T CELL ANTIIDIOTYPIC ANTIBODIES REVEAL DIFFERENCES BETWEEN TWO HUMAN LEUKEMIAS

BY D. N. POSNETT, R. D. BIGLER, Y. BUSHKIN*, D. E. FISHER, C. Y. WANG*, L. F. MAYER, N. CHIORAZZI, AND H. G. KUNKEL

From The Rockefeller University and *Memorial Sloan-Kettering Cancer Center, New York, New York 10021

A T cell membrane antigen-recognition structure has recently been described in several laboratories, including our own (1–6). This molecule is a heterodimer of approximately 80–90 Kd, composed of two disulfide-linked chains. It fulfills many of the requirements for the T cell receptor for antigen, such as major histocompatibility complex-restricted antigen recognition (2–4). This molecule has been studied on murine cells (1, 2, 4), human T cell clones (3), and human T cell leukemia cells (5).

The latter study, from this laboratory, described monoclonal antibodies reactive with idiotype-like determinants on a human T cell leukemia (5). Of two antibodies, one reacted with a private idiotype-like determinant and another cross-reacted with 1–2% of normal T cells by immunofluorescence. Both antibodies immunoprecipitated the same ~80 Kd disulfide-linked heterodimer from the membrane-iodinated leukemic cells, and this molecule comodulated with the T3 molecule. Thus, strong indirect evidence suggested that these monoclonal antibodies were recognizing a T cell antigen receptor. The presence of private idiotypic determinants and shared determinants on this molecule suggested a general structure similar to that of immunoglobulin molecules (7).

This paper compares two T cell antiidiotypic antibodies each specifically reactive with their respective leukemia T cell clone. Immunoprecipitation with these antibodies revealed major differences between the two idiotype-bearing heterodimer molecules. In addition, proliferation studies in response to Sepharose-linked antiidiotypic antibody revealed marked differences between the two T cell leukemias.

Materials and Methods

**Cell Preparations.** Cells derived from the peripheral blood of a patient with Sezary syndrome, patient SU, and another patient with T cell chronic lymphocytic leukemia

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This paper is dedicated to Dr. Henry G. Kunkel, who died on December 14, 1983.

This work was supported in part by U. S. Public Health Service grant No. 1 R23 CA 35463-01 awarded by the National Cancer Institute, grant No. AI 10811-19 (H. G. K.), and a research grant from the National Leukemia Association Inc. D. N. P. is a Special Fellow of the Leukemia Society of America. Correspondence should be addressed to D. N. Posnett, M.D., The Rockefeller University, 1230 York Avenue, New York, NY 10021.
(CLL),

patient FF, were obtained by Ficoll-Hypaque density centrifugation and used for immunizations of mice to develop monoclonal antibodies. Other cell preparations used were obtained from blood or teased tissues by similar means. Endothelial cell cultures were obtained from Dr. E. A. Jaffe (8). The cell lines used for immunofluorescence included the following T cell lines: MOLT 4, KE 37, HPB-ALL, 1301, CEM-T, Jurkat, SKW 3, GM 3671, GM 3639, GM 4155, HUT 102, HUT 78 and three different human T-T cell hybridomas. The B cell lines included: GM 1899, RPMI 8866 P, ARH-77, JESS, JOSH 7, U 266, two Epstein-Barr virus (EBV)-derived cell lines from normal donors and an EBV-derived cell line from patient FF. Mitogen-activated T cells were obtained after stimulation with phytohemagglutinin (PHA, Gibco Laboratories, Grand Island, NY), pokeweed mitogen (PWM, Gibco), concanavalin A (Con A, Sigma Chemical Co., St. Louis, MO), phorbol acetate (TPA, Consolidated Midland Corp.), and staphylococcus protein A (SPA, Pansorbin brand, Calbiochem-Behring Corp., La Jolla, CA). Mitogen-activated B cells were obtained using TPA, SPA, and tuberculin-purified protein derivative (PPD, Connaught Lab Ltd.).

