

# STIMULATION OF A SUBSET OF NORMAL RESTING T LYMPHOCYTES BY A MONOCLONAL ANTIBODY TO A CROSSREACTIVE DETERMINANT OF THE HUMAN T CELL ANTIGEN RECEPTOR

BY ROBERT D. BIGLER, DAVID N. POSNETT, AND NICHOLAS CHIORAZZI

*From The Rockefeller University, New York 10021*

The structure of the putative T cell antigen receptor has been defined using monoclonal antibodies (mAb)<sup>1</sup> prepared against various monoclonal T cell populations in several laboratories (1–3), including our own (4, 5). These reagents have defined an 80–90 kilodalton (kD) disulfide-linked heterodimer usually composed of a 49–53 kD alpha chain of acidic pI and a 41–43 kD beta chain of more basic pI. These heterodimeric molecules fulfill certain anticipated characteristics for the T cell antigen receptor, i.e., the presence of idiotype (1–6), homology of the gene sequences to immunoglobulin (7–10), and the capacity to block the function of antigen-specific T cell lines or hybridomas when complexed with mAb that detect private idiotypes on the molecule (2, 3, 6). An additional functional characteristic expected of the T cell receptor is the ability to specifically mediate stimulation of resting T cells after interaction with antigen. This issue has been approached previously by using mAb that bind to the variable region of the antigen receptor on activated cells, such as interleukin 2 (IL-2)-dependent T cell lines, T-T hybridomas, or human T cell leukemias that express the Tac antigen. When present either as soluble or Sepharose-bound antibody, these mAb have been shown (2, 3, 5, 6) to specifically increase IL-2 production or DNA synthesis in these previously activated cells. However, since these antibodies bind to private idiotypic specificities, they have not been useful in demonstrating a comparable pattern of activation with normal resting T cells. Recently, heteroantisera (11, 12) and mAb (4, 13) have been described that detect common determinants on the T cell antigen receptor expressed on normal T cells. Although these reagents confirm the presence of this molecule on normal T cells, they have not been reported to stimulate the reactive cells.

In a previous report (4), we described an antibody reacting with the T cell antigen receptor on a subpopulation of normal circulating human T cells. This mAb, S511, was produced using a human T cell leukemia as the immunogen and was shown to immunoprecipitate an 80 kD, disulfide-linked heterodimer

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<sup>1</sup> *Abbreviations used in this paper:* DNP, dinitrophenyl; FITC, fluorescein isothiocyanate; GAMG, goat anti-mouse Ig; IL-2, interleukin 2; mAb, monoclonal antibody; PHA, phytohemagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

composed of a 43 kD chain and a 38 kD chain on the leukemic cells. In addition to reacting with the leukemic T cells, this antibody was shown to react with 1–2% of circulating T cells in all individuals tested. The current study was initiated to investigate the nature of these reactive cells and to test the hypothesis that binding of mAb S511 to these cells would result in the activation of resting T lymphocytes. The data demonstrate that the population of normal T lymphocytes reactive with S511 is composed of resting cells and that both soluble and Sepharose-bound antibody induce proliferation of these cells. Additionally, the S511 molecules on the normal S511-bearing T lymphocytes appear to be similar to those on the original leukemic cells. A preliminary report of these findings has been presented (14).

### Materials and Methods

*Monoclonal Antibodies.* Preparation and characterization of the murine mAb S511 has been previously described (4). The S511 mAb, an IgG2b $\kappa$  antibody, was purified from ascites by fractionation with 50% ammonium sulfate and elution from DEAE cellulose (DE-52; Whatman, Inc., Clifton, NJ) using a 0–0.2 M NaCl gradient. This antibody preparation was coupled to cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Piscataway, NJ) at a ratio of 6 mg protein bound to 0.5 g beads using standard conditions. A control mAb reacting with the hapten dinitrophenyl (DNP) (provided by Dr. D. H. Katz, Medical Biology Institute, La Jolla, CA) was prepared in the same manner as S511. Antibodies OKT-3, OKT-4, and OKT-8 were obtained commercially (Ortho Diagnostic Systems, Inc., Raritan, NJ) and mAb 9.6 was the gift of Dr. John Hansen, Fred Hutchinson Cancer Research Center, Seattle, WA.

