circulation of normals (30)
	>95% of thymocytes in suspensions of thymus (8)
	27-35% of lymphocytes in suspensions of spleen (4)
	5-10% of lymphocytes in suspensions of tonsils (3)
	>95% of PBL stimulated *in vitro* with PHA (12)
Did *not* stain:	
	CLL lymphocytes
	Human RBCs
	Plasma cells in marrows of MM patients
	Macrophages (glass- or plastic-adherent cells)
Stained *only* T-dependent areas of cryostat sectioned normal lymph nodes	

Numbers of specimens tested are given in parentheses.

cein-conjugated polyvalent anti-Ig serum or with the anti-T-cell serum as described in Materials and Methods. Lymphocytes forming HEAC rosettes stained only with the polyvalent anti-Ig serum. Further, when HEAC rosettes were removed by Ficoll-Hypaque centrifugation, the majority of lymphocytes left at the interface (presumably T cells) simultaneously stained with the anti-T cell serum and formed T rosettes.

The distribution of cells stained with the anti-T-cell serum in a section of normal human lymph node is shown in Figure 1. As can be seen, only T-dependent paracortical regions of the node are stained, while follicular areas are negative. Figure 2 shows a section through human thymus with intensely and uniformly stained thymocytes. Finally, Figure 3 is an example of the kind of surface staining that is obtained if PBLs are stained with the anti-T cell serum. It is our impression that two distinct subsets of positive cells can be detected with the antiserum: strongly stained cells (about one fourth or one third of all positive) and weakly stained cells. Similar observation was reported by Brown and Greaves.[16]

These data point to a high degree of specificity of the antiserum for T lymphocytes.

Distribution of T Cells in Lymphoproliferative Disorders

The anti-T cell serum was used for studying the distribution of T cells in the circulation and lymph nodes of patients with various lymphoproliferative diseases. The T cells were identified by spontaneous rosette formation in suspensions of PBLs and/or lymph nodes and by positive immunofluorescence with the anti-T cell serum. Table 4 summarizes our data on the T cell distribution in various blood dyscrasias.

Patients with acute lymphocytic leukemia (ALL) generally fall into one of several categories: those with neither marker detectable, those with one

Table 4—Distribution of T Cells in Patients With Blood Dyscrasias

Disorder	In circulation		In lymph nodes	
	T marker	T rosettes	T marker	T rosettes
Acute lymphocytic leukemia (6)	0 or N	0 or N	ND	ND
Chronic lymphocytic leukemia (30)	↑	↑	Large masses	ND
Hairy cell leukemia (7)	↑	⇊	Diffused	↓
Sézary's syndrome (2)	⇈	⇈	ND	ND

N = normal, ND = not determined. The number of patients studied is given in parentheses.

marker at a normal proportion and the other missing, and those with decreased proportions of either one or both markers.

Chronic lymphocytic leukemia (CLL) patients were studied with the anti-T cell serum which was *not* absorbed with CLL cells. These patients had increased numbers of lymphocytes bearing the T marker: 60 to 90% of circulating cells stained with the antiserum. At the same time, the majority of these cells stained with labeled anti-Ig sera, an indication that leukemic lymphocytes, unlike the normal B cells, share antigenic markers with the T lymphocytes.[25] The total number of cells rosetting with SRBCs was increased in all CLL patients.[25] In addition, distribution of the T cells in their lymph nodes was altered, with large compact masses of T-positive lymphocytes infiltrating the node. Figure 4 illustrates such a mass in a node obtained from a CLL patient.

Hairy cell leukemia (HCL) was characterized by a marked depletion of the T-rosetting subpopulation in blood as well as in lymph nodes and a significant increase in the T-marker-bearing circulating lymphocytes. Controls with trypsin-treated cells as well as staining of fixed cell preparations confirmed that the T marker was synthesized by the cells and was not absorbed to the "hairy" cell surfaces from the serum. In lymph nodes, T-marker-bearing cells were densely scattered throughout, so that the normal organization into T- and B-dependent areas was obliterated by diffusing T-positive cells.

In patients with Sézary's syndrome, both T-marker and T-rosetting lymphocyte subpopulations in peripheral blood were characteristically elevated. For example, in 1 patient, over 85% of PBLs formed T rosettes and over 95% stained with the anti-T cell serum.

