

IDIOTYPE-LIKE MOLECULES ON CELLS OF A HUMAN T
CELL LEUKEMIA

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Progress in defining the nature of T cell receptors for antigen has been slow and many conflicting results have been reported (reviewed in reference 1). Recently more success has been achieved by developing monoclonal antibodies, directed against T cell clones, that show unique specificities for the clones (2-4). In this study we have utilized a similar approach and have screened monoclonal antibodies made against cells from a human T cell leukemia for idiotypelike specificity. Two such monoclonal antibodies were found that reacted with the same membrane molecule; one showed absolute specificity for the immunizing cells, the other also reacted with a small population of normal T cells.

Materials and Methods

Leukemic cells were obtained from a male from the Dominican Republic. He presented with typical clinical and laboratory findings of Sezary Syndrome. Peripheral leukocyte counts ranged from 30,000 to 97,000 with ~85% Sezary cells. The patient was leukopheresed and leukemic cells obtained by Ficoll-Hypaque separation. Surface markers on the leukemic cells were: sheep erythrocyte rosette +, OKT3 +, OKT4 +, OKT8 -, Ia -, and surface immunoglobulin -. The leukemic cells were cryopreserved until use.

Cryopreserved cells were thawed and 2×10^7 cells in alum were injected intraperitoneally into BALB/c mice (The Jackson Laboratories, Bar Harbor, ME). 3 wk later the mice were boosted with an equal number of cells, and splenic leukocytes were obtained for fusion 3 d later. Spleen cells were fused with SP 2/0 cells by the technique of Kennett et al. (5). Supernatants from viable hybridoma cultures were screened by standard indirect immunofluorescence technique by the use of fluorescein-conjugated Fab₂ fragments of goat anti-mouse IgG antiserum (Tago Inc., Burlingame, CA) and a Cytofluorograf 30-H (Ortho Diagnostic Systems Inc., Westwood, MA). Results obtained by flow cytometry were correlated by visual immunofluorescence. Hybridomas of interest were cloned in soft agar. Cloned cells were injected into pristane-primed BALB/c mice and the monoclonal antibody was isolated from the ascites by ammonium sulfate precipitation and ion exchange chromatography.

Normal peripheral blood mononuclear cells were subjected to rosetting with neuraminidase-treated sheep erythrocytes. Rosetting and nonrosetting populations plus granulocytes, erythrocytes, and platelets were used to screen the reactivity of the cloned hybridomas. Human T cell lines (MOLT 4, CEM-T, Jurkat, KE 37, 1301, 3639, and HUT 102), B cell lines (8866, Cess, and Josh 7), and myeloid cell lines (K 562, HL 60, and U 937) were also evaluated by flow cytometry. T cell blasts were generated by stimulation with concanavalin A at 10 μ g/ml (Sigma Chemical Co., St. Louis, MO), phytohemagglutinin 1:100, or pokeweed mitogen 1:100 (Gibco Laboratories, Grand Island, NY) and stained with the monoclonal antibodies. Samples of peripheral blood from patients with various T cell malignancies were also analyzed by indirect immunofluorescence. The malignancies included two samples of blood from patients with T-acute lymphocytic leukemia, four samples from T-chronic lymphocytic leukemia, three samples of leukemic

cells in T cell lymphomas, one sample of adult T cell leukemia, and seven samples of cutaneous T cell lymphoma (five with Mycosis Fungoides and 2 with Sezary Syndrome). Some of the malignant cell samples were the generous gift of Dr. Barton Haynes.

Cell surface molecules were radioiodinated using the lactoperoxidase technique. Immunoprecipitation was performed using a modification of the technique of Allison et al. (2). The immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described (2). Two-dimensional gel electrophoresis was performed using a modification of the technique of O'Farrell (6).

Experiments to detect modulation of surface antigens were performed as previously described (6). Enrichment of the population of normal T cells reactive with the monoclonal S511 was obtained using the indirect rosetting technique of Mayer et al. (7).