*Stimulation of Normal T Cells.* Peripheral blood mononuclear cells were obtained and separated by rosetting with neuraminidase-treated sheep erythrocytes. Autologous non-rosetting cells were irradiated at 3,000 rad and mixed with rosetting cells at a 1:10 ratio when indicated. These cells were cultured in RPMI 1640 medium supplemented with 20% human AB serum and serial dilutions of purified mAb, phytohemagglutinin (PHA) (Gibco Laboratories, Grand Island, NY), or 10% lectin-free IL-2 (Electro-Nucleonics Inc., Fairfield, NJ). Cells were cultured for 9 d in flat-bottom microtiter plates (Linbro; Flow Laboratories, Inc., McLean, VA) and then analyzed for [<sup>3</sup>H]thymidine incorporation on alternate days. Proliferation was calculated from the mean thymidine incorporation of quadruplicate samples of cells cultured in the presence of mAb compared with cells cultured in media alone. Data are expressed as a proliferation index: (antibody culture mean cpm)/(media culture mean cpm).

*Simultaneous Surface Immunofluorescence and DNA Cell Cycle Analysis.* T cells were obtained by sheep erythrocyte rosetting and were stained by indirect immunofluorescence with mAb and fluorescein isothiocyanate-conjugated (FITC) goat anti-mouse immunoglobulin (Tago, Inc., Burlingame, CA) in saturating conditions. For DNA cell cycle analysis, these cells were fixed overnight in 70% ethanol, washed, and suspended at  $5 \times 10^5$ /ml in phosphate-buffered saline containing 18  $\mu$ g/ml propidium iodide and 40  $\mu$ g/ml RNase (Sigma Chemical Co., St. Louis, MO) (15). After 30 min the cells were analyzed for simultaneous surface FITC fluorescence and DNA fluorescence using a 50-H Cytofluorograf (Ortho Diagnostic Systems).

*Generation of S511-reactive T Cell Lines.* Five million peripheral blood T cells were suspended in 0.5 ml of RPMI 1640 medium with 10% fetal calf serum, to which an equal volume of 2% S511-Sepharose 4B beads was added. The suspension was centrifuged and then incubated at 4°C for 30 min. The pellet was gently resuspended and the S511-Sepharose was allowed to settle in a conical tube for 15 min. After settling, the S511-Sepharose and any bound cells were removed from the bottom of the tube and placed in medium containing 10% IL-2. Proliferating cells were expanded in this medium with addition of new S511-Sepharose every 7–10 d. The surface molecules on a cell line obtained using this method and on the original leukemic cells were <sup>125</sup>I-labeled using

lactoperoxidase, immunoprecipitated with mAb S511, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (4, 5).

## Results

*Induction of T Cell Proliferation by Soluble S511.* Our previous study (4) demonstrated that 1–2% of circulating peripheral blood T cells from all individuals tested were S511<sup>+</sup>. If the S511 molecule represents the antigen receptor on these normal T lymphocytes, then S511 antibody binding may specifically induce activation and proliferation of these normal cells in a manner similar to specific antigen-induced activation. This hypothesis was evaluated by obtaining peripheral blood T cells from three individuals with 1.5–2.6% circulating S511-reactive T cells. These cells were cultured for 9 d in the presence of soluble mAb, either S511 or anti-DNP, and irradiated nonrosetting cells. Cultures from all three individuals demonstrated T cell proliferation when cultured with S511 and no proliferation when cultured with anti-DNP. The results of the proliferation induced on sequential days by mAb S511 and anti-DNP at 1.6  $\mu\text{g}/\text{ml}$  are displayed in Fig. 1. They demonstrate that, over 9 d, the proliferation index for anti-DNP was between 0.6 and 2.0 for all three individuals, while the S511 mAb consistently induced proliferation starting on day 3 with an index of 3.0–17.2. The stimulation on day 3 continued and reached a maximum on day 7, with the proliferation index ranging between 41.6 and 185.1. After this peak of activation on day 7, the amount of thymidine incorporated on day 9 decreased, presumably due to extensive cell proliferation and limitations of the culture conditions. Additional experiments were also performed using the previously described mAb S160 (4) as the control antibody. This mAb detects a private idiotope of the antigen receptor molecule on the same leukemic cells used to generate mAb S511. As with anti-DNP, mAb S160 also failed to activate normal T cells (data not shown).

