T CELL-ACTIVATING PROPERTIES OF AN ANTI-THY-1 MONOCLONAL ANTIBODY

Possible Analogy to OKT3/LEU-4

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With the development and widespread investigative use of monoclonal antibodies (MAb),[1] it has been possible to characterize and define a number of cell surface differentiation antigens that play critical roles both in the regulation of the immune response and in the process of intercellular communication. Specifically, MAb that define characteristic T cell antigens and T cell subsets have been described in human (1, 2) and murine (3) systems. Functional studies have demonstrated that many of these cell surface antigens which previously served only as markers are involved in specific immune functions. For example, the human pan-T cell antigen, T3, defined by MAb OKT3, Leu-4, and UCHT-1, has been shown to play a critical role in the T cell antigen recognition process (4) and may exist as a complex on the T cell surface with the antigen-specific T cell receptor (5). In addition, Lyt-2 and OKT8/Leu-2 have been postulated to act as auxiliary recognition structures for class I-restricted T cells in mice and humans (6–8); L3T4 and OKT4/Leu-3 antigens are thought to function in a similar manner for class II-restricted T cells (7–9). These molecules are thought to increase the affinity of the antigen-specific T cell receptor for its target antigen.

We have attempted to further elucidate roles for cell surface antigen involvement in T cell activation by producing MAb to T cell hybridomas and screening these MAb for their ability to modulate (stimulate or inhibit) T cell functions. This report describes the production and initial characterization of one such stimulatory rat monoclonal antibody, G7, that recognizes the murine pan T cell antigen, Thy-1. G7 is a potent mitogen for resting T cells, and also induces interleukin 2 (IL-2) secretion from normal T cells and T cell hybridomas. The possible relationship of Thy-1 to the T3 molecular complex on human T

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lymphocytes is discussed.

Materials and Methods

Experimental Animals. Outbred Swiss mice, outbred *nu/nu* mice, BALB/c mice, AKR mice, and Lewis rats were obtained from the Animal Production Unit, National Institutes of Health.

Media and Culture Conditions. Dulbecco's modified Eagle's medium (DMEM) with 4.5 g glucose/liter (M. A. Bioproducts, Walkersville, MD) supplemented with nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), glutamine (300 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), 5-fluorocytosine (1 μg/ml), 2-mercaptoethanol (5 × 10^{-5} M), and 10–20% fetal calf serum (FCS) (KC Biologicals, Lenexa, KS) was used for all cell cultures.

Cell Lines. The properties of the murine B cell lymphoma M12.4.1, an Ia-bearing tumor (10) and the interleukin 2 (IL-2)-dependent HT-2 cells (11) have previously been described. The nonsecretor hybridoma cell line, SP2/0-Ag-14 (SP2/0), was used for all B cell fusions. BW 5147, a murine AKR thymoma line was used for all T cell fusions.

Monoclonal Antibodies. The following culture supernatant fluids containing rat MAb to murine lymphocyte surface antigens were used in this study: 53.7.3 (anti-Lyt-1) was derived by Ledbetter and Herzenberg (3); 15E (anti-Thy-1.2), derived by Sarmiento et al. (12) and J1j (anti-Thy-1.2), derived by Bruce et al. (13) were obtained from Dr. M. Norcross, NIH. A purified rat MAb to murine Thy-1.2, 30-H12, was obtained from Becton, Dickinson & Co., Sunnyvale, CA. A mouse MAb to Thy-1.2 was obtained in ascites form from New England Nuclear, Boston, MA. This reagent was used to deplete T cells from spleen cell populations. A rat MAb to the murine IL-2 receptor, 7D4, has been previously described (14). The MAR 18.5 monoclonal antibody to rat kappa chain was produced by Lanier et al. (15).

