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Immunology

Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor–ligand complex

(lymphokine/T lymphocyte/hormone–receptor complex)

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ABSTRACT **Xenogeneic monoclonal antibodies were prepared to the murine interleukin 2 (IL-2)-dependent HT2 cell line. One rat IgM monoclonal antibody (7D4) was identified that inhibited proliferation of the HT2 cells and of IL-2-dependent CTLL cells in the presence of crude rat IL-2 as well as of purified human IL-2. The level of inhibition was dependent on both antibody and IL-2 concentration. Cell distribution studies using a fluorescence-activated cell sorter showed that the antigen identified by 7D4 is expressed at a high density on HT2 cells and on concanavalin A (Con A)-induced T-cell blasts and at a substantially lower density on lipopolysaccharide-induced B-cell blasts; 7D4 binding was not detected on >95% of nonactivated thymocytes, T cells, or B cells. Competition binding studies indicated that 7D4 fails to inhibit the binding of ^{3}H-labeled human IL-2 to CTLL cells. However, 7D4 specifically immunoprecipitated ^{3}H-labeled human IL-2 from detergent extracts of HT2 cells or Con A-induced T-cell blasts that had been pulsed with [^{3}H]IL-2; in contrast, 7D4 did not react with free [^{3}H]IL-2. Initial biochemical analysis of immunoprecipitates with 7D4 of detergent extracts from surface-iodinated Con A-activated spleen cells showed a major band having apparent molecular weight of 48,000–62,000. Collectively, these results suggest that 7D4 detects an epitope on the IL-2 receptor distal to the ligand binding site or another molecule that physically associates with the receptor.**

Interleukin 2 (IL-2)$^+$, a glycoprotein of M_r 15,000 (1, 2), is secreted by T lymphocytes after stimulation with mitogen, antigen, or alloantigen (3) and functions as a hormone-like growth factor for many subsets of T cells. The use of IL-2 has permitted the establishment of long-term lines and clones of untransformed T cells that have distinct biological function and antigen specificity (4). Furthermore, the development of several murine cell lines—e.g., HT2 (5) and CTLL (6)—that solely depend on an exogenous source of IL-2 for growth has provided a definitive assay cell population to assess the presence of IL-2 in crude culture fluids containing many biologically active molecules and has greatly aided the purification and biochemical characterization of this lymphokine.

IL-2 exerts its growth-promoting properties after interaction with specific membrane binding sites (IL-2 receptors) expressed on activated, but not on resting, T cells (7–9). After IL-2 associates with its receptor, it is internalized and undergoes lysosomal degradation (9). The relationship between the degradation and biological function of IL-2 is unclear. Further delineation of the molecular structures and pathways involved in the IL-2 growth mechanism should be facilitated as monoclonal antibodies reactive with the specific components of the IL-2 hormone–receptor complex become available. Recently, monoclonal antibodies to IL-2 (10, 11) and the human IL-2 receptor (12) have been described. In the present paper, we report the characterization of a rat monoclonal antibody (7D4) that is reactive with an epitope either on the murine IL-2 receptor distal to the IL-2 hormone binding site or to another molecule physically complexed with the IL-2 receptor.

MATERIALS AND METHODS

Animals. Inbred strain 2 guinea pigs, C57BL/6 and BALB/c mice, and Lewis rats were obtained from the Animal Production Section, Division of Research Services, National Institutes of Health (Bethesda, MD).

Cell Lines. The properties of the murine IL-2-dependent HT2 cells and of CTLL cells have been described by Watson (5) and by Baker *et al.* (6), respectively. The nonsecretor hybridoma cell line SP2/0-Ag-14 (SP2/0) was used for all fusions.