The dependence of this stimulation on antibody concentration and the pres-

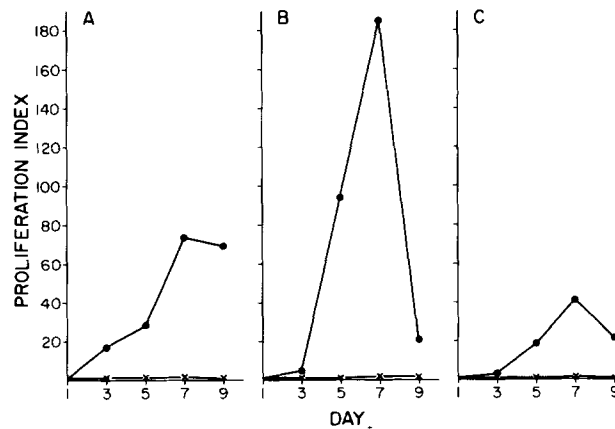


FIGURE 1. Proliferation of normal resting T cells induced by mAb S511 (●) or mAb anti-DNP (×) at 1.6  $\mu\text{g}/\text{ml}$  of soluble antibody. T lymphocytes from three different normal individuals were analyzed for [<sup>3</sup>H]thymidine incorporation. Data are presented as a proliferation index: (mean cpm of cells cultured in antibody)/(mean cpm of cells cultured in media alone).

ence of autologous nonrosetting cells was evaluated. Initial experiments used the same culture conditions as above, which contained 10% irradiated nonrosetting cells. Table I demonstrates that, over a range of antibody concentration from 0.01 to 10  $\mu\text{g/ml}$ , proliferation was not induced by anti-DNP. However, over

TABLE I  
*Normal T Cell Proliferation Induced by Monoclonal Antibody S511*

	T cells + non-T cells*		T cells alone <sup>‡</sup>	
	Day 3	Day 7	Day 3	Day 7
Anti-DNP				
10 $\mu\text{g/ml}$	1.1	1.6	0.9	1.5
1	1.2	1.2	1.0	1.2
0.1	1.1	1.8	0.8	1.0
0.01	1.0	0.9	0.9	1.4
S511				
10 $\mu\text{g/ml}$	3.3	61.3	1.7	79.9
1	2.8	49.3	2.0	126.8
0.1	1.1	2.0	1.1	14.3
0.01	7.9	1.0	1.2	2.6
IL-2 <sup>§</sup> 10%	2.4	22.0	ND <sup>†</sup>	ND
PHA 1:100	221.8	9.9	213.6	20.6

\* Mean proliferation indices of three experiments. Cultures contained 10% irradiated nonrosetting cells.

<sup>‡</sup> Mean proliferation indices of two experiments using sheep erythrocyte-rosetted cells without the addition of nonrosetting cells.

<sup>§</sup> Lectin-poor, IL-2-supplemented media without the addition of mAb.

<sup>†</sup> Not done.

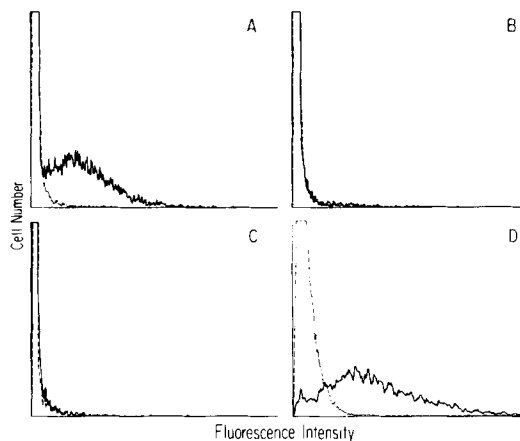


FIGURE 2. Fluorescence histograms of normal T cells stimulated by mAb or IL-2. (A-C) T cells were cultured for 7 d with 10% irradiated non-T cells and antibody. After two additional days of growth in media containing 10% IL-2 without antibody, the cells were washed and stained by indirect immunofluorescence with FITC GAMG alone (---) or mAb S511 plus FITC GAMG (—). (A) Cells cultured in S511 at 1.6  $\mu\text{g/ml}$ . (B) Cells cultured in anti-DNP at 1.6  $\mu\text{g/ml}$ . (C) Cells cultured in 10% IL-2 alone. (D) An IL-2-dependent T cell line activated by S511-Sephadex.

this same range mAb S511 induced about a threefold increase in proliferation on day 3 and a concentration-dependent maximum proliferation on day 7 of 49–61 times greater than background. This same concentration range was examined for stimulation of T cells without the addition of non-T cells as accessory cells. As before, no stimulation occurred with anti-DNP. The S511 cultures, however, showed a twofold increase on day 3 and a pronounced concentration-dependent proliferation on day 7. The proliferation on day 7 in these T cell cultures was comparable to that observed in cultures to which irradiated nonrosetting cells were added. The extensive proliferation induced by mAb S511 is striking when one compares the proliferation index of S511 cultures (127 on day 7), which activates 2% of the T cells in culture, and the proliferation index of PHA (214 on day 3), which activates the majority of T cells in culture.