Patients with lymphomas were arbitrarily divided into four groups (Table 5). Only small numbers of patients were investigated so far. The numbers and distribution of T lymphocytes in nodes and circulation of patients with Hodgkin's varied with the stage of disease, the later stages

Table 5—Distribution of T Cells in Lymphoproliferative Disorders

	In circulation		In lymph nodes	
Lymphomas	T marker	T rosettes	T marker	T rosettes
Hodgkin's disease	↓ or N	↓	Depleted or diffused	↓ or ↑
Nodular	↓ or 0	↓	Small islands	↓
Diffuse	N to ↓	⇊	Small islands or diffused	↓
Histiocytic	N to ↓	N to ↓	0 to ⇊	↓

N = normal.

being characterized by decreased numbers of T-marker and T-rosetting–bearing lymphocytes in both circulation and lymph nodes. In some Hodgkin's nodes the T-marker lymphocytes were depleted, while those forming T rosettes were elevated. This lack of correlation between T-marker and T-rosetting populations was also seen in peripheral circulation of some Hodgkin's patients.

In non-Hodgkin's malignant lymphomas, either nodular or diffuse, circulating lymphocyte pool was generally depleted in both kinds of T subpopulations. The most pronounced changes were seen in the lymph nodes. Small nodule-like deposits of cells positive for T marker were scattered throughout the node of a patient with nodular lymphoma (see Figure 5B). These foci of T cells were much smaller than the nodules of abnormal lymphocytes identified in paraffin-embedded sections. In fact, a nodule, such as that seen in Figure 5B, contained several foci of T-positive cells.

In lymph nodes from patients diagnosed to have diffuse malignant lymphomas, two types of T-cell topography were seen. Either the T-positive cells were limited to randomly scattered small islands of strongly fluorescing lymphocytes or they were seen infiltrating the entire node, a more common picture in our experience (Figure 6).

Discussion

Attempts to prepare anti-T cell sera have, by and large, been limited to thymocytes as a source of human T antigen. Ablin and co-workers [26] prepared anti–human thymocyte serum which reacted in immunodiffusion and immunofluorescence with an antigen present only on fetal thymus cells but absent from PBL. Smith *et al.*,[27] using thymus cells

obtained from fetuses and small infants, prepared a rabbit antiserum which caused selective lysis of thymocytes and other cells of probable thymic origin. The cells sensitive to this antiserum all expressed an antigenic marker tentatively designated as the human thymic lymphocyte antigen (HTLA) by the authors. Several other investigators raised anti-thymocyte sera in various animals, including primates,[19] and each of the resultant anti-T cell sera had a somewhat different reactivity in terms of cytotoxicity, inhibition of spontaneous T rosettes, and effects on responses to mitogens and to allogeneic stimulation.[28,29] These studies indicate that such reactivity of antithymocyte sera is a reflection of heterogeneity within the T-cell population.[28] The situation in humans may be roughly equivalent to that in the mouse where antisera directed against thymocytes detect the antigen, and antisera to leukemia antigen and to lymphocyte-defined antigen identify various subpopulations among T cells.[30,31]

Several years ago, T cell–specific antisera were first produced by heteroimmunizations with mouse brain.[32] There is a reason to believe that the same approach may be used for preparation of anti–human T cell sera. Takada *et al.*[33] have recently reported that human brain has antigenic determinants identical or very similar to those found on the thymus-derived Molt cell line. Brown and Greaves [16] used anti–human brain serum, made T-cell specific by appropriate absorptions, to label the cell population that formed spontaneous rosettes with sheep erythrocytes. We have prepared antiserum reacting with human thymocytes and T lymphocytes, but not B cells, by injecting rabbits with human cerebrum emulsified in incomplete Fruend's adjuvant. Many investigators failed to produce a specific anti–T cell serum by heteroimmunization with human brain (e.g., the work of Schlesinger and Galili [34] and that of Thiele *et al.*[35]). It could be that this failure is due to differences in brain preparations and/or different immunization schedules. The use of complete and incomplete Freund's adjuvant for immunization of our animals and differences in reactivities of the resulting sera illustrates the difficulty. The distribution and concentration of T–cell antigens in brain may vary between individuals. In addition, some rabbits have been known to respond well and others poorly in terms of antibody synthesis to human brain antigens.[36]