Results

Screening of Monoclonal Antibodies. Six fusion experiments were performed, yielding 610 viable hybrids. After initial screening by immunofluorescence and flow cytometry, 28 were found to show some specificity for the immunizing cells. Further screening with T cells and non-T cells yielded two antibodies that appeared to react primarily with the immunogen. These two hybrids, called S160 and S511, were cloned and the supernatants of clone cultures (S160-1 and S511-2) were used for further study by indirect immunofluorescence. One lot of supernatant from each clone was determined to be saturating on the immunizing cells in indirect fluorescence studies when diluted to 1:20, however, undiluted supernatants were always used. Ouchterlony analysis of the clone culture supernatants revealed that S160-1 was an IgG₁k antibody and S511-2 was IgG_{2b}k antibody.

The percentage of positive cells reacting with the monoclonals was always within 5% of the visual leukocyte differential that showed ~85% of the mononuclear cells in the leukopheresis to be leukemic cells. Analysis by flow cytometry consistently revealed a population of nonreactive cells that correlated with nonmalignant cells observed by visual immunofluorescence that were present in the sample. Table I shows some of the results for the two monoclonals with a wide variety of cell types including other T cell leukemias and long term T cell lines. These were all negative with the exception of leukemia S cells, which were the immunizing antigen. The S leukemia was a Sezary type T cell and negative for the human T cell leukemia virus (HTLV). Other samples of Sezary T cells plus two cells known to be positive for HTLV, HUT 102 and the patient with adult T cell leukemia, were negative with these antibodies.

S511-2 showed a definite reactivity with a small population of normal T cells, which was not seen with S160-1. 15 different preparations of normal T cells were studied by flow cytometry and the range of positivity for S511-2 was 0.8–2.7%, with a mean value of 1.6%. Fig. 1 shows a comparison of the fluorescence histograms of the two antibodies under identical conditions with normal T cells. The irregular nature of the histogram of S511 is due to the small number of reactive cells and the scale used to identify this population. Visual counts of normal T cells stained with S511 gave a mean of 1.9% positive with a range of 1.0–2.5%, while cells stained with S160 were entirely negative. Marked enrichment of the minor S511 positive normal T cells was achieved by utilizing the specific antibody in an indirect rosetting procedure. One fraction showing 19% positivity with S511 is shown in Table I; the reaction with S160 remained negative.

TABLE I
Percentage of Cells Stained by the Two Fluorescent Monoclonal Antibodies in the Survey of Various Types of Cells

	T cell leukemias		Cell lines				Normal cell preparations			
	Leukemia S	Others	T cell	B cell	Myeloid	B + adh.	T cells	T blasts	Thym.	T511 [‡]
No.	1	17	7	3	3	10	15	6	3	1
S160-1	>80	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0
S511-2	>80	<0.5	<0.5	<0.5	<0.5	<0.5	1.6*	1.3*	0.7*	19

No. refers to number of different leukemias, cell lines, or preparations of normal cells.

* Mean value.

[‡] T cells enriched with the S511 antibody.

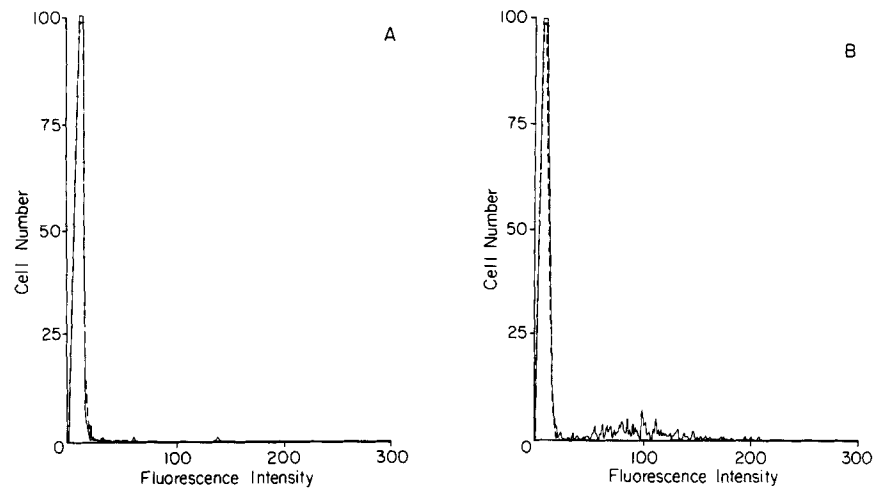


FIGURE 1. Cytofluorometric patterns for isolated normal T cells with monoclonal antibody S160 in (A) and S511 in (B). Reactivity of the latter antibody with a small population of normal T cells is apparent.