The thymidine incorporation observed in the S511 cultures could represent either specific proliferation of the S511-bearing cells or nonspecific expansion of recruited cells. The latter was especially possible since some proliferation occurred in the IL-2 cultures without the addition of antibody (Table I). Thus, the nature of the proliferating cells in IL-2 containing cultures and antibody-containing cultures was examined. T cells were cultured in the presence of either S511, anti-DNP, IL-2, or PHA and additional irradiated non-T cells, washed twice in media on day 7, and then cultured for two additional days in media containing 10% IL-2. The results of immunofluorescence staining at this time showed that, in S511-stimulated cultures, 34% of the cells were S511<sup>+</sup> (Fig. 2A), 88% were OKT-3<sup>+</sup>, and 40% were Ia<sup>+</sup>. This expansion of the S511 population compares with 0.9% S511<sup>+</sup> cells in the anti-DNP cultures (Fig. 2B) and 1.9% in the media control cultures. Fluorescence staining of cells activated by PHA for either 3 or 7 d also showed only 2.2 or 1.2% S511-bearing cells, respectively. Those cells activated by the IL-2 preparation were also analyzed for S511 expression. As shown in Fig. 2C, only 0.6% of these proliferating cells were S511<sup>+</sup>. Thus, the proliferating cell in the S511-stimulated culture is primarily the S511-bearing lymphocyte rather than nonspecifically recruited cells, and the proliferation observed in the IL-2-containing cultures (Table I) is not due to selective expansion of S511-reactive cells.

*Cell Cycle Analysis of Circulating S511-bearing T Lymphocytes.* The preceding experiments indicate that mAb S511 could specifically activate the circulating S511-reactive cell in normal individuals. Previous studies (2, 3, 5, 6) in which cells were stimulated with mAb detecting the T cell antigen receptor have used cells that were previously activated. To investigate whether the S511-bearing cell was resting or was previously activated and therefore analogous to these cell lines, T lymphocytes reacting with mAb S511 were studied by cell cycle analysis.

T lymphocytes were stained by indirect immunofluorescence with either the second antibody, FITC-conjugated goat anti-mouse immunoglobulin (GAMG), alone or with mAb 9.6, which detects the sheep erythrocyte receptor, or mAb S511. After surface staining and fixation, the cells were analyzed for DNA content measured by propidium iodide fluorescence. To discriminate between single cells in the S+G<sub>2</sub>/M phase of the cell cycle and clumps of cells artifactually induced by the fixation procedure, the initial gating for flow cytometry compared pulse width vs. pulse area of the red DNA fluorescence signal. For a clump of

cells, such as a doublet with a diploid chromosome number in each cell, the total DNA fluorescence will be the same as a single dividing cell with a tetraploid chromosome number; however, the time of flight across the laser beam, as measured by pulse width, frequently will be longer for the doublet than the single cell, while the total area under the signal peak could be similar. Thus, to discriminate between single cells with S+G<sub>2</sub>/M DNA content and the artifactual clumps with doubled or higher multiple DNA content, these gating parameters were useful. The gate that was used extended along the pulse area axis to include at least the region where single cell G<sub>2</sub>/M fluorescence would occur. For this reason, a few of these clumped doublets were necessarily included in the gate, while most of the clumps with a pulse width greater than that detected for the majority of single cells were excluded. This artifact can be seen in the last column of Table II and will be discussed below.

The analysis of the peripheral blood T cells of one individual studied in this manner is displayed in Fig. 3. The FITC-GAMG antibody control, Fig. 3A, demonstrated that almost all counts are clustered in the region where there is no FITC surface fluorescence or G<sub>0</sub>/G<sub>1</sub> in the cell cycle (region 1). Analysis with mAb 9.6, Fig. 3B, showed that almost all of the cells possessed the sheep