In order to use the antiserum for tagging of T cells in disease states, it is imperative to ascertain its specificity. The prepared antiserum was extensively absorbed to remove nonspecific antibodies directed to human species antigens, immunoglobulins, and other lymphocyte antigens. Further, the specificity of the antiserum for T cells was checked by several criteria as detailed in Tables 2 and 3. Unfortunately, working with human cells,

we were restricted by the availability of pure T-cell populations that can be used for testing. Thymocytes represent the best source of human T cells. However, recent evidence pointing to a high degree of heterogeneity among T cells suggests that thymocytes constitute but one subpopulation of T cells. Thus, in evaluating specificity, it appears more important to show that B cells (CRLs and SIg-bearing lymphocytes) do not react with the antiserum than to demonstrate positive reactivity with all T cells. However, it must be remembered that mitogen-stimulated [22] and antigen-activated [37] T cells may express immunoglobulins on their surfaces.

It has been emphasized that the tests for specificity should demonstrate reciprocity and coincidence with other membrane markers such as receptors for complement and for SRBC.[38] While the antiserum we prepared did not stain normal peripheral lymphocytes forming HEAC rosettes, the overlap between cells rosetting with SRBC and T marker-positive cells was not complete. This indicated that more T cells in circulation carried the T marker than receptors for SRBC. In contrast to the small discrepancy between the two T-cell markers in the normal peripheral circulation, striking differences in their distribution were observed in the circulation and lymph nodes of patients with various lymphoproliferative disorders (Tables 4 and 5).

Little is known about the qualitative and quantitative distribution and about functional significance of the different T antigens in humans. Several lines of evidence indicate, for example, that thymocytes and PBL have distinct T determinants in addition to the ones they share.[19] Using the antiserum to human thymic lymphocyte antigens, Woody *et al.*[28] demonstrated that con A–responsive PBL, the responder cells in MLC, and killer T cells react with the antiserum, while the peripheral lymphocytes responding to PHA and pokeweed mitogen and those producing MIF and TF are not affected in their functions by treatments with the antiserum. Our results indicate that different T-cell subpopulations may predominate in the circulation and lymphoid organs in the course of a lymphoproliferative disease. It is likely that these changes may reflect altered functional capabilities of the infiltrating cells. In order to define how these changes affect the course and progression of disease, we are beginning to correlate clinical manifestations with the distribution of T cells in the circulation and lymphoid organs of patients with lymphoproliferative diseases.

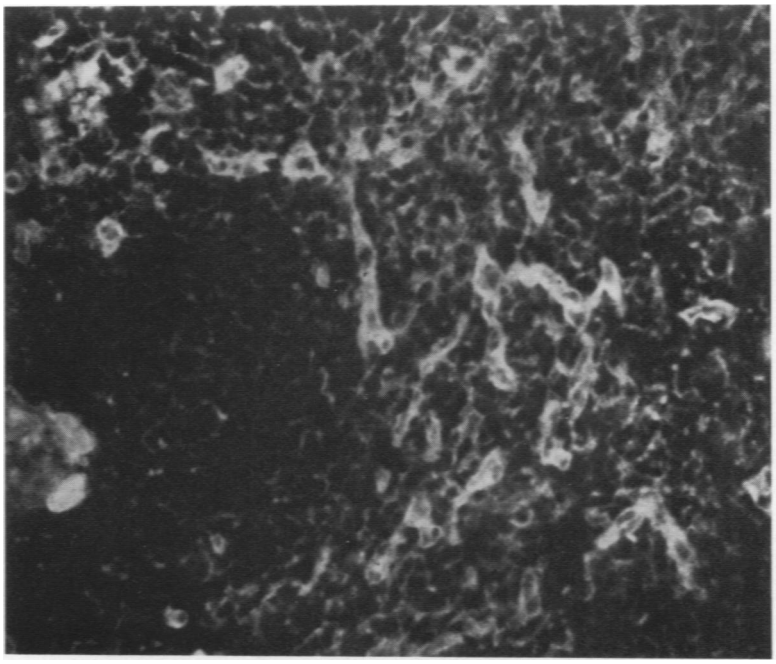
References

1. Cone RE, Spent J, Marchalonis JJ: Antigen-binding specificity of isolated cell-

surface immunoglobulin from thymus cells activated to histocompatibility antigens. Proc Natl Acad Sci USA 69:2556–2560, 1972