Membrane Immunoprecipitation Analyses. The S leukemia cells were labeled with ¹²⁵I and SDS-PAGE patterns with the two antibodies were obtained following immunoprecipitation. Fig. 2 illustrates these results. Very similar patterns were obtained with the two antibodies. Under nonreducing conditions a clear band was observed at a position of ~80 kilodaltons. Under reducing conditions this disappeared and two smaller bands at positions of ~43 kdaltons and ~38 kdaltons were observed. The 43-kdalton band was always weaker in intensity than the 38-kdalton band. Both smaller bands were shown to arise from the 75-kdalton protein by the cutting of the larger band out of a gel, reduction of the protein, and resolution on a second gel (data not shown). Two-dimensional gel patterns showed microheterogeneity in both of the reduced peptides (Fig. 2B). The subfractionation of the 38-kdalton band was sometimes observed under optimal separation conditions in unidimensional experiments. Concentrated preparations of the normal T cell population reacting with the S511 antibody also showed similarities to the bands observed with the leukemia cells.

Modulation Experiments. Table II shows that monoclonal Leu-4 (T3) antibody modulated the antigens reactive with both the S160 and S511 antibodies. Control antigens detected with antibodies Leu-1 and T-11 were not affected. Experiments

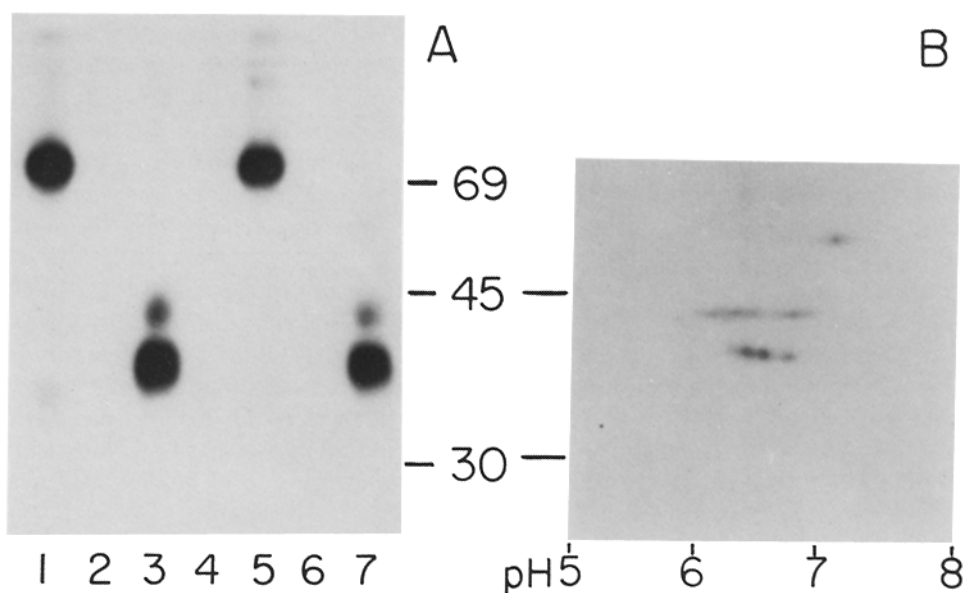


FIGURE 2. (A) SDS gel pattern of the immune precipitate obtained from ^{125}I labeled Leukemia S cells by the two monoclonal antibodies. Lane 1, before reduction and lane 3, after reduction for antibody S160; lanes 5 and 7, before and after reduction for antibody S511. Lanes 2, 4, and 6 represent control precipitations with these antibodies against the KE37 T cell line. The numbers in the area between A and B represent the position of marker proteins of known molecular weight. (B) Results of two-dimensional gel analysis for S511 on the reduced samples from labeled Leukemia S cells. The upper and lower bands correspond to the upper and lower band in the reduced sample of A. The single spot in the upper part of B is a marker spot.

TABLE II
Modulation of the Antigen Detected by Monoclonal Antibodies S-160 and S-511 by Leu-4(T3) Antibody

Antibody used to induce modulation	Percentage of positive cells after restaining with:				
	Leu-4	S-160	S-511	Leu-1	T-11
			%		
Leu-4	11	13	15	93	87
S-160	37	23	20	93	92
S-511	68	52	46	92	93

in the reverse direction with the leukemia antibodies show only a partial effect on the Leu-4 antibody reaction.