IL-2 Production Assay. Assays of IL-2-driven proliferation were performed by measuring [^{3}H]thymidine ([^{3}H]TdR) incorporation of 5 × 10^3 HT-2 cells in triplicate stimulated by a 33% dilution of IL-2-containing supernatant fluids at 37°C for 28 h in complete medium. [^{3}H]TdR was added to the culture for the final 4 h. The amount of IL-2 measured in this assay in these experiments was always limiting (less than maximal IL-2-driven proliferation using concanavalin A (Con A)-stimulated rat spleen cell supernatants). The SEM was always <20% of the mean in all proliferation assays, and for simplicity, only the mean is reported.

Production and Characterization of T Cell Hybridomas. Autoreactive T cell hybridomas were made in the course of antigen-specific T hybridoma production, as previously described (16). Briefly, BALB/c mice were immunized in the footpads with pork insulin emulsified in complete Freund's adjuvant (CFA); 7 d later, draining lymph nodes were removed and restimulated with pork insulin and syngeneic antigen-presenting cells (APC) in vitro for 72 h. Recovered blasts were fused to BW 5147 cells in supplemented DMEM containing hypoxanthine, aminopterin, thymidine, and 20% fetal calf serum (FCS). After 7–10 d, growing hybrids were expanded and tested for IL-2 production when challenged with antigen and syngeneic APC. Positive hybrids were cloned at least once at limiting dilution and frozen in large quantities.

The hybridomas C6/G8 and F7/G3 were produced in the same fusion, from a BALB/c mouse immunized to pork insulin. Both were found to produce IL-2 when challenged with the syngeneic APC M12.4.1 in the presence or absence of the immunogen, pork insulin.

Production of MAb G7. A single Lewis rat was immunized intraperitoneally with 40 × 10^6 C6/G8 T hybridoma cells emulsified in CFA. 3 wk later the rat was boosted intraperitoneally with an additional 40 × 10^6 T hybridoma cells. After an additional 3 wk, the rat was boosted with 40 × 10^6 C6/G8 cells i.v. and i.p. Three days later the spleen was removed and cells fused to SP2/0 by established methods (17). Supernatants from 341 wells containing growing hybridomas were screened for their ability to either augment or inhibit IL-2 secretion by the T hybrid C6/G8 (1 × 10^5 cells per microtiter well) in the presence of irradiated M12.4.1 stimulator cells (1 × 10^4 cells per microtiter well). Three

supernatants were detected, which on initial screening augmented T hybridoma IL-2 production, but only one of these (G7) maintained this property after cloning at limiting dilution. G7 ascites was produced by injecting 5×10^6 hybridoma cells into outbred nu/nu mice that had been previously primed with pristane (Aldrich Chemical Co., Milwaukee, WI). G7 was purified by affinity chromatography on a Sepharose-Protein A column. The monoclonal antibody was determined to be of IgG_{2c} class by Ouchterlony analysis with class-specific typing antisera (Miles Laboratories, Elkhart, IN).

Cell Purification and Proliferation Assays.   Splenic T cell-enriched cells were prepared by passage over nylon wool columns. For proliferation assays, T cell-enriched cells were resuspended in medium containing 2.5% human serum and distributed into 96-well microtiter plates at 4×10^5/well (Costar, Cambridge, MA) with the indicated concentration of MAb in 200 μl of media for 4 d unless otherwise specified. The cells were pulsed with [^{3}H]TdR for the final 18 h of culture. T cell-depleted spleen cells were prepared by treating cell suspensions with anti-Thy-1.2 in the presence of guinea pig serum as a complement source.