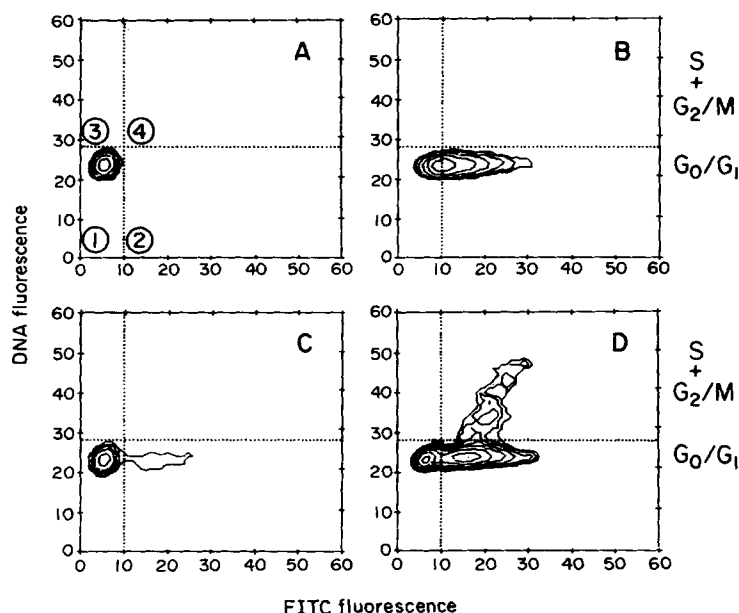


FIGURE 3. Simultaneous determination of DNA fluorescence using propidium iodide for cell cycle analysis and FITC fluorescence for indirect immunofluorescence of surface antigens of T lymphocytes. All samples were stained with saturating amounts of propidium iodide and different mAb against surface antigens as described in Materials and Methods. In each panel the lines discriminating between G<sub>0</sub>/G<sub>1</sub> and S+G<sub>2</sub>/M cells are included. Additionally, the line separating surface fluorescence-positive and -negative regions is included to define the four possible compartments: (1) G<sub>0</sub>/G<sub>1</sub> nonfluorescent, (2) G<sub>0</sub>/G<sub>1</sub> fluorescent, (3) S+G<sub>2</sub>/M nonfluorescent, and (4) S+G<sub>2</sub>/M fluorescent. (A) Normal T cells stained with the second antibody, FITC GAMG, alone. (B) Normal T cells stained with mAb 9.6, which reacts with the sheep erythrocyte receptor. (C) Normal T cells stained with mAb S511. (D) S511-enriched T cell line derived from the same individual stained with mAb S511.

erythrocyte receptor and were present exclusively in the  $G_0/G_1$  state (region 2), confirming the data obtained with the FITC-GAMG control. When T cells were stained with mAb S511 (Fig. 3C), 1.5% of the cells were surface fluorescence positive and all were  $G_0/G_1$  (region 2). No S511 fluorescent cells were detected in the region of activated cells (region 4). To confirm the ability of this technique to detect cycling cells, an IL-2-dependent cell line enriched for S511 antigen expression, which had been derived from the same donor as the normal T cells, was analyzed in an identical manner (Fig. 3D). This cell line demonstrated that S511<sup>+</sup> cells can be detected in the S+ $G_2/M$  phase of the cell cycle as well as in the  $G_0/G_1$  phase.

All three individuals used for the initial stimulation studies (Fig. 1) were analyzed using this technique to ensure that no difference existed in the cell cycle of the various S511 populations. These data are presented in Table II and confirm the results described above. All samples failed to demonstrate any cells in the FITC-nonfluorescent cycling cell compartment. As before, the FITC-GAMG control and the mAb 9.6 demonstrated that FITC-fluorescent and -nonfluorescent cells were present only in  $G_0/G_1$ . The mAb S511 stained 1.5–2.6% of the circulating T lymphocytes, all of which were in  $G_0/G_1$ . This percentage of S511-reactive cells correlated very well with our previous report of the amount of circulating S511-reactive cells. For all three individuals and all three surface stains, a population of 0.9–1.4% was consistently detected in the surface FITC fluorescence-positive cycling cell compartment. This percentage is related to the small number of doublets included in the initial gate described above and thus represents nonspecific background counts. This interpretation is

TABLE II  
*Surface Immunofluorescence and Simultaneous Cell Cycle Analysis of Normal T Lymphocytes*

Sample*	Antibody	Percentage of cells in compartment:			
		Surface fluorescence (–) $G_0/G_1$	Surface fluorescence (+) $G_0/G_1$	Surface fluorescence (–) S+ $G_2/M$	Surface fluorescence (+) S+ $G_2/M$
1	FITC-GAMG <sup>‡</sup>	97.9	0.9	0.1	1.0
	mAb 9.6 <sup>§</sup>	10.5	88.4	0	1.1
	mAb S511	96.3	2.6	0.1	0.9
2	FITC-GAMG	98.4	0.1	0.1	1.4
	mAb 9.6	10.6	88.2	0	1.2
	mAb S511	97.2	1.8	0.1	0.9
3	FITC-GAMG	98.5	0.4	0.1	1.0
	mAb 9.6	8.6	90.4	0	1.0
	mAb S511	97.5	1.5	0	0.9
4	mAb S511	20.8	50.7	0.7	27.4

\* Samples 1–3 are normal T cells; sample 4 is an IL-2-dependent T cell line derived from donor of sample 3.