2. Roelants GE, Ryden A, Hagg LB, Loor F: Active synthesis of immunoglobulin receptors for antigen by T lymphocytes. Nature [Lond] 247:106–108, 1974
3. Mitchison NA, Taylor R, Rajewsky K: Co-operation of antigenic determinants in the induction of antibodies. Developmental Aspects of Antibody Formation and Structure. Edited by J Sterzl. Prague, Academic, 1970, p 547
4. Kontiainen S, Andersson LC: Antigen-binding T cells as helper cells: Separation of helper cells by immune rosette formation. J Exp Med 142:1035–1039, 1975
5. Cerottini J-C, Nordin AA, Brunner KT: Cellular and humoral response to transplantation antigens. I. Development of allo-antibody-forming cells and cytotoxic lymphocytes in the graft-versus-host reaction. J Exp Med 134:553–564, 1971
6. Goldstin, P, Wigzell H, Blomgren H, Svedmyr EAJ: Autonomy of thymus-processed lymphocytes (T cells) for their education into cytotoxic cells. Eur J Immunol 2:498–501, 1972
7. Gershon RK: T cell control of antibody production. Contemp Top Immunobiol 3:1–40, 1974
8. Rich RR, Pierce CW: Biological expressions of lymphocyte activation. II. Generation of a population of thymus-derived suppressor lymphocytes. J Exp Med 137:649–659, 1973
9. Dutton RW: Inhibitory and stimulating effects of concanavalin A on the response of mouse spleen cell suspensions to antigen. II. Evidence for separate stimulatory and inhibitory cells. J Exp Med 138:1496–1505, 1973
10. Fathman CG, Small M, Herzenberg LA, Weissman IL: Thymus cell maturation. II. Differentiation of three "mature" subclasses *in vivo*. Cell Immunol 15:109–128, 1975
11. Cantor, H. Simpson E, Sato VL, Fathman CG, Herzenberg LA: Characterization of subpopulations of T lymphocytes. I. Separation and functional studies of peripheral T cells binding different amounts of fluorescent anti-Thy 1.2 (theta) antibody using a fluorescence activated cell sorter (FACS). Cell Immunol 15:180–196, 1975
12. Stobo JD: Phytohemagglutinin and concanavalin A: Probes for murine "T" cell activation and differentiation. Transplant Rev 11:60–86, 1972
13. Stout RD, Herzenberg LA: The Fc receptor on thymus-derived lymphocytes. II. Mitogen responsiveness of T lymphocytes bearing the Fc receptor. J Exp Med 142:1041–1051, 1975
14. Jondal M, Holm G, Wigzell, H: Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J Exp Med 136:207–215, 1972
15. Wybran J, Carr MC, Fudenberg HH: The human rosette-forming cells as a marker of a population of thymus-derived cells. J Clin Invest 51:2537–2543, 1972
16. Brown G, Greaves MF: Cell surface markers for human T and B lymphocytes. Eur J Immunol 4:302–310, 1974
17. Owen FL, Fanger MW: Studies on the human T-lymphocyte population. II. The use of T-cell specific antibody in the partial isolation and characterization of the human lymphocyte receptor for sheep red blood cells. J Immunol 113:1138–1144, 1974
18. Luecker RD, Abdin ZH, Williams RC Jr: Peripheral blood T and B lymphocytes during acute rheumatic fever. J Clin Invest 55:975–985, 1975
19. Williams, RC Jr, DeBoard JR, Mellbye OJ, Messner RP, Lindstrom FD: Studies of T- and B-lymphocytes in patients with connective tissue diseases. J Clin Invest 52:283–295, 1973
20. Bentwich Z, Douglas SD, Siegal FP, Kunkel HG: Human lymphocyte sheep