Fluorescent Staining.   The cell population (2×10^6) selected for analysis was first incubated with an excess of G7 culture supernatant fluid for 30 min at 4°C, and then washed three times with Hank's balanced salts solution (Biofluids, Inc., Rockville, MD) containing 0.1% NaN_3 and 3% FCS. These cells were then stained with an excess of fluorescein isothiocyanate (FITC) (Calbiochem-Behring Corp., La Jolla, CA)-conjugated monoclonal mouse anti-rat kappa chain antibody (MAR 18.5) and then analyzed on a fluorescence-activated cell sorter, FACS II (B-D FACS Systems, Becton, Dickinson & Co., Mountain View, CA). For analysis of IL-2 receptor expression, splenic T cell-enriched cells were cultured at 2×10^6/ml with either Con A (5 μg/ml) or G7 (1:500 ascites dilution) monoclonal antibody. After 72 h of culture, a monoclonal antibody to the murine IL-2 receptor, 7D4, which had been conjugated to FITC, was added in excess to 2×10^6 of G7-stimulated, Con A-stimulated, or unstimulated T-enriched spleen cells.

Cell Surface Labeling, Immunoprecipitation, and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). $400-600 \times 10^6$ BALB/c thymocytes from 3–6-wk-old mice or $40-80 \times 10^6$ C6/G8 T hybridoma cells were labeled with $Na^{125}I$ (Amersham Corp., Arlington Heights, IL) by lactoperoxidase (Worthington Biochemical Corp., Freehold, NJ)-catalyzed cell surface iodination without carrier $K^{127}I$ (18). Labeled membrane proteins were then extracted with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS), in a 0.05 M Tris, 0.005 M EDTA, and 0.03 M NaCl (pH 7.4) buffer containing 1 mM phenylmethylsulfonyl fluoride, 50 μg/ml soybean trypsin inhibitor, and 50 μg/ml aprotinin.

The detergent extracts were precleared by serial incubation with Sepharose 4B, first coupled with mouse IgG, then coupled with either protein A-rabbit anti-rat Ig (Miles Laboratories) or MAR 18.5 for 30 min at 4°C. To couple antibodies to a solid-phase reactant, purified G7 was reacted with protein A-Sepharose; purified 30-H12 was reacted with rabbit anti-rat Ig-protein A-Sepharose; and culture supernatants from 15E were incubated with MAR 18.5-Sepharose for 45 min at 25°C. These antibody-coupled Sepharose beads were then washed and reacted with the previously precleared extract for 60 min at 4°C with continuous agitation. Immunoprecipitates were then washed three times through the same buffer used for membrane protein solubilization, which contained 0.2% CHAPS, 0.2% deoxycholate, and 30% glycerol. The precipitated samples were then boiled for 5 min in buffer containing 5% SDS and 0.1 M dithiothreitol and then subjected to SDS-PAGE containing 0.2% SDS in 12.5% acrylamide slab gels (19). After electrophoresis, the gel was fixed in 20% trichloroacetic acid, stained with Coomassie blue, destained, and dried, and autoradiographs were produced using a Kodak X-Omatic intensifier screen at -70°C for 1-7 d.

Results

Induction of IL-2 Secretion by MAb G7.   To produce xenogenic monoclonal antibodies to an autoreactive T cell hybridoma, a single rat was immunized with

the autoreactive T cell hybridoma, C6/G8. After fusion to the murine SP2/0 line, the resulting supernatants were screened for their ability to augment or suppress IL-2 production by the C6/G8 T hybridoma induced in the presence of the syngeneic B lymphoma, M12.4.1. After limiting dilution cloning, one MAb, G7, was found to be able to induce IL-2 secretion from C6/G8 in the presence or absence of stimulator cells (Fig. 1). However, the stimulating properties of G7 were not specific for the immunizing hybridoma as another T cell hybridoma (F7/G3) had an identical dose response curve (Fig. 1). In fact, G7 has been shown to induce IL-2 secretion from any T cell hybridoma tested that is known to be capable of IL-2 secretion as judged by its response to specific antigen plus APC, or Con A (data not shown). Also it should be noted that G7 did not stimulate HT-2 cell thymidine incorporation in multiple determinations (data not shown).