<sup>‡</sup> FITC goat anti-mouse Ig, used alone or as the second antibody in indirect immunofluorescence.

<sup>§</sup> mAb 9.6 recognizes the sheep erythrocyte receptor.

supported by the finding that this value does not change for any of the surface stains and, specifically, does not increase with the S511 stain, as would be the case if any S511<sup>+</sup> cells were activated. Thus, the S511-reactive T cells exist in the G<sub>0</sub>/G<sub>1</sub> phase. These cell cycle studies of the circulating S511-bearing cells, plus the lack of preferential expansion of S511-bearing cells when activated by PHA or IL-2 described in the immunofluorescence studies above, indicate that the S511-reactive T lymphocyte does not demonstrate evidence of prior activation.

*Isolation and Expansion of S511-reactive T Lymphocytes.* The S511-bearing T cell population from normal individuals was isolated by binding to S511-Sepharose. Five normals were studied and, in each case, the S511-bearing T cells proliferated after S511-Sepharose exposure. These S511-reactive cells were expanded in IL-2-containing media and analyzed between days 14 and 21 by immunofluorescence. In each case at least 60% S511<sup>+</sup> cells could be demonstrated. When these lines were examined for expression of OKT-4 and OKT-8, both surface markers were detected (data not shown), suggesting that these early cell lines are polyclonal with respect to surface phenotype and that the S511 marker is not restricted to a specific T cell subset. One line was passaged for >2 months in IL-2-containing medium with periodic addition of S511-Sepharose. An aliquot of cells removed from this culture at that time was freed of S511-Sepharose and grown in IL-2 medium for 24 h to avoid modulation of the antigen. When these cells were stained with the S511 mAb and analyzed by flow cytometry, >90% of the cells in culture were S511<sup>+</sup> (Fig. 2D). This enrichment and expansion of the reactive cells permitted the analysis of the surface molecules bearing the S511 epitope.

*Initial Characterization of the S511 Antigen Present on a Polyclonal T Cell Line.* This cell line was analyzed before cloning to investigate two questions regarding the structure of the normal S511 molecule. First, since the molecule expressed on the original leukemic T cells differed slightly from most other molecules described, did normal cells express a similar molecule or did the malignant clone synthesize a defective molecule? Second, did the S511 molecule on these normal cells exhibit molecular heterogeneity in this uncloned population? The molecule present on the S511-bearing leukemic T cells was compared with the molecules present on the normal cell line, using immunoprecipitation and SDS-PAGE analysis (Fig. 4). The mobility of the unreduced molecule on the leukemic cells was 77 kD (compared with human transferrin as a molecular weight marker) which is comparable with our previous reports. After reduction this molecule was shown to be a heterodimer composed of two chains, with mobilities of 43 and 38 kD. In contrast to the single molecule on the leukemic cells, the unreduced molecules immunoprecipitated by S511 from the cell line migrated as a diffuse band between 77 and 90 kD. When these molecules were reduced, the heterodimeric structure was present; however, the mobility of the two chains was not identical to that of the chains present on the leukemic cell. The heavier chain showed a greater heterogeneity of mobility, migrating between 43 and 55 kD, while the lighter chain migrated between 38 and 40 kD. Thus, by immunoprecipitation and SDS-PAGE analysis, it appears that normal S511-



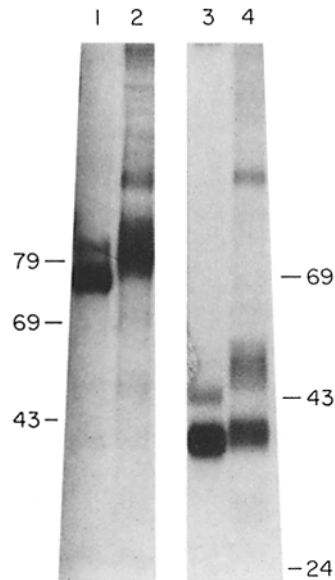


FIGURE 4. SDS-PAGE analysis after immunoprecipitation using mAb S511 of  $^{125}\text{I}$ -labeled surface molecules from the original leukemic cells (lanes 1 and 3) or a polyclonal IL-2-dependent, S511-bearing T cell line from a normal individual (2 and 4). Samples were electrophoresed using nonreducing (1 and 2) or reducing (3 and 4) conditions. A band migrating at 105 kD in 2 and 4 was not influenced by reduction and its significance is unclear. The position of the molecular weight markers is indicated. These were human transferrin (79 kD), bovine albumin (69 kD), ovalbumin (43 kD), and trypsinogen (24 kD).

bearing T cells possess a surface receptor molecule comparable in structure to that on the leukemic cells, but with greater molecular variability.