erythrocyte rosette formation: Some characteristics of the interaction. Clin Immunol Immunopathol 1:511–522, 1973
21. Whiteside TL, Berardi RS, Rabin BS: Quantitation of human peripheral blood T and B lymphocytes. Int Arch Allergy Immunol 48:731–738, 1975
22. Whiteside TL, Rabin BS: Surface immunoglobulin on activated human peripheral blood thymus-derived cells. J Clin Invest 57:762–771, 1976
23. Mendes NF, Tolnai MEA, Silveira NPA, Gilbertsen RB, Metzgar, RS: Technical aspects of the rosette tests used to detect human complement receptor (B) and sheep erythrocyte-binding (T) lymphocytes. J Immunol 111:860–867, 1973
24. Amos B: HLA typing-cytotoxicity test. Manual of Tissue Typing Techniques. Edited by JG Ray, DR Harse, RP Pedersen, DE Kayhoe. Publication No. (NIH) 75-545. Washington, DC, US Department of Health, Education, and Welfare. 1974, pp 23–26
25. Whiteside TL, Winkelstein A, Rabin BS: Immunologic characterization of chronic lymphocytic leukemia cells. Cancer, 1976 (In press)
26. Ablin RJ, Morris AJ: Immunofluorescent identification of a human thymic specific antigen. Blut 29:32–36, 1974
27. Smith RW, Terry WD, Buell DN, Sell KW: An antigenic marker for human thymic lymphocytes. J Immunol 110:884–887, 1973
28. Woody, JN, Ahmed A, Knudsen RC, Strong DM, Sell KW: Human T-cell heterogeneity as delineated with a specific human thymus lymphocyte antiserum: In vitro effects on mitogen response, mixed lymphocyte culture, cell-mediated lymphocytotoxicity and lymphokine production. J Clin Invest 55:956–966, 1975
29. Rodt H, Thierfelder S, Thiel E, Götze D, Netzel B, Huhn D, Eulitz M: Identification and quantitation of human T-cell antigen by antisera purified from antibodies cross-reacting with hemopoietic progenitors and other blood cells. Immunogenetics 2:411–430, 1975
30. Reif AE, Allen JMV: The AKR thymic antigen and its distribution in leukemias and nervous tissues. J Exp Med 120:413–433, 1964
31. Leckband E, Boyse EA: Immunocompetent cells among mouse thymocytes: A minor population. Science 172:1258–1260, 1971
32. Golub ES: Brain-associated theta antigen: Reactivity of rabbit anti-mouse brain with mouse lymphoid cells. Cell Immunol 2:353–361, 1971
33. Takada A, Takada Y, Ito U, Minowada J: Shared antigenic determinants between human brain and human T-cell line. Clin Exp Immunol 18:491–498, 1974
34. Schlesinger M, Galili U: Antigenic differences between T and B lymphocytes in man. Isr J Med Sci 10:715–724, 1974
35. Thiele HG, Stark R, Keeser D, Zimpel H: Lack of antigenic correlations between brain and thymus. Lancet 2:1447, 1973
36. Kongshavn PAL, Gold P, Shuster J, Colquhoun B, Freedman SO: Ability of anti-brain heteroantisera to distinguish thymus-derived lymphocytes in various species. Clin Immunol Immunopathol 3:1–15, 1974
37. Yoshida TO, Andersson B: Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. Scand J Immunol 1:401–408, 1972
38. Aiuti F, Cerrotini J-C, Coombs RRA, Cooper M, Dickler HB, Froland SS, Fudenberg HH, Greaves MF, Grey HM, Kunkel HG, Natvig JB, Preud'homme J-L, Rabellino E, Ritts RE, Rowe DS, Seligmann M, Siegal FP, Stjernsward J, Terry WD, Wybran J: Special technical report: Identification, enumeration and isolation of B and T lymphocytes from human peripheral blood. Scand J Immunol 3:521–532, 1974
39. Baxley G, Bishop GB, Cooper AG, Wortis HH: Rosetting of human red blood cells to thymocytes and thymus-derived cells. Clin Exp Immunol 15:385–392, 1973