G7 was also able to induce IL-2 production from nylon column-purified splenic T lymphocytes (Fig. 2). As in the case of the T cell hybridomas, there was a concentration-dependent production of IL-2. The proliferation of HT-2 cells was generally lower when supernatants from G7-activated spleen T cells were assayed than when T hybridomas were activated by G7. One likely explanation for this finding is that the growth of T cell hybridomas is IL-2 independent while activated spleen cells both produce and consume IL-2. The T cell hybridoma supernatants allow a measurement of total IL-2 produced, while the spleen cell supernatants only indicate excess unbound IL-2 in the culture at a given point in time.
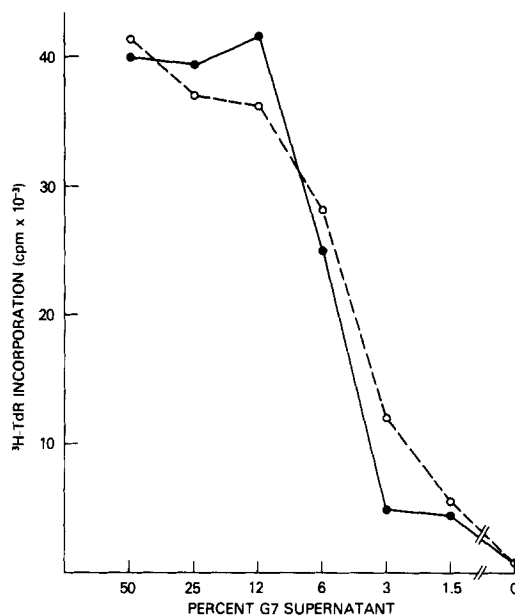

FIGURE 1. G7-induced IL-2 secretion from T cell hybridomas. Dilutions of G7 cell culture supernatant were added to 1×10^5 (C6/G8 (●) or F7/G3 (O) T hybridomas. Supernatants were removed after 48 h and added in 33% final concentration to HT-2 cells. Proliferation was measured by [^{3}H]TdR incorporation.
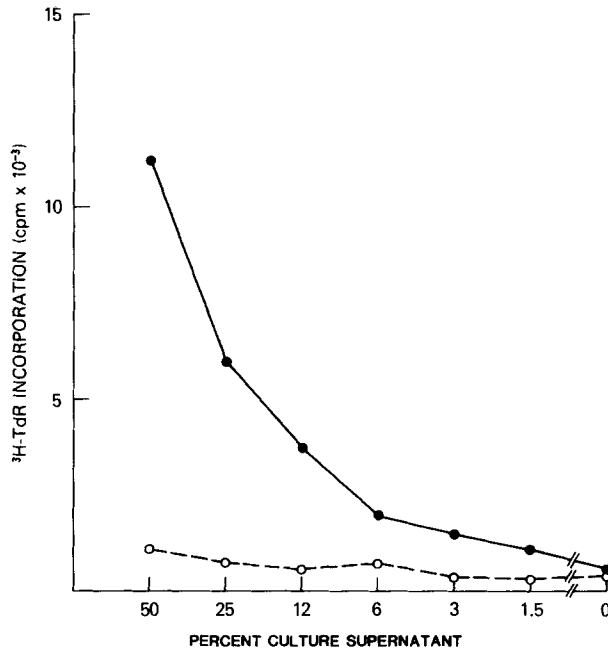
FIGURE 2.   G7-induced IL-2 secretion from T cell-enriched spleen cells. T cell-enriched spleen cells (4 × 10⁵) were treated with varying concentrations of G7 (●) or SP2/0 (○) cell culture supernatant. Supernatants were removed after 24 h and added in 33% final concentration to HT-2 cells. Proliferation was measured by [³H]TdR incorporation.

Induction of T Cell Proliferation and IL-2 Receptor Expression by MAb G7.   When T cell-enriched spleen cells were cultured for 4 d with varying concentrations of protein A-purified G7, a vigorous proliferative response was observed that plateaued at an antibody concentration of 25–50 μg/ml (Fig. 3). Kinetic studies revealed that this response peaked at 4 d of culture (Fig. 4). In general, the proliferative response to G7 was equal or greater than that observed with optimal concentrations of Con A or phytohemagglutinin (results not shown).