### Discussion

The present study used mAb S511 previously shown (4) to crossreact with a determinant of the T cell antigen receptor expressed on ~2% of circulating T cells from all individuals tested to date. Since this epitope is present on cells from all normal individuals, the mAb does not appear to bind to an allotypic marker like that described for the murine T cell antigen receptor (13). Our experiments attempted to answer several questions about the normal S511-bearing T lymphocytes. First, could these cells be specifically stimulated by the antibody and did they show evidence of activation before antibody exposure? Second, how did the T cell antigen receptor of the normal S511<sup>+</sup> T cells compare with that of the leukemic T cells?

The ability of mAb S511 to activate normal S511-reactive T cells in a manner analogous to antigen stimulation was investigated using soluble S511 antibody. Proliferation was induced in a time- and concentration-dependent manner by culturing normal T cells in the presence of S511 mAb and irradiated non-T cells as accessory cells. In contrast, an anti-DNP mAb and a previously described mAb (S160) that reacts with a private idiotope on the S511-bearing leukemic cells (4), failed to induce proliferation under these same conditions. Several observations suggested that mAb S511 was acting directly on S511<sup>+</sup> T cells to cause their

proliferation. The small proliferation index on day 3, when compared with that of PHA, implies that only a few cells were proliferating at this point. Extensive proliferation of S511<sup>+</sup> cells was suggested when the peak proliferation index of S511 cultures (61 on day 7) was compared with the peak value obtained with PHA (222 on day 3). Immunofluorescence analysis demonstrated that 34% of the cells in these S511 cultures were S511<sup>+</sup> after 7 d. T cells nonspecifically activated by PHA or by IL-2 failed to demonstrate preferential expansion of these S511-bearing T lymphocytes. Thus, a selective expansion of the S511 population, rather than nonspecific cell recruitment, appears to be responsible for most of this brisk proliferative response.

Activation of normal T lymphocytes by the antigen receptor has not been previously reported using either mAb or antisera reacting with the antigen receptor (1-6, 11-13). Normal T lymphocytes, however, have been induced to proliferate by OKT-3 (16, 17). Since the T3 molecule co-modulates with the antigen receptor molecule (2, 4, 5) and is closely associated with the 43 kD beta chain (18), this nonclonally restricted, T3-induced activation probably uses the same mechanism as antigen receptor-induced proliferation. Thus, the major differences between the activation of normal T cells by OKT-3 and S511 are the number of cells activated and the molecule within the antigen receptor complex that is bound by the mAb. The different kinetics of S511 activation compared with OKT-3 activation probably reflect the small number of cells activated, similar to the proliferation of small populations in antigen-specific systems (19-21) or mixed lymphocyte reactions (22, 23).

Our initial studies of S511-induced activation used T cells and a small percentage of irradiated nonrosetting cells to function as accessory cells. The requirement for these additional cells was investigated by culturing T cell preparations alone with antibody. Results indicated that there were no major differences in the extent of proliferation generated with or without added accessory cells. T cells alone were also used in developing S511-reactive lines by S511-Sepharose activation. Previous studies (5) in this laboratory have demonstrated that leukemic T cells containing <1% monocytes can be induced to proliferate by an anti-T cell receptor mAb. Studies using T cell lines and hybridomas also may not require the addition of accessory cells to induce clonal proliferation by clonotypic mAb (2, 6, 24). Similarly, an alternate pathway of T cell activation using mAb reacting with the T11 molecule may not require the presence of monocytes (25). Since the rosetted T cells used in our experiments probably contain a few monocytes, and since the S511 mAb is IgG2b and would be expected to bind fairly well to Fc receptors of accessory cells, the absolute requirement for these cells cannot be determined from our studies. However, the above-mentioned observations together suggest that accessory cell populations may not be essential for antibody-induced activation.