Discussion

The two monoclonal antibodies described above clearly show idiosyncratic specificity for a membrane component of the leukemic T cells used for immunization. Both antibodies failed to react with the non-malignant cells of the S leukemia, any other leukemic T cells, T cell lines, B cells, or any other cell type tested. Antibody S160-1 also failed to react with normal T cells. Antibody S511-2, however, did react with a small population of normal T cells, ~2%. The latter could be markedly enriched with the monoclonal antibody making it easier to demonstrate the specific reactivity. Immune precipitation of labeled membrane

components of the leukemic T cells followed by gel separation showed that both monoclonal antibodies brought down very similar molecules. In the unreduced state they showed bands with the same mobility of ~ 80 kdaltons. On reduction two bands were observed with mobility of ~ 43 and ~ 38 kdaltons. The larger 43-kdalton band was always fainter than the 38-kdalton band, but both clearly arose from the 80-kdalton protein after reduction. The exact reason for the difference in intensity of the two bands is not apparent. It may be a result of differential membrane labeling with ^{125}I .

Two-dimensional gels showed some further separation of each of the bands. The 38-kdalton band gave a series of spots resembling those of multiply glycosylated derivatives. Thus each monoclonal antibody recognized a similar disulfide-linked protein that separated on reduction into two nonidentical polypeptide chains. Further evidence that these antibodies recognized the same component was obtained from serial precipitation studies; initial precipitation with one antibody removed antigen for the other. Also, in the immunofluorescence studies each antibody blocked the other.

Although no direct evidence was obtained that these antibodies recognized a type of T cell receptor on these leukemic T cells, considerable indirect evidence strongly suggests that this is indeed the case. The molecules described above are similar to those described both in the mouse and the human for cytotoxic T cell clones in which inhibition of the specific activity with the antibody was observed (3, 4). Minor differences were observed, especially with the previous human study (3) in which the reduced bands were reported as larger. In each case however, two disulfide-linked polypeptide chains were involved. Allison and co-workers (2) have emphasized the rarity of other molecules on T cell membranes that consist of disulfide-linked polypeptides. In addition, Leu-4 (T3) antibodies were found to modulate the receptor for both antibodies used in the present study. The close relationship of the T3 antigen and various T cell receptors has been emphasized (3, 8). Perhaps the strongest evidence for a relationship to T cell receptors stems from the private idiotypelike antigen reacting with the one antibody and the slightly public antigen among T cells reacting with the other, even though both antigens are present on the same protein molecules. This strongly suggests an antibodylike system.

The relationship between the two polypeptide chains observed and the two antigenic sites detected by the monoclonal antibodies remains to be determined. The one antigenic site appears to involve a variable region with a private "idiotypic." The other antigenic site, found on some normal T cells, could also derive from variable regions and represent a cross-reactive "idiotypic" or a framework antigen. However, it is also possible that this represents a constant region antigen that is characteristic of a small percentage of T cells with a specific function.

A distinct advantage of the use of human leukemic T cells for the study of T cell receptors for antigen is the large amount of material available for the isolation and characterization of the receptors. Sufficient progress has been made, especially with the specific receptors on IL-2-dependent T cell clones mentioned above, to enable this work to proceed successfully with leukemic cells and T cell lines even when no biological activity is apparent. In some instances

however, T cell leukemias have been shown to have specific recognition properties and these will be all the more useful. Experiments parallel to those in the present studies are currently underway in our laboratory with one such T cell leukemia that shows specific stimulation by defined alloantigens. These antiidiotypic antibodies also may prove useful in the future therapy of these leukemias.

Summary

Two monoclonal antibodies were obtained that showed unique specificities for the leukemic T cells used for immunization. One antibody, S160, was totally specific for the antigen. The other antibody, S511, also reacted with a small population of normal T cells. This was made especially evident by concentrating these normal T cells with the antibody. Considerable evidence was obtained that both antibodies reacted with the same membrane molecules. In the unreduced state a major component of ~80 kdaltons was observed; after reduction this split into two components of ~43 and ~38 kdaltons. The reaction of the two antibodies with different antigenic sites on the same molecule, one representing a private site and the other a more cross-reactive site, strongly suggests an antibodylike molecule, but composed of polypeptide chains differing from immunoglobulins.

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