Antisera and Monoclonal Antibodies. Heterologous class-specific antisera to rat IgM, IgG, and IgA were obtained from Cappel Laboratories (Cochranville, PA) and used to determine the isotypes of rat monoclonal antibodies by Ouchterlony analysis of concentrated culture supernatant fluids. The following culture supernatants containing rat monoclonal antibodies to murine lymphocyte surface antigens were used: 53.7.3 (anti-Lyt-1) and 53.6.7 (anti-Lyt-2), developed by Ledbetter and Herzenberg (13) and obtained from J. Ashwell (National Institutes of Health); Bet-2 (anti-IgM), developed by and obtained from John Kung (National Institutes of Health; ref. 14); J1j (anti-Thy-1), developed by Bruce *et al.* (15) and obtained from R. Germain (National Institutes of Health). A murine monoclonal antibody to human IL-2 was prepared by R. Robb. The MAR 18.5 monoclonal antibody is a murine antibody to rat κ chains prepared by Lanier *et al.* (16) and obtained from John Kung. A cytotoxic mouse monoclonal antibody to Thy-1.2 was obtained from New England Nuclear and used to prepare B-cell populations.

Production of Monoclonal Antibody. Lewis rats were immunized by intraperitoneal injection of 60×10^6 HT2 cells in complete Freund's adjuvant (Difco). After 2 mos, each animal received a second injection of 60×10^6 HT2 cells intraperitoneally in RPMI 1640 medium (Biofluids, Rockville, MD). Four days later, the spleen was removed and fused to hybridoma SP2/0 by using polyethylene glycol 4000 (E. Merck, Darmstadt, Germany) as described by Oi and Herzenberg (17). After fusion, the cell suspension was distributed in flat-bottomed microtiter plates (Costar 3596) at 1×10^6 cells per well in hy-

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Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; IL-2, interleukin 2; LPS, lipopolysaccharide.

poxanthine/aminopterin/thymidine (HAT)-containing Dulbecco's modified Eagle's medium supplemented with glucose at 4.5 g/liter (Gibco) and with nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), glutamine (300 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), 5-fluorocytosine (1 μg/ml), and 20% fetal calf serum. After sufficient growth (10–14 days), culture supernatant fluids were screened in a functional assay that assessed the ability of antibodies to inhibit proliferation of the HT2 cells to IL-2. Since HAT medium itself inhibited this assay, it was necessary to wean cultures to HAT-lacking medium prior to screening for antibodies. Hybrids secreting antibodies of the desired specificity were cloned and recloned at least once by limiting-dilution methods at one-hybrid cell per well in 0.2 ml of medium containing 0.5–1 × 10^6 BALB/c thymocytes per well.

Assays of IL-2-Driven Proliferation. Unfractionated supernatant fluids containing guinea pig or rat IL-2 as well as purified human IL-2 and ^{3}H-labeled human IL-2 were prepared as described (9, 18). Assays of IL-2-driven proliferation were carried out by measuring [^{3}H]thymidine incorporation of the test cell population [4 × 10^3 of HT2 and CTLL; 5 × 10^4 of concanavalin A (Con A)-induced T-cell blasts] stimulated by IL-2-containing supernatant fluids at various dilutions at 37°C for 24–48 hr in RPMI 1640 medium supplemented with glutamine (300 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), 5-fluorocytosine (1 μg/ml), α-methyl mannoside (20 mg/ml), 50 μM 2-mercaptoethanol, and 10% fetal calf serum as described (18). The results of triplicate cultures are expressed as Δcpm—i.e., cpm with IL-2 minus cpm with medium alone. The percentage inhibition was calculated from the formula: % inhibition = [1 − (Δcpm with antibody/Δcpm without antibody) × 100]. The SEM was generally <10% of the mean and for simplicity only means are reported.

Fluorescence Staining. The cells (1 × 10^6) were first incubated with excess culture supernatant containing the monoclonal antibody for 30 min at 4°C and then washed three times with Hanks' balanced salt solution containing 0.1% NaN_3 and 3% fetal calf serum. These cells were stained with excess purified fluorescein-conjugated monoclonal mouse anti-rat κ chain antibody (MAR 18.5) and then analyzed on a fluorescence-activated cell sorter (FACS II; Becton Dickinson).