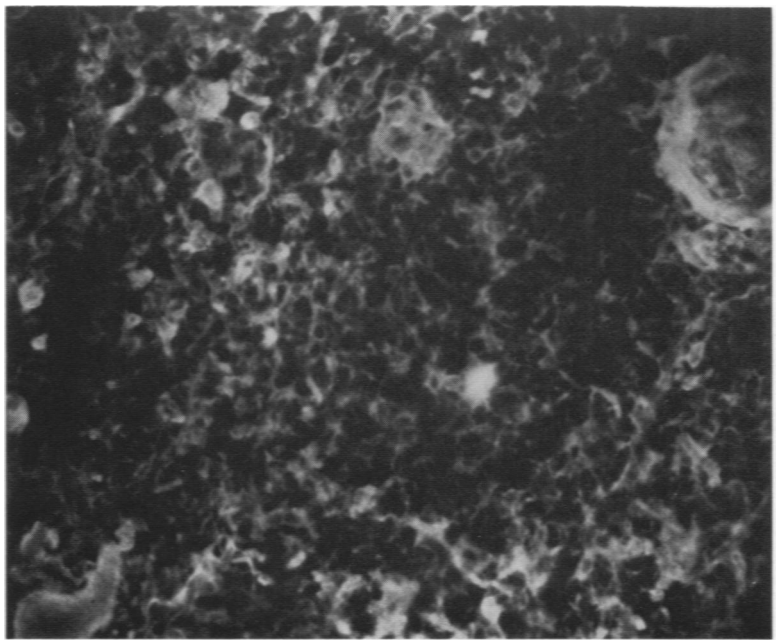
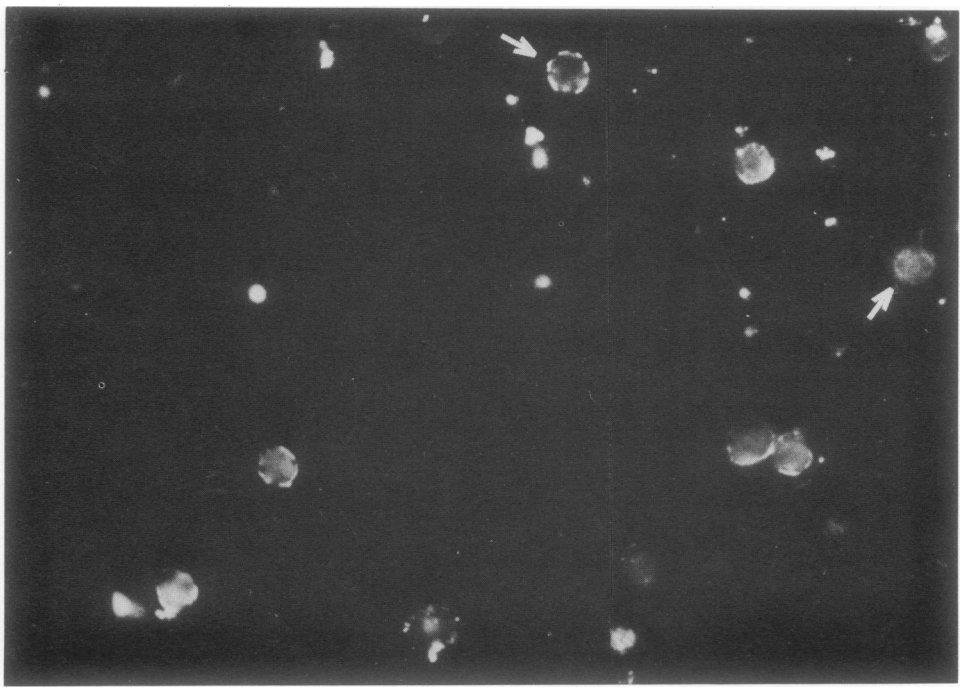


Figure 1—A cryostat section of a normal human lymph node stained with the anti-human brain serum. The follicular area is negative, while the T-dependent paracrotical region is stained. (Indirect immunofluorescence, × 400) **Figure 2**—A cryostat section of a normal human thymus stained with the anti-human brain serum (Indirect immunofluorescence, × 400).