**T Cell Antiidiotypic Monoclonal Antibodies.** The monoclonal antibodies S160 and S511 have been described (5) and recognize the idiotype-bearing molecule Tid SU. S160 reacts with a private idiotypic determinant and S511 reacts with a shared determinant, since it stains 1–2% of normal T cells. Cells from patient FF were used to generate murine monoclonal antibodies as described elsewhere (9). Hybridomas of interest were cloned on soft agar. Culture supernatants or appropriate dilutions of murine ascites containing monoclonal antibodies in saturating dosages were used for indirect immunofluorescence and immunoprecipitations. A fluorescein-conjugated F(ab')2 goat anti-mouse Ig antiserum (Tago Inc., Burlingame, CA) was used for immunofluorescence, which was evaluated with a cytofluorograph (30-H, Ortho Diagnostic Systems Inc., Westwood, MA); results were checked visually on a fluorescent microscope. For modulation experiments and proliferation experiments the monoclonal antibodies S511, S160, and FFA26 were purified by 45% ammonium sulfate precipitation and gradient DEAE ion exchange chromatography and then coupled to Sepharose 4B (Pharmacia). 1 g of Sepharose beads bound 9.8 mg S511, 5.7 mg S160 and 9.0 mg FFA26 antibody.

**Modulation.** The methods used have been described (10). Briefly, cells were cultured in the presence of saturating concentrations of monoclonal antibodies in regular culture medium (RPMI 1640, 10% fetal calf serum, 1% glutamine, 1% penicillin, and 1% streptomycin) for ~24 h, washed three times, and stained by direct (Leu-4a, Leu-3a antibodies; Becton, Dickinson & Co.) and indirect immunofluorescence (all other antibodies; Becton, Dickinson & Co.) and indirect immunofluorescence (all other antibodies). Modulation also was induced by monoclonal antibodies bound to Sepharose.

**Stimulation of Leukemic T Cells by Antiidiotypic Antibodies.** Leukemic T cells were cultured at 10^6 cells/ml in Linbro flat-bottom 96-well plates for various durations in the presence of different concentrations of Sepharose-coupled antiidiotypic antibodies with or without purified human IL-2 (Electro-Nucleonics, Inc.) and ultra-pure human IL-1 (Genzyme). Proliferation of cells was assayed for by [3H]thymidine uptake. Binding of the cells to their respective antiidiotypic antibodies coupled to Sepharose was determined visually with an inverted microscope.

**Immunoprecipitation and Gel Electrophoresis.** Cell membranes were iodinated using lactoperoxidase (Sigma) at 10 μg/5 × 10^7 cells and glucose oxidase (Calbiochem) at 0.1 U/5 × 10^7 cells in the presence of glucose and 125I Na iodide (New England Nuclear, Boston, MA). Immunoprecipitations and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were performed as described elsewhere (1, 5). Two-dimensional gel electrophoresis was performed according to O'Farrel (11), with some modifications. First-dimensional resolution was carried out on an isoelectric focusing slab gel using pH 3.5–10 ampholines (LKB Instruments, Gaithersburg, MD). The slabs were then cut into strips for the second dimension resolution by SDS–PAGE. The isoelectric focusing strip

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1. **Abbreviations used in this paper:** CLL, chronic lymphocytic leukemia; Con A, concanavalin A; EBV, Epstein-Barr virus; PHA, phytohemagglutinin; PPD, purified protein derivative; PWM, pokeweed mitogen; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SPA, staphylococcus protein A; TPA, phorbol acetate.
was placed on top of an 11.5% acrylamide gel. Molecular weight standards were run on each gel. Gels were then stained, destained, dried on filter paper, and autoradiographed on Kodak X-Omat R film.

Results

Production of an Anti-T Cell Idiotype Monoclonal Antibody. Human T lymphocytes from a patient with T cell CLL (FF) were used to immunize mice and generate monoclonal antibodies specific for this malignant clone of T cells. These cells had the following membrane phenotype revealed by indirect immunofluorescence: T1+, T3+, T4+, T11+, T6−, T9−, T10−, Ia−, Leu7−, Tac+. Cells from a Sézary cell leukemia (SU), also examined in this study, differed slightly in membrane phenotype: T1+, T3+, T4+, T11+, T6−, T9−, T10−, Ia−, Leu7−, Tac−.