Cell Surface Labeling, Immunoprecipitation, and NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. From 40 to 60 × 10^6 Con A-induced blast cells were labeled with Na^{125}I (New England Nuclear) by lactoperoxidase (Sigma)-catalyzed cell surface iodination without carrier K^{127}I (19). Labeled membrane proteins were extracted with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, Calbiochem) in 0.05 M Tris·HCl/0.3 M NaCl/5 mM EDTA, pH 7.4, containing 0.02% NaN_3 1 mM phenylmethylsulfonyl fluoride, soybean trypsin inhibitor (50 μg/ml), and aprotinin (50 μg/ml). The detergent extracts were cleared by serial incubation with Sepharose 4B, first containing mouse IgG and then containing MAR 18.5, for 30 min at 4°C. The MAR 18.5-coupled Sepharose beads were allowed to react with culture supernatants containing the monoclonal antibody, washed four times, and then used for immunoprecipitation by incubation with the previously cleared extract for 30 min at 4°C with periodic mixing. Immunoprecipitates were washed three times with 0.05 M Tris·HCl/0.3 M NaCl/5 mM EDTA, pH 7.4, containing 0.2% CHAPS/0.2% deoxycholate/0.1% NaDodSO$_4$/1 M sucrose. The samples were boiled for 5 min in buffer containing 5% NaDodSO$_4$/0.1 M dithiothreitol and then analyzed by 0.2% NaDodSO$_4$/polyacrylamide gel electrophoresis in 7–12.5 linear gradient slab gels (20). After electrophoresis, the gel was fixed with 20% trichloroacetic acid, stained with Coomassie blue, destained, and dried, and autoradiographs were produced using a Kodak X-Omatic Intensity Screen at −70°C for 1–5 days.

RESULTS

Production of Rat Monoclonal Antibodies to the Murine HT2 Cell Line. Lewis rats were immunized with the murine cloned IL-2 dependent HT2 cell line in complete Freund's adjuvant and, after a booster injection, the spleen cells from these animals were fused to the nonsecretor SP2/0 hybridoma line. To identify antibodies that might react with the IL-2 receptor or other cell surface antigens that participate in the IL-2-driven growth mechanism, a functional screening assay was used that tested for antibodies that inhibited proliferation of HT2 cells in the presence of IL-2. In one fusion, 8 of 238 positive wells contained antibodies that significantly inhibited (>60%) IL-2-driven proliferation of HT2 cells. Of these 8, one monoclonal antibody (7D4) was obtained that consistently inhibited this response. Monoclonal antibody 7D4 was cloned and recloned once at one cell per well by the limiting-dilution technique. Ouchterlony analysis of concentrated culture fluids indicated that 7D4 was a rat IgM monoclonal antibody.

The Effect of Antibody 7D4 on IL-2-Dependent Proliferation. The culture fluid containing monoclonal antibody 7D4 was tested in several assays for its ability to inhibit IL-2-driven

Table 1. Effect of 7D4 on IL-2-driven T-cell proliferation

Exp.	IL-2	Antibody	[^{3}H]Thd incorporation, Δcpm	% inhibition
		HT2 cells		
1	Rat (1:80)	—	61,900	—
		7D4 (1:4)	11,000	82
		(1:100)	28,800	53
		(1:500)	58,600	5
		Control (1:4)	73,600	0
	Guinea pig (1:8)	—	112,900	—
		7D4 (1:100)	1,400	99
		(1:500)	900	99
		(1:2,500)	18,900	83
		CTLL cells		
2	Purified human (0.08 ng)	—	9,300	—
		7D4 (1:8)	1,300	86
		anti-IL-2 (1:8)	2,000	78
		anti-Lyt-1 (1:8)	9,700	0
		C57BL/6 Con A-activated blasts		
3	Rat (1:80)	—	107,000	—
		7D4 (1:8)	17,800	88
		anti-Lyt-2 (1:8)	111,200	0
		anti-IgM (1:8)	86,600	19
		anti-Lyt-1 (1:8)	114,500	0
		Control (1:8)	107,500	0
		Strain 2 guinea pig Con A-activated blasts		
4	Guinea pig (1:8)	—	80,200	0
		7D4 (1:8)	89,000	0