It has been shown (20, 21) that resting T cells cannot respond to IL-2 unless they first express IL-2 receptors. Thus, although G7 stimulated IL-2 secretion from resting T cells, this mechanism alone should not account for the proliferation of resting T cells. To test the hypothesis that G7 was also able to stimulate IL-2 receptor expression, T cell-enriched splenocytes were incubated with an optimal dilution of the G7 MAb or Con A for 72 h. After this time period the cultures were harvested, washed, and stained with an FITC-conjugated anti-IL-2 receptor antibody, 7D4. As a control, unstimulated T cell-enriched spleen cells were first incubated with G7 at 4°C, then stained with FITC-7D4. T cells activated by G7 were strongly positive for the IL-2 receptor while unstimulated T cells showed little reactivity with FITC-7D4 (Fig. 5). In this particular study, G7-activated T cells expressed a higher density of the IL-2 receptor than Con A-activated cells (Fig. 5), although in other studies both agents induced a similar level of IL-2 receptor expression (data not shown).

Tissue Distribution of the Antigen Recognized by G7.   In addition to positive
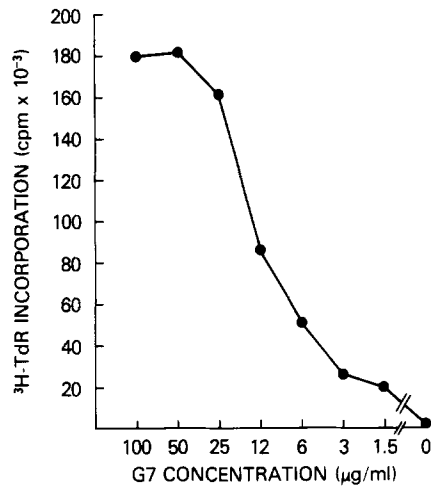
FIGURE 3. G7-induced T cell proliferation. T cell-enriched spleen cells (4×10^5) were treated with varying concentrations of protein A-purified G7 MAb. Proliferation was measured in a 4-d culture by [^{3}H]TdR incorporation.
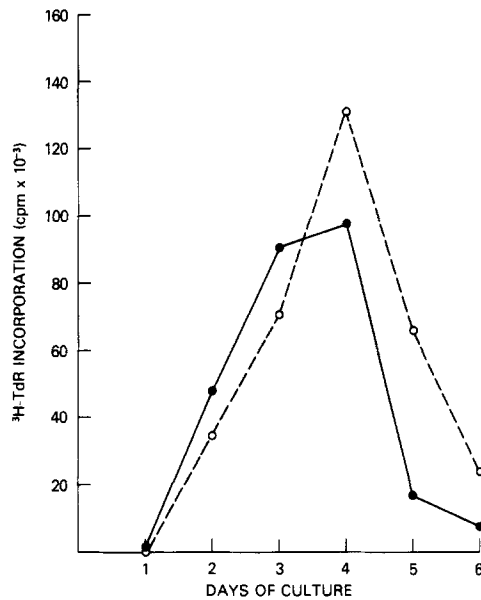

FIGURE 4. Kinetics of G7-stimulated T cell proliferation. G7 in ascites form at 1:100 (●) or 1:500 (○) dilution was added to cultures of 4×10^5 T cell-enriched spleen cells. Proliferation was measured after the indicated time period by [^{3}H]TdR incorporation.

reactivity with the immunizing T cell hybridoma (C6/G8) (Fig. 6), G7 appeared to recognize a pan-T cell antigen. FACS analysis showed the antigen recognized by G7 to be present on all T cells as well as thymocytes from outbred mice, and to be absent from splenic T cell-depleted populations and bone marrow. G7 also reacted with the BW 5147 T hybrid fusion partner (data not shown).