From 90 monoclonal antibodies, one revealed total specificity for the FF cells used for immunization. This antibody (FFA26) failed to react with all other cell types tested (Table I), including a number of T cell leukemias, normal T cells, thymocytes, T cell lines, and mitogen-activated T cells. Other tested cell types are listed in Table I and include normal, activated, and malignant B cells, and several different sources of lymphohematopoietic cell types and cell lines. An EBV-derived B lymphoblastoid cell line from the patient FF also failed to stain

| Cell type (No. of samples) | % cells staining |
|----------------------------|-----------------|
| FF, T cell CLL             | 90              |
| FF, EBV-derived B cell line| 0               |
| Other T cell leukemias (9) | 0               |
| B cell leukemias (4)       | 0               |
| Normal T cells (10)        | 0               |
| Normal non-T mononuclear cells (7) | 0 |
| Normal activated T cells (3)* | <2 |
| Normal activated B cells (1)* | <2 |
| T cell lines (15)*         | <2              |
| B cell lines (8)*          | <2              |
| Spleen (2)                 | 0               |
| Thymus (1)                 | 0               |
| Bone marrow (2)            | 0               |
| Cord mononuclear cells (1) | 0               |
| Granulocytes (1)           | 0               |
| Platelets (2)              | 0               |
| Cultured endothelial cells (1) | 0 |
| Cell lines (HL60, U937, K562)* | <2 |

* Because of the larger background fluorescence observed with the negative control antibody in these cell populations, small numbers of weakly stained cells cannot be assessed and negative results are therefore expressed as <2%. Negative control antibodies were the same as those mentioned in Table II.
with FFA26, thus eliminating the possibility that this antibody reacted with a rare allotypic determinant of an HLA molecule, which would be expected to be present on both T and B cells. The cell line HUT 102, which harbors human T cell leukemia virus and its associated membrane antigens, did not react with FFA26. The other T cell leukemias included three cases of Sezary syndrome, a case of mycosis fungoides, one adult T cell leukemia, and four T cell acute lymphoblastic leukemias. All but two of these T cell leukemias had a T3+, T4+, T8− membrane phenotype. The other two, however, expressed a suppressor phenotype: T3+, T4−, T8+. Thus, the monoclonal antibody FFA26 is specific for the malignant clone of cells from patient FF. This specificity is idotype-like and resembles the specificity of previously described antibodies (1, 6), including those generated against SU cells (5).

Comodulation of T3 Antigen with the T Cell Idiotypic Antigen. Previous studies have demonstrated a close association of the T3 molecule with the T cell idioype-bearing structure on T cell clones (3) and on human T leukemia cells (5), demonstrated by comodulation experiments. In a similar manner the T cell idioype-bearing molecule of FF cells (Tid FF) and the T3 molecule comodulate (Table II). Modulation could be induced by both soluble and Sepharose-linked FFA26 antibody. Comodulation was specific for the antigens recognized by FFA26 and the Leu 4a antibody which detects the T3 molecule. Other surface antigens on FF cells did not comodulate, including T4 and T11 (data not shown) and two other membrane antigens present on the FF cells (Table II). In addition, reappearance of T3 and Tid FF antigens was demonstrated 24 h after continued culture of FF cells in the absence of the modulating antibody, thus indicating that FF cells in culture are able to re-express the Tid FF molecule. These modulation properties of FF cells are the same as for SU cells with their respective antiidiotypic antibodies (5). Thus, the antiidiotypic antibodies reacting with their respective T cell leukemias appeared to be very similar. However, further studies mentioned below, comparing these two systems side by side, revealed major differences.

Stimulation of Leukemic Cells by Sepharose-linked Antiidiotypic Antibody. In proliferation studies the two leukemic cell types, FF and SU, behaved quite differently. FF cells that express the Tac antigen proliferated in response to IL-2.

| Antibody used to induce modulation | Percentage of cells staining with |
|------------------------------------|----------------------------------|
|                                   | Leu 4a | FFA26 | FFB2* | FFA85* | Control* |
| Leu 4a                            | 3      | 11    | 98    | 92     | 6        |
| FFA26-seph 0.1%                    | 8      | 11    | 99    | 99     | 4        |
| FFA26-seph 0.001%                  | 90     | 96    | 98    | 93     | 1        |
| Control seph 0.1%                  | 96     | 93    | 96    | 89     | 1        |

* FFB2 and FFA85 are murine monoclonal antibodies generated against FF cells and reactive with antigens on T cells as well as other cell types.