HT2 or CTLL cells (4 × 10^3) or Con A-induced blasts (5 × 10^4) were cultured with the indicated dilutions or amount of IL-2 and supernatants containing rat anti-mouse monoclonal antibodies for 24–48 hr. The following antibodies were used: anti-Lyt-1 (53.7.3), anti-Lyt-2 (53.6.7), anti-IgM (Bet-2), and control (supernatant from SP2/0). Radioactivity (cpm) of cells cultured in medium alone was: HT2, <1,000; CTLL, <1,000; C57BL/6 Con A-activated blasts, 4,100; strain 2 guinea pig Con A-activated blasts, 10,200.

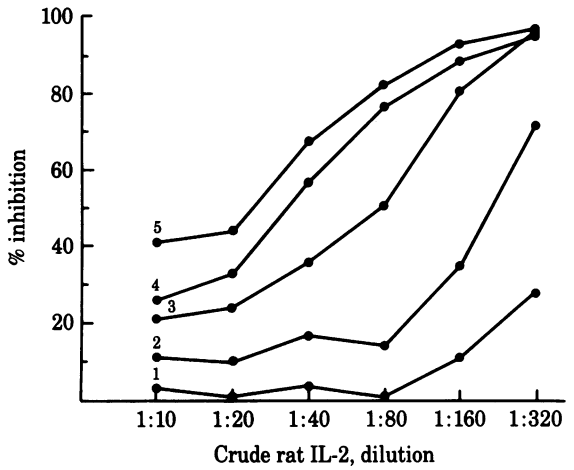

FIG. 1. Inhibition of IL-2 driven proliferation of HT2 cells by purified 7D4. HT2 cells (4×10^3) were cultured with graded doses of crude rat IL-2 and various amounts 7D4. Curves: 1, 15 ng; 2, 60 ng; 3, 250 ng; 4, 1 μg; 5, 4 μg of 7D4, respectively.

proliferation (Table 1). Antibody 7D4 inhibited proliferation of HT2 cells in the presence of crude rat or guinea pig IL-2 (experiment 1). Interestingly, ≈600-fold more 7D4 was required for 82–83% inhibition of proliferation of HT2 cells when IL-2 obtained from rat rather than guinea pig T lymphocytes was used. 7D4 as well as a monoclonal antibody to human IL-2 inhibited proliferation of the murine IL-2-dependent CTLL cell line in the presence of human IL-2 that had been purified on an immunoaffinity column containing a monoclonal antibody to human IL-2 (experiment 2). Thus, 7D4 was capable of inhibiting IL-2-dependent proliferation in a situation in which purified human IL-2 was the only exogenous lymphokine present. 7D4 also inhibited proliferation of C57BL/6 Con A-induced blast cells in the presence of crude rat IL-2; rat monoclonal antibodies to Lyt-2 (53.6.7) or Lyt-1 (53.7.3) that bind to T-cell blasts as well as a rat monoclonal antibody to mouse IgM or culture fluids from the SP2/0 line had no significant inhibitory effect (experiment 3). Thus, inhibition of IL-2-driven proliferation does not occur by antibody merely binding to an IL-2-responsive cell. Culture supernatants containing 7D4 did not inhibit the ability of guinea pig Con A-induced blast cells to respond to guinea pig IL-2 (experiment 4). This result also suggests that it is unlikely that 7D4 inhibits IL-2-driven proliferation by functionally inactivating free IL-2 or by reacting with cell-bound IL-2 because only murine HT2 cells, and not guinea pig Con A-induced blasts, were inhibited by 7D4 when guinea pig IL-2 was used.