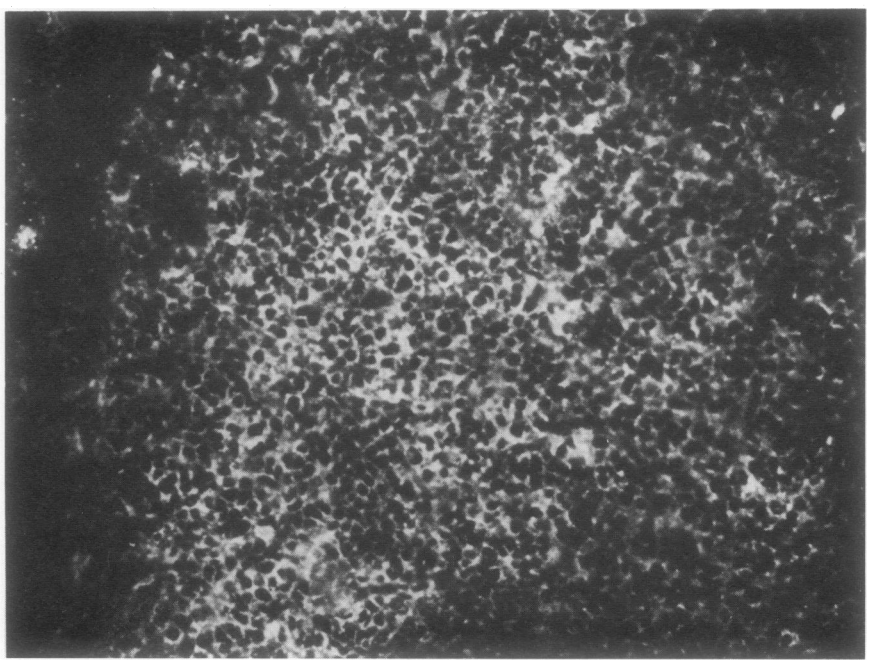


Figure 3—Peripheral blood lymphocytes stained with the anti-human brain serum. Note that some cells are stained more strongly than others (*white arrows*). (Indirect immunofluorescence, × 400) **Figure 4**—A cryostat section of a lymph node from a patient with chronic lymphocytic leukemia. The node is infiltrated by masses of anti-human brain-positive cells. (Indirect immunofluorescence, × 200)