* Control antibodies were murine monoclonal antibodies, specific for an IgM kappa private idiotypic determinant and for the hapten dinitrophenol. Neither of the negative control antibodies has reacted with any cell types tested.
However, the Tac-negative SU cells did not incorporate $[^3H]$thymidine in the presence of IL-2, and a response of these cells could not be induced by further addition of IL-1 to the culture medium or by the addition of S511-seph antibody with or without IL-1 and IL-2. The failure of S511-seph to activate SU cells was not due to loss of antibody activity, since these Sepharose beads clearly bound to the SU leukemia cells in a specific manner (Fig. 1). Likewise FFA26-seph beads bound specifically to the FF leukemia cells. Fig. 1 illustrates clusters of FF cells typically seen in association with FFA26-seph beads, indicating that these cells were proliferating in response to stimulation by FFA26-seph. This was clearly demonstrated in a dose response titration of FFA26-seph, which shows a marked increase in $[^3H]$thymidine incorporation at optimal concentration of FFA26-seph over the baseline incorporation due to IL-2 alone (Fig. 2). This stimulation was specifically induced by FFA26-seph and not by S511-seph. In addition, further experiments, using other Sepharose-linked antibodies that bound to FF cells (FFB2 and FFA85, see Table II), demonstrated absence of stimulation by these antibodies in contrast to FFA26-seph. SU cells failed to respond to IL-2, S511-seph, or FFA26-seph (Fig. 2). The possibility that S511-seph was toxic for lymphocytes was ruled out, since S511-seph did not prevent FF cells from proliferating in response to IL-2 (Fig. 2) and did not affect cell viability (Fig. 1), and >88% of SU cells excluded trypan blue during the 2-d culture period. In addition, other experiments showed that S511-seph could support and increase

![Figure 1](https://example.com/figure1.jpg)

**Figure 1.** Photomicrographs of leukemic T cells in culture with Sepharose-linked antidiotypic antibodies. SU cells are shown in panels A and B, FF cells in C and D. FFA26-seph was added to the cultures in A and C, while S511-seph was added in B and D. The two different leukemic cell types were bound by the appropriate antidiotypic antibody in B and C, but not by the unmatched antibody in A and D. In addition, FF cells are clustered in panel C, suggesting that they are proliferating.
the proliferation of a S511-positive, IL-2-dependent T cell line, derived from a normal donor. This cell line is currently under further investigation (Bigler, R. D., D. N. Posnett, and N. Chiorazzi, manuscript in preparation), because it represents the small population of normal T cells identified by the S511 antibody (5).

FFA26-seph is capable of stimulating FF cells to proliferate in the absence of exogenously added IL-2 (Fig. 3). This response is amplified by addition of IL-2 to the culture medium. The maximal point of proliferation occurs after 2–3 d of culture. We questioned whether FF cells produce IL-2 in response to FFA26-seph. Such an autocrine secretion of IL-2 could result in the proliferation observed with FFA26-seph by interaction of IL-2 with IL-2 receptors constitutively present on these cells. Supernatants were thus obtained from proliferating FF cells on day 2 of culture with optimal concentrations of FFA26-seph or PHA. At that point, mean [3H]thymidine incorporation was 11,760 +/- 238 cpm for FFA26-seph-stimulated cells, 27,112 +/- 1133 cpm for PHA-stimulated cells, and 744 +/- 130 cpm for the culture medium control. Using a previously described assay for IL-2 (12), these supernatants were all found to contain <0.05 U IL-2 activity. This suggested that FF cells are incapable of producing IL-2 under the conditions tested. However, these results do not exclude the possibility
that small amounts of IL-2 may have been produced and immediately consumed by the FF cells, which express membrane IL-2 receptors.

**Cells of Two Different T Cell Leukemias Bear Different Tid Molecules.** Immunoprecipitation after cell membrane iodination and NP40 cell lysis revealed clear differences and some similarities between the two idiotype-bearing T cell molecules (Tid). Immunoprecipitates of both molecules and their respective antiidiotypic antibodies are shown on a 5–20% acrylamide gradient gel (Fig. 4). Unreduced TidFF was clearly larger than the TidSU molecule. The TidFF band was observed at a position of 90–95 Kd, while the TidSU band was at a position of ~78 Kd. In the reduced state both molecules were composed of two distinct chains: (a) a heavily iodinated chain and (b) a lightly iodinated chain. The latter type of chain from both molecules co-migrated at 43 Kd, but the heavily labeled chains were quite different in relative mobility (lanes 3 and 4, Fig. 4). The heavily labeled Tid FF band has a position of 49 Kd, while the heavily labeled Tid SU band has a position of 38 Kd. Despite these differences, both molecules are similar, in that both were disulfide-linked heterodimers, with molecular weights similar to those reported for clonotypic molecules in mouse and man (1–6).