7D4 was purified on an immunoaffinity column containing a mouse anti-rat κ chain monoclonal antibody (MAR 18.5) and tested for its ability to inhibit proliferation of HT2 cells in the presence of graded doses of crude rat IL-2 (Fig. 1). At suboptimal concentrations of crude rat IL-2, as little as 60 ng of purified 7D4 significantly inhibited proliferation. However, at maximal concentrations of crude rat IL-2, 4 μg of purified 7D4 was required for partial (≈40%) inhibition of proliferation. Thus, it appears that the inhibition of IL-2-dependent proliferation is related to the concentration of antibody as well as to the concentration of rat IL-2.

Cell Distribution of Antigen Detected by Antibody 7D4. The cell distribution of the surface antigen defined by 7D4 was determined by indirect immunofluorescence staining of a variety of murine cell populations and subsequent analysis on a FACS (Fig. 2). These cells were also examined for the expression of Thy-1 (monoclonal antibody J1j) and IgM (using monoclonal antibody Bet-2) (data not shown). The antigen defined by 7D4 was not detected on unfractionated spleen cells, thymocytes, or nylon wool-purified T cells (>90% Thy-1$^+$). 7D4 reactivity was easily detected on HT2 cells and Con A-induced T-cell blasts (>95% Thy-1$^+$) and was also found at a substantially lower intensity on essentially all lipopolysaccharide (LPS)-induced B-cell blasts (>95% IgM$^+$). Thus, 7D4 appears to define a surface antigen of activated lymphocytes.

Anti-7D4 Reacts with the IL-2 Receptor–Ligand Complex. Based on the inhibition of IL-2-driven proliferation and on the cell distribution studies, one candidate for the antigen defined by 7D4 is the IL-2 receptor. We attempted to determine whether 7D4 was able to block the binding of ^{3}H-human IL-2 to murine CTLL cells in a ligand–receptor binding assay (9). Significant inhibition of IL-2 binding by 7D4 was not consistently observed even under conditions of high antibody (≈3 mg of purified 7D4) and low [^{3}H]IL-2 (≈1 ng) concentrations. Conversely, ≈100 ng of unlabeled human IL-2 failed to block the binding of ≈1 ng of ^{125}I-labeled purified 7D4 (data not shown).