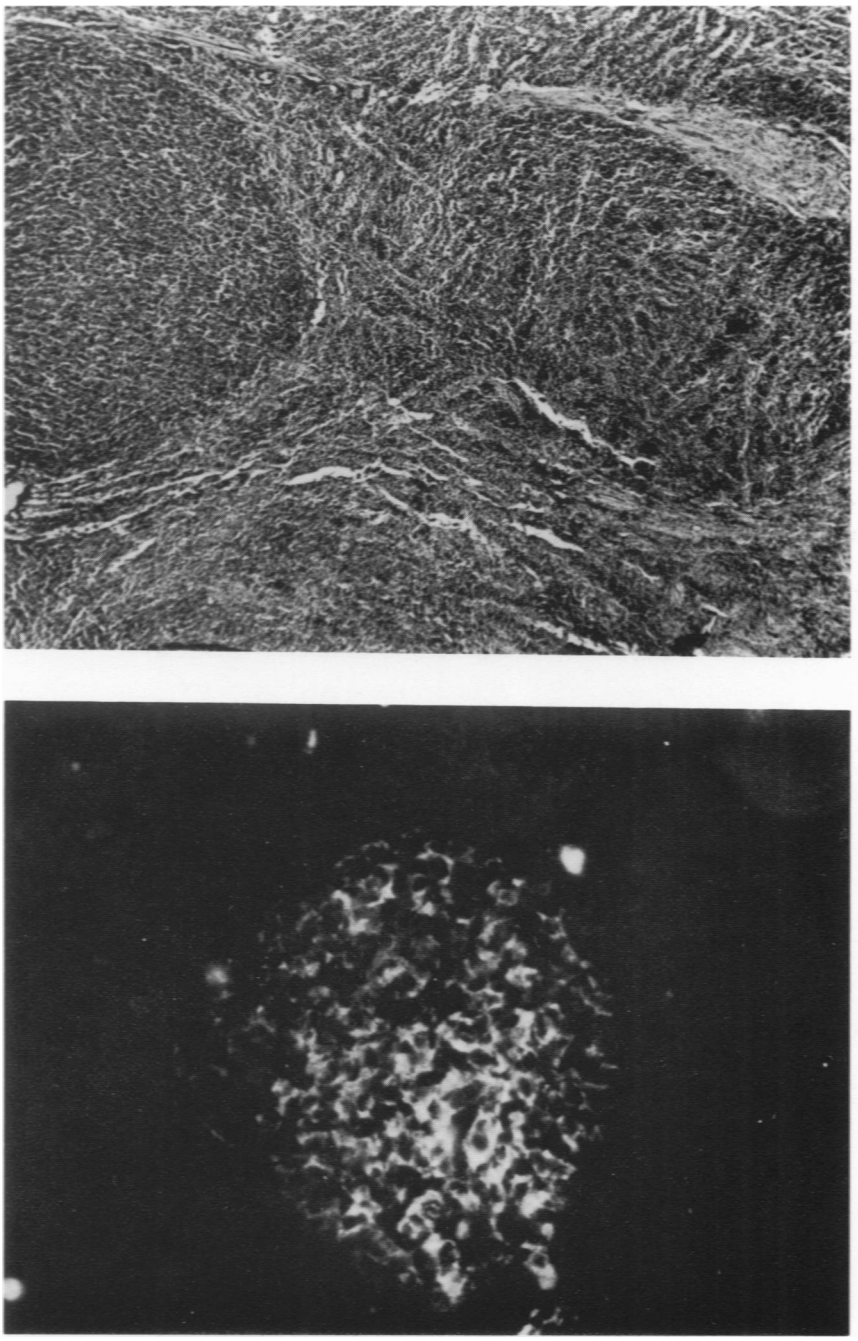


Figure 5—Paraffin (**A**) and cryostat (**B**) sections of a lymph node from a patient with nodular lymphoma. The nodules of abnormal lymphocytes seen in the paraffin section (**A**) contain scattered focal deposits of T-positive cells such as the one in the cryostat section (**B**). (**A**, light microscopy, × 50; **B**, indirect immunofluorescence, × 200)

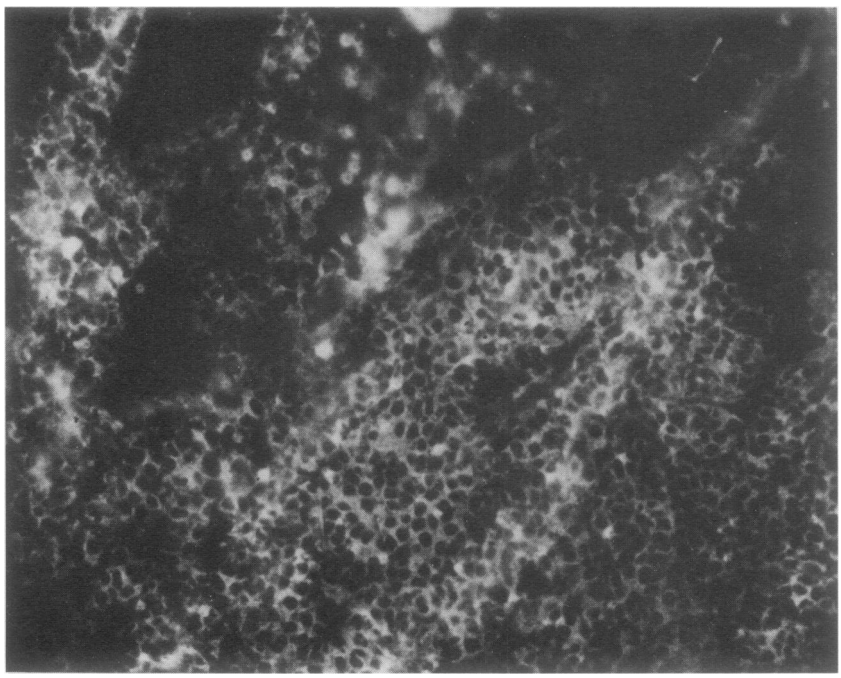


Figure 6—A cryostat section of a submandibular lymph node from a patient with diffuse malignant lymphoma stained with the anti-human brain serum. The node is infiltrated by the T-positive cells. (Indirect immunofluorescence, × 200)