Several laboratories, including our own, have shown (2, 3, 5, 6, 13) that, when T cell lines, hybridomas, or leukemias were cultured in the presence of either soluble or Sepharose-bound anti-T cell receptor mAb, a specific proliferative response could be generated. In each of these systems, the target T cells were previously activated. The S511-bearing normal cells were studied to determine if these cells were similarly activated. By cell cycle analysis at least 98% of

circulating T cells were present in the  $G_0/G_1$  phase, and all S511-reactive cells were in  $G_0/G_1$ . These findings, based on DNA analysis of individual S511-bearing cells, are in agreement with studies by Darzynkiewicz et al. (26, 27) and previous work (reviewed in 28) that simultaneously analyzed DNA and RNA content of cells and demonstrated that circulating lymphocytes are normally in the  $G_0$  or  $G_{1Q}$  phase (27). Since the Tac antigen has been shown (29) to be expressed 8–16 h after activation even without an increase in cellular DNA content, cells were cultured in IL-2-containing media as a different method of detecting activation. Immunofluorescence analysis demonstrated that no preferential increase in the S511-reactive cells occurred. These two results suggest that the S511-reactive cells are not activated *in vivo*. The possibility of a nonquantifiable degree of preactivation, however, cannot be totally excluded, since the assays used sheep erythrocyte-rosetted T cells and, as mentioned above, the erythrocyte receptor has been implicated as an alternate pathway of T cell activation (25). Thus, the present study extended our prior observations on the presence of a small S511-reactive population in the circulation of normals, demonstrating the lack of prior quantifiable activation of these cells.

The ability to stimulate normal S511-reactive T lymphocytes was used to develop IL-2-dependent cell lines expressing the S511 epitope. The leukemic S511 molecule has been shown to differ from most other antigen receptor molecules in the mobility of the two chains (43 and 38 kD) and the isoelectric points (pI) of each chain (near neutral pI) (1–6). SDS-PAGE analysis of the S511-bearing normal T cell line demonstrated that the relative mobility of the S511 molecule that was derived from normal cells was comparable to that of the molecules on the leukemic cells. Thus, a subset of normal T lymphocytes routinely synthesizes this form of antigen receptor molecule, implying that the disparity in mobility cannot be ascribed to a biosynthetic defect present in the malignant cells. One possible explanation for these differences is the presence of differences in glycosylation. That glycosylation accounts for a large portion of the molecular weight of the T cell antigen receptor has become evident from recent studies (7–10, 30). If this is the basis for the mobility differences in the S511 molecule, then S511<sup>+</sup> normal cells glycosylate either one or both polypeptide chains differently than most other antigen receptor molecules described. Since differential glycosylation can modulate the function of a common IgE-binding factor (31) and sugar moieties play a role in immune regulation by suppressor T cells (32, 33), differences in glycosylation of the antigen receptor may have a functional significance. Another explanation for the differences in mobility is the synthesis of a polypeptide different than those previously described. Although current evidence does not suggest the existence of T cell receptor isotypes, recent studies indicate that this possibility cannot be completely excluded (34, 35). Both of these possible explanations are under study.

### Summary

A previous study from this laboratory described a monoclonal antibody, S511, that reacted with the T cell antigen receptor on a human T cell leukemia and also on 1–2% of circulating T lymphocytes in all normal individuals tested. The data presented in the present study demonstrate that, when normal T lympho-

cytes are cultured with or without irradiated non-T cells in the presence of soluble S511 antibody, a concentration- and time-dependent proliferation of the S511-reactive population occurred. Proliferation indices as high as 184 times greater than control were observed, which represents a major stimulatory effect on the initially minor S511<sup>+</sup> subset. When S511<sup>+</sup> cells were studied for evidence of prior activation, they were shown to be unresponsive to interleukin 2 (IL-2) unless exposed to S511 antibody, and were shown to be in the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle. Thus, the S511 antibody activated resting normal T cells in a manner analogous to specific antigen binding to the T cell antigen receptor.

The leukemic S511 molecule has been shown previously to differ from most other antigen receptors in the mobility of the two chains at 43 and 38 kD and the neutral isoelectric point of each chain. Expansion of reactive normal cells by S511-Sepharose permitted the development of IL-2-dependent T cell lines enriched for S511-bearing cells. The antigen receptor molecules on one such polyclonal S511-enriched T cell line were immunoprecipitated with S511 antibody and shown to have comparable mobility to that present on the leukemic cells, but to possess a greater heterogeneity of mobility. Thus, the leukemic cells and normal cells express similar T cell receptor molecules. The differences in the S511 T cell antigen receptor molecule possibly relate to differences in glycosylation or polypeptide structure.

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