Since the binding affinity of IL-2 to its receptor is very high

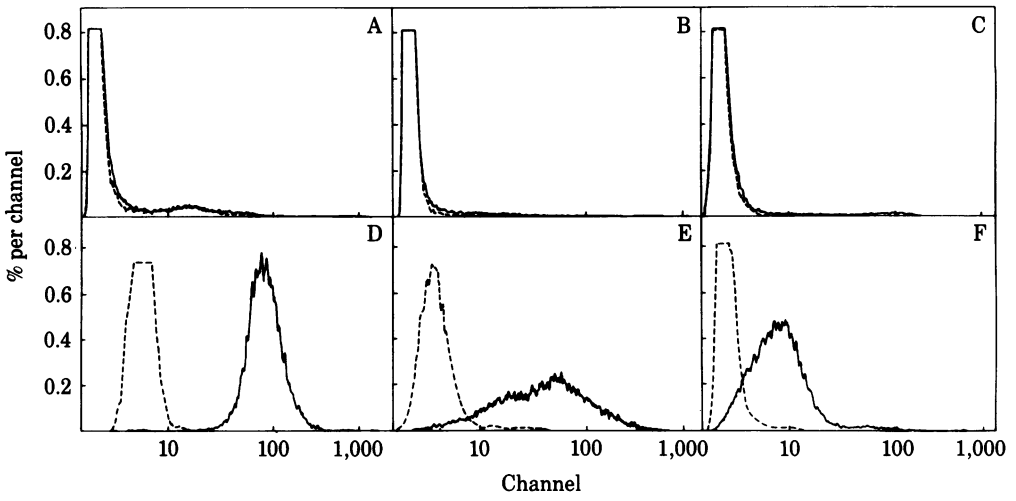

FIG. 2. Cell distribution of the antigen defined by antibody 7D4 on the FACS. Various cell populations were allowed to react with control supernatant (----) or 7D4 supernatant (——) and then stained with excess fluorescein isothiocyanate-conjugated mouse anti-rat κ monoclonal antibody (MAR 18.5). (*A*) C57BL/6 spleen cells. (*B*) C57BL/6 T cells. (*C*) BALB/c thymocytes. (*D*) HT2 cells. (*E*) C57BL/6 Con A-induced T-cell blasts. (*F*) C57BL/6 LPS-induced B-cell blasts.

[$K_d \approx 10^{-11}$ to 10^{-12} M (9)], we hypothesized that the ligand–receptor interaction would be stable in detergents that do not disrupt noncovalent antibody–antigen interactions. Therefore, the ability of 7D4 to precipitate the IL-2 ligand–receptor complex was assessed. Murine HT2 cells or C57BL/6 Con A-stimulated spleen cell blasts were pulsed with ^{3}H-labeled human IL-2; ≈90% of the input [^{3}H]IL-2 was cell bound and this binding was specific because unlabeled human IL-2 inhibited >98% of this binding. Nonidet P-40 extracts were prepared from these cells and subjected to immunoprecipitation analysis with 7D4 and other rat monoclonal antibodies to murine lymphocyte cell surface antigens (Table 2). Only 7D4 specifically immunoprecipitated a significant amount (16–29%) of the cell-associated [^{3}H]IL-2. In contrast, 7D4 immunoprecipitated <1% of free [^{3}H]IL-2. If the detergent CHAPS was used instead of Nonidet P-40, 56% of the [^{3}H]IL-2 extracted from pulsed Con A-induced blasts was precipitated by 7D4. As an additional specificity control for this assay, radiolabeled IL-2 was not precipitated by 7D4 when mock immunoprecipitations were carried out with [^{3}H]IL-2-containing detergent extracts from cell lines that did not contain the cell surface antigen defined by 7D4 (data not shown). Thus, although 7D4 does not effectively compete for the binding of IL-2 with its receptor, it appears that 7D4 is reactive with a molecular species that is associated with IL-2 binding capacity.

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis Characterization of Antigen Defined by Antibody 7D4. The molecule(s) reactive with 7D4 was further characterized by NaDodSO$_4$/polyacrylamide gel electrophoresis analysis of precipitates prepared from detergent extracts of C57BL/6 Con A-induced blast cells that had been ^{125}I-labeled by lactoperoxidase-catalyzed iodination. Under reducing conditions, 7D4 immunoprecipitated material that appeared as a diffuse band of apparent molecular weight 48,000–62,000 (Fig. 3, lane A). Under nonreducing conditions and similar electrophoresis, this material migrated to a similar position, suggesting that it is not comprised of disulfide-bonded subunits (data not shown). No bands were observed on NaDodSO$_4$/polyacrylamide gel electrophoresis when the extract was precipitated with MAR 18.5 treated with SP2/0 control supernatant (Fig. 3, lane B). 7D4 did not precipitate any material from extracts of ^{125}I-labeled thymocytes (data not shown).