Characterization of these molecules by two-dimensional gel electrophoresis demonstrated further differences. The co-migrating 43 Kd chains from the two molecules appeared to be different (Fig. 5). Both chains of the SU molecule had slightly acidic and overlapping pI values. In contrast, the FF molecule contained an acidic chain (49 Kd) and a neutral chain (43 Kd). In both molecules micro-
heterogeneity of both chains was observed, suggesting that these molecules are glycosylated proteins. However, the observed differences in molecular weight between TidSU and TidFF are unlikely to be solely due to glycosylation differences.

Discussion

In order to compare the putative receptor for antigen on two different T cell leukemias, monoclonal antibodies with idiotype-like specificity were produced. Such antibodies were obtained with each of the two different T cell leukemias and demonstrated (a) idiotypic specificity, (b) comodulation of the T3 antigen and the idiotype-bearing molecule, and (c) immunoprecipitation of a disulfide-linked heterodimer from the respective leukemic cells. These results are remarkably similar to recently reported data on the putative T cell receptor for antigen (1-6).

FFA26 is an antibody that recognized a private idiotypic determinant of FF leukemia cells. In addition, this antibody selectively induced FF cells to proliferate, quite as antigen may induce T cells to proliferate by reacting with the major histocompatibility–restricted antigen receptor. Antidiotype antibodies may thus mimic antigen by inducing specific proliferation of the idiotype-bearing T cells. Both proliferation and IL-2 secretion have been observed with T cell clones stimulated by Sepharose-linked anticlonotypic antibodies (14, 15). However, it
Figure 5. Two-dimensional gel analysis of reduced TidFF (upper panel) and TidSU (lower panel). The isoelectric point of the upper band of TidFF is acidic, while that of the lower band is neutral. Both bands of TidSU are slightly acidic. The co-migrating 43 Kd chains of TidFF and TidSU are different when analyzed by isoelectric focusing. All chains consist of multiple spots, indicating that they are glycosylated.

remains unclear to what extent the observed proliferation is due to autocrine production of IL-2 and whether this is the primary mechanism by which these T cell clones are induced to proliferate.

The effects of antiidiotypic antibodies on two human T cell leukemias were compared. While FF cells could easily be induced to proliferate by FFA26-seph, SU cells consistently failed to respond to S511-seph, in spite of visible binding of the SU cells to the antibody-coated beads. This difference was also reflected by a general responsiveness of FF cells to IL-2 and various mitogens and a general unresponsiveness of SU cells to IL-2 and mitogens. A possible interpretation of the difference between SU and FF cells is that they represent leukemic transformation of T cells at different stages of differentiation. Morphologically, SU and FF cells differ significantly. SU cells are typical Sezary cells with multiple nuclear lobulations and it is likely that they home to skin, since the patient demonstrated leukemic infiltration of the epidermis. FF cells in contrast are small lymphocytes typical of CLL residing primarily in the peripheral blood, spleen, lymph nodes, and bone marrow of the patient. A possible interpretation is that SU cells are
end-stage cells in T cell differentiation, similar to plasma cells in B cell differentiation. The inability of SU cells to respond to S511-seph can also be compared to leukemic B lymphocytes, such as CLL cells, that frequently fail to respond to cross-linking of their membrane immunoglobulin, in contrast to normal B cells (12).