Immunoprecipitation Studies. BALB/c thymocytes or the C6/G8 T cell hybri-
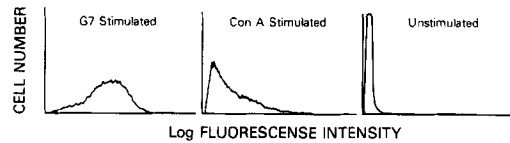
FIGURE 5. The G7 MAb stimulates IL-2 receptor expression. Resting, Con A-, or G7-activated T cell-enriched spleen cells were stained directly with FITC-conjugated 7D4 antibody.
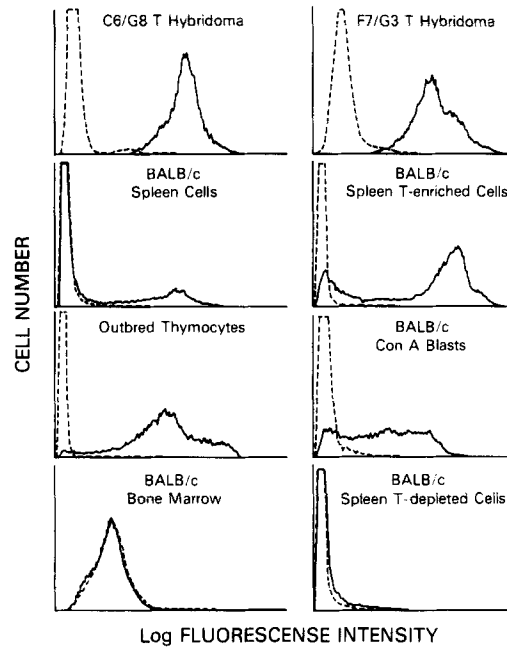

FIGURE 6. Tissue distribution of the antigen recognized by G7. Cellular populations were prepared as described in Materials and Methods. The indicated cells were either stained with G7 cell culture supernatant and a FITC-conjugated mouse anti-rat kappa chain MAb, MAR 18.5 (——), or MAR 18.5 alone (– – –), then analyzed by FACS.

doma were labeled with ^{125}I by lactoperoxidase-catalyzed iodination, followed by lysis in CHAPS, immunoprecipitation, and SDS-PAGE (Fig. 7). In thymocyte lysates, G7 precipitated a 28–32 kD protein and a less intense 50 kD (referred to as p50) protein (Fig. 7D). When extracts from thymocytes were analyzed, the lower 28–32 kD band appeared to be composed of multiple (four) discrete components. When the T hybridoma lysate was precipitated with G7, the lower molecular weight material appeared as a single band (Fig. 7, F vs. D). Under nonreducing conditions, the migration distances did not change, indicating that the proteins recognized by G7 were not subunits connected by interchain disulfide bonds (results not shown).

Because of the presence of a very prominent band of ~28 kD in the G7 immunoprecipitates and because G7 identified a pan-T cell antigen, the possibility existed that G7 recognized at least, in part, the Thy-1 antigen. When thymocyte lysates prepared with CHAPS were analyzed with two monoclonal
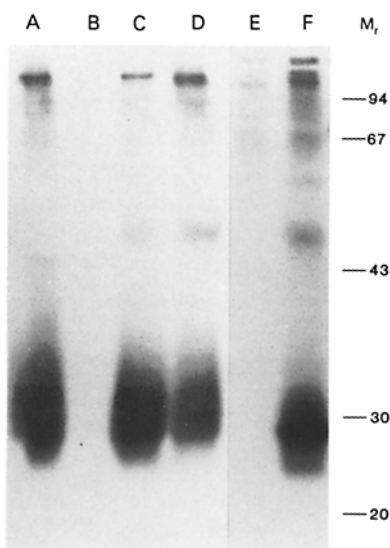
FIGURE 7.   Molecules precipitated by the G7 MAb. ^{125}I-surface-labeled BALB/c thymocytes
(*A–D*) or C6/G8 T hybridoma (*E–F*) were extracted in CHAPS and immunoprecipitated with
G7 (*D*, *F*), the anti-Thy-1.2 MAb 30-H12 and 15E (*A* and *C*, respectively), or protein A-
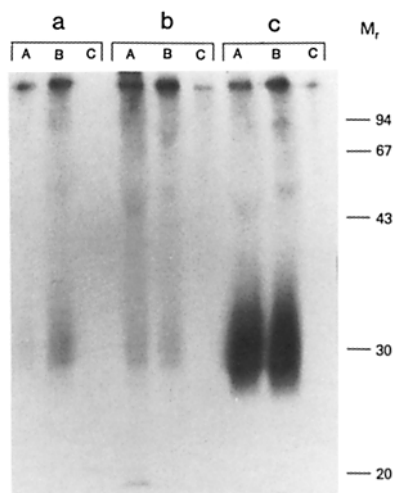Sepharose beads alone as controls (*B*, *E*).