Table 2. 7D4 specifically precipitates cell-associated [^{3}H]IL-2

	% [^{3}H]IL-2 precipitated				
	IL-2-pulsed HT2		IL-2-pulsed C57BL/6 Con A blasts		
Antibody	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 3
7D4	29	16	17	21	56
Anti-Thy 1	—	<1	2	—	—
Anti-Lyt-1	<1	<1	2	<1	—
Anti-Lyt-2	—	—	<1	<1	—
Anti-IgM	—	<1	<1	<1	—
Anti-rat κ	—	<1	1	<1	<1

HT2 or C57BL/6 Con A-induced blasts ($20–60 \times 10^6$) were pulsed with 0.1 unit of ^{3}H-labeled human-IL-2 for 45 min at 4°C. Detergent extracts (experiments 1 and 2, Nonidet P-40; experiment 3, CHAPS) were prepared from these cells and immediately subjected to immunoprecipitation with the indicated monoclonal antibodies, which had been adsorbed on Sepharose beads containing a mouse anti-rat κ monoclonal antibody (MAR 18.5). Data represent percentage precipitated and are means of duplicate values that varied by <1%. The input cpm for each determination varied from 400 to 800 cpm. The following antibodies were used: anti-Thy-1 (J1j), anti-Lyt-1 (53.7.3), anti-Lyt-2 (53.6.7), anti-IgM (Bet-2), and anti-rat κ (MAR 18.5). In two experiments, 7D4 precipitated <1% of free [^{3}H]IL-2.

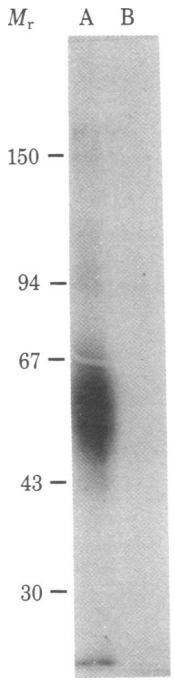

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoresis of material immunoprecipitated by 7D4. C57BL/6 Con A-induced blast cells were labeled with ^{125}I by the lactoperoxidase method and extracted with CHAPS. The extracts were subjected to immunoprecipitation with MAR 18.5-Sepharose beads coated with 7D4 (lane A) or with SP2/0 culture supernatant (lane B). The autoradiogram represents a 7–12.5% linear gradient gel run under reducing conditions.

DISCUSSION

Four lines of evidence have been presented that support the hypothesis that antibody 7D4 is reactive with the IL-2 receptor of murine T lymphocytes. First, 7D4 binds to activated, but not to resting, lymphocytes. Others have shown that only activated, but not resting, T cells are capable of either absorbing IL-2 activity (7, 8) or binding radiolabeled IL-2 (9). Second, 7D4 inhibited IL-2-driven proliferation of HT2 and CTLL cells, two cell lines that are widely used as indicators for IL-2 and that do not appear to require any other exogenous lymphokines for continued growth. Third, after ^{3}H-labeled human IL-2 was bound to HT2 cells or Con A-induced blasts, it was possible to specifically precipitate the radiolabeled IL-2 from detergent extracts of these cells with 7D4. Presumably, this reflects immunoprecipitation of the radiolabeled human IL-2 that was associated with IL-2 receptors in the extract because 7D4 was incapable of reacting with [^{3}H]IL-2 itself. Lastly, NaDodSO$_4$/polyacrylamide gel electrophoresis of the material precipitated by 7D4 and of the human IL-2 receptor (12) revealed similar characteristics (see below). Although the simplest interpretation of our data is that 7D4 reacts with an epitope on a polypeptide chain of the IL-2 receptor, we cannot exclude the possibility that 7D4 identifies a molecular species that is distinct from, yet physically associated with, the IL-2 receptor.

Dose-response experiments showed that inhibition of IL-2-driven proliferation by 7D4 was dependent on the concentration of both the antibody and IL-2 with inhibition maximal at low IL-2 and high 7D4 concentrations. This concentration dependence for inhibition suggests that 7D4 directly interferes with the IL-2 growth signal rather than affecting some generalized growth requirement of T cells.