The mechanism of proliferation induction of FF cells by FFA26-seph does not appear to primarily involve IL-2 secretion. FF cell culture supernatants contained no measurable IL-2, when stimulated under optimal conditions. In addition, FFA26-seph could induce increased proliferation, in spite of the presence of excess IL-2 (Fig. 2). These results indicate that cross-linking of the T cell receptor for antigen may cause T cell proliferation by mechanisms other than induction of IL-2 secretion. The results do not exclude the possibility that small amounts of IL-2 may have been produced and immediately consumed by the FF cells, which express membrane IL-2 receptors. However, the recent report by Arya et al. (16) of failure to find IL-2 mRNA in virus-transformed T lymphocytes, supports the notion that IL-2-independent mechanisms may operate in normal or malignant T cell proliferation. In addition, the response of FF cells to cross-linking of their putative membrane antigen receptor by the FFA26-seph beads is quite like the response of human B cells to cross-linking of membrane immunoglobulin, the B cell antigen receptor, by anti-immunoglobulins. In both systems proliferation peaks after 2 d of culture and can be achieved in the absence of growth factors (BCGF, IL-2). In the human B cell system this requires a high concentration of soluble anti-immunoglobulin (12) or insolubilized anti-immunoglobulin (17). FF cells also proliferated in response to insolubilized antidiotypic antibody, but failed to show such a response with soluble antibody in the form of hybridoma supernatant (data not shown).

The antidiotypic antibodies revealed a further major difference between the two leukemic cell types and their respective Tid molecules. Immunoprecipitation studies showed a relatively large difference in molecular weight between the two Tid molecules. This was possibly due to a difference in one of the two disulfide-linked chains. Thus, the upper band of TidFF and the lower band of TidSU were both heavily labeled by iodination in contrast to the two lightly labeled bands, both migrating at 43 Kd. The heavily labeled bands of TidFF (49 Kd) and TidSU (38 Kd) are sufficiently different in molecular weight to explain the size difference of the two unreduced molecules. The alpha and beta chain as described previously (1–6, 14, 18) migrate as the upper and lower bands, respectively on SDS–PAGE analysis and are usually acidic (alpha) and basic (beta) on two-dimensional analysis. It is possible that, in the case of TidSU, the position of these chains is inverted (Fig. 4).

A possible explanation for the differences observed between TidSU and TidFF is that they belong to different classes of T cell receptors for antigen. Classes of T cell receptors for antigen are expected, based upon analogy with the immunoglobulins. They have, however, not yet been clearly demonstrated in mouse or man. Constant region antigenic determinants will be useful for defining classes of the T cell receptor for antigen. In this respect, it is of interest that the antibody S511, which cross-reacts with a small subpopulation of normal T cells by immunofluorescence and immunoprecipitates a disulfide-linked heterodimer from
these normal T cells, does not stain FF cells or immunoprecipitate a molecule from FF cells (Bigler, R. D., D. N. Posnett, and N. Chiorazzi, manuscript in preparation). These results lend further support to the contention that TidSU and TidFF represent two different groups of the presumptive T cell antigen receptor.

Recently, the cDNA sequence of a rearranged T cell gene encoding for a T cell–specific protein chain has been described in mouse and man (19–21). This molecule has variable, joining, and constant regions and an estimated molecular weight of 35,000. The features of cell specificity, variable and constant portions of the molecule, and the molecular weight of the single chain, are all similar to those of the TidFF and TidSU molecules, further supporting the suggestion that they represent the T cell receptor for antigen.

Summary

Two different human T cell leukemias were compared, using antiidiotype-like murine monoclonal antibodies. In each case these antibodies immunoprecipitated disulfide-linked heterodimer molecules from their respective leukemic cells. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the two idiotype-bearing molecules a major difference in molecular weight was observed, which could be attributed to a similar difference in size of the heavily iodinated chain of either heterodimer. The lightly iodinated chains of both molecules co-migrated at 43 Kd, but appeared to have different isoelectric points on two-dimensional gel analysis. The possibility that these two different heterodimers correspond to different classes of the putative T cell receptor for antigen is discussed.

Assays of proliferation of the leukemic cells using Sepharose-bound antiidiotype-like monoclonal antibody showed that one of the leukemic cell types proliferated readily in response to its antiidiotypic antibody. This proliferation was not associated with measurable production of IL-2 and appeared to be a direct effect of the antiidiotypic antibody, which may mimic antigen in its interaction with the T cell receptor for antigen. The other leukemic cell type did not respond to Sepharose-bound antiidiotypic antibody and was generally unresponsive to lymphokines and mitogens. It is possible that the two leukemic cell types represent different stages of T cell differentiation.

Rosanne Wisniewolski, Robert Folkl, and David Solomon provided technical assistance. Pamela Bolton prepared the manuscript.

Received for publication 2 April 1984.

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