FIGURE 8.   The G7 MAb recognizes the Thy-1 antigen. ^{125}I-surface-labeled BALB/c thymo-
cytes were extracted in CHAPS and first immunoprecipitated with 30-H12 (*a*), G7 (*b*), or
protein A-Sepharose beads control (*c*). The supernatant from each of the first immunoprecip-
itations was split into three equal parts and immunoprecipitated a second time with 30-H12
(*A*), G7 (*B*), or protein A-Sepharose beads (*C*).

anti-Thy 1.2 reagents, 30-H12 and 15E, a precipitation pattern identical to that
of G7 was observed (Fig. 7 *A* and *C*). To confirm that G7 recognized the Thy-1
antigen, a sequential immunoprecipitation study was performed on CHAPS
extracts of BALB/c thymocytes (Fig. 8). In this study, aliquots of the lysate were
first treated with an excess of G7, 30-H12, or protein A-Sepharose beads. The

supernatants from each of these three precipitates were then subjected to a second precipitation with G7, 30-H12, or protein A-Sepharose. Pretreatment with G7 removed the bands reactive with 30-H12 and pretreatment with 30-H12 markedly decreased reactivity with G7 (Fig. 8). Precipitation with protein A-Sepharose had no effect on subsequent precipitation by either G7 or 30-H12. The band represented by p50 was only faintly present in these experiments and it is difficult to state with certainty whether it was precleared by either G7 or 30-H12. The p50 band was also present in G7 immunoprecipitates performed in other detergents, and was not seen in immunoprecipitation performed with non–anti-Thy-1 MAb (data not shown).

Effects of Other Anti-Thy-1 MAb on Resting and G7-activated T Cells. Taken together the tissue distribution studies and the immunoprecipitation experiments strongly suggest that G7 recognizes the Thy-1 antigen and perhaps an additional membrane antigen (p50). To determine whether the ability of G7 to activate T lymphocytes was a unique property of this antibody, a panel of several monoclonal anti-Thy-1 antibodies was tested on both spleen T cell-enriched cells for proliferative effects and on a T cell hybridoma for IL-2 production (Table I). In these

TABLE I

Comparison of Anti-Thy-1 MAb in T Cell Proliferation and IL-2 Production Assays

Antibody*	[^{3}H]TdR Incorporation‡			
Exp. 1: T Cell Proliferation§				
	Supernatant dilution			
	1:8	1:16	1:32	1:64
G7	42,300	20,200	7,100	2,100
30-H12	1,700	900	2,700	1,500
15E	1,700	1,100	1,000	1,100
J1j	2,700	1,600	1,800	1,200
SP2/0	1,900	900	1,400	900

Exp. 2: HT-2 Proliferation (T hybrid IL-2 Production)‖				
	Supernatant dilution			
	1:4	1:8	1:16	1:32
G7	54,400	66,200	35,900	23,100
30-H12	300	100	900	1,400
15E	1,900	1,100	900	300
J1j	600	400	300	800
SP2/0	1,200	1,000	900	300