The mechanism of inhibition of T-cell proliferation by 7D4 is not yet known. It must be emphasized that the inhibition

apparently does not occur by direct competition with IL-2 for IL-2 receptor binding sites. Other experiments indicated that 7D4 did not block the internalization and lysosomal degradation of radiolabeled human IL-2, suggesting that functional inhibition does not occur at this level. Taken together, our results are most compatible with the hypothesis that 7D4 binds to the IL-2 receptor distal to the ligand binding site with subsequent functional impairment of the growth signal. Such binding might result in lowering the affinity of IL-2 for the receptor such that a higher concentration of IL-2 is necessary to achieve cellular proliferation. Alternatively, 7D4 may lead to capping and shedding of the cell-bound IL-2 with impairment of signal transmission or 7D4 may interfere with a second signal that is dependent on the IL-2 concentration and is also required for growth. We have no information to distinguish between these possibilities. The recent description of another cell surface molecule present on guinea pig T-cell blasts distinct from the IL-2 receptor that functions in IL-2-driven proliferation suggests that the mechanism controlling T-cell growth may be complex (18).

It appears that the anti-TAC monoclonal antibody recognizes the human IL-2 receptor. It has been shown that anti-TAC not only identifies an antigen exclusively expressed on activated T cells and inhibits proliferation of IL-2-dependent cell lines but also blocks the binding of radiolabeled IL-2 to an IL-2 receptor-positive cell line (12). Although one criterion for antireceptor antibodies is their ability to inhibit hormone binding to the receptor, all antireceptor antibodies do not exhibit this property. Some antisera and monoclonal antibodies to the receptors for acetylcholine (21), insulin (22, 23), transferrin (24), and catecholamine (25) failed to block ligand binding although inhibition (21) or stimulation (22, 25) of biological function was sometimes noted. 7D4 appears to be a reagent of this type.

Biochemical characterization of the putative human IL-2 receptor from ^{35}S-labeled HUT102B cells by using anti-TAC antibody showed the presence of two major proteins of apparent $M_r \approx 113{,}000$ and 47,000–53,000. When surface-iodinated material was examined, only the M_r 47,000–53,000 protein was detected (12). $NaDodSO_4$/polyacrylamide gel electrophoresis of material immunoprecipitated with 7D4 from surface-iodinated Con A-induced blast cells revealed a major broad band from M_r 48,000–62,000. We do not know whether this diffuse band represents several distinct polypeptide chains or several molecular species of a single chain with charge or size heterogeneity and whether this material is analogous to that detected on the surface of HUT102B cells. Some of the different properties of TAC and the antigen defined by 7D4 may be related either to the species being studied or to the cells used for biochemical analysis.

In receptor–ligand binding studies using radiolabeled human IL-2, it has been reported that several murine and human IL-2-dependent cells contained 3,000–10,000 IL-2 binding sites (9). We have not yet specifically determined the number of molecules on the surface of HT2 cells or T-cell blasts that react with 7D4. However, the level of fluorescence staining observed for these two cell populations was somewhat greater than we expected for cells containing ≈10,000 receptors per cell. One explanation for this discrepancy is that the IL-2 binding assay as previously carried out might have detected only receptors that contained high-affinity binding sites.

There is some controversy concerning the role of IL-2 in B-cell growth (26–28). It has been reported that IL-2 receptors are not detected on murine LPS-induced B-cell blasts since these cells fail to bind radiolabeled IL-2 (9). However, it is clear from the FACS studies that 7D4 binds to essentially all LPS-induced B-cell blasts, although the intensity of staining is generally 4–10% of that of T-cell blasts or HT2 cells. 7D4 does not react with normal resting B lymphocytes as judged by the failure to detect any specific staining of unfractionated spleen cells. At present, it is premature to conclude that 7D4 is reacting with IL-2 receptors on B-cell blasts. It is possible that 7D4 cross-reacts with a unique differentiation antigen present on activated B cells or that it reacts with a shared molecular component of T-cell and B-cell growth factor receptors. Comparison of the molecular structures of the material immunoprecipitated by 7D4 from normal B- and T-cell blasts should help clarify this issue.

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