* Described in Materials and Methods.
‡ Mean rounded to nearest hundred.
§ 4 × 10^5 nylon wool-purified BALB/c spleen cells were incubated with the indicated dilution of anti-Thy-1 or SP2/0 control cell culture supernatants. After 3 d, the cultures were pulsed with [^{3}H]TdR and harvested 18 h later.
‖ 1 × 10^5 C6/G8 T hybridoma cells were cultured with the indicated dilution of anti-Thy-1 or SP2/0 control cell culture supernatant. After 48 h, supernatant was removed and added to IL-2-dependent HT-2 cells as described in Materials and Methods.

experiments, none of the other anti-Thy-1 monoclonal antibodies showed the T cell-activating properties of G7 at the dilutions tested. A mouse monoclonal antibody to the Thy-1.2 allele also had no activating effects (data not shown). In addition, MAb specific for the Thy-1.2 allele were able to block G7-mediated proliferation of T lymphocytes from BALB/c but not AKR mice (Table II).

Discussion

The Thy-1 antigen of mice was one of the first differentiation antigens to be discovered on thymocytes by Reif and Allen in 1964 (22) as alloantigens later designated Thy-1.1 and Thy-1.2. The Thy-1 antigen is coded for by a gene on chromosome nine of the mouse (23) and is a glycoprotein of 25,000–30,000 daltons found on thymocytes, peripheral T cells, fibroblasts, epithelial cells, and neurons in the mouse (22, 23). This molecule is of interest because it has amino acid sequence homology to immunoglobulin (25), implying that Thy-1 may be involved in immune recognition function. During the process of screening rat monoclonal antibodies to murine autoreactive T cell hybridomas, we identified a single monoclonal antibody to Thy-1, G7, which was a potent inducer of IL-2 production from all functioning T cell hybridomas as well as from normal T cells. G7 also was a potent mitogen for normal T cells and was as effective as Con A in its ability to induce expression of the IL-2 receptor on normal cells.

No other mitogenic monoclonal antibodies to nonidiotypic determinants on murine T lymphocytes have been identified, including a number of anti-Thy-1 reagents. However, certain anti-Thy-1.2 MAb were able to block G7-mediated T cell proliferation on Thy-1.2$^+$ cells. This implies that the epitopes recognized by these reagents are in close proximity to the G7-binding site. Alternatively, binding of the anti-Thy-1.2 reagents may induce conformational and/or allosteric changes in the molecule preventing subsequent binding of G7 irrespective of the proximity of epitopes. The failure of certain anti-Thy-1 sera, anti-Thy-1 monoclonal antibodies, or anti-Thy-1 alloantisera to activate T cells is not secondary to a failure of these antibodies to cross-link Thy-1 molecules, since Jones and Janeway (26) have demonstrated that the addition of a second layer of anti-Ig antibody sufficient to cap Thy-1 failed to stimulate the T cells to divide. On the

TABLE II
Effects of T Cell-Specific MAb on G7-Mediated T Cell Proliferation *

Antibody	[³H]TdR incorporation‡	
	AKR	BALB/c
None	161,100	227,500
Rat anti-Thy-1.2§	162,900	9,700
Mouse anti-Thy-1.2‖	180,900	1,100
Rat anti-Lyt-1¶	199,100	258,100

* 4 × 10⁵ nylon wool-purified AKR or BALB/c spleen cells were incubated with a 1:400 dilution of MAb G7 ascites and the indicated anti-Thy-1.2 or anti-Lyt-1 antibodies. The cultures were harvested as in Table I.
‡ Mean rounded to nearest hundred.
§ MAb 15E cell culture supernatant at 1:4 dilution.
‖ 1:400 dilution of ascites.
¶ 1:4 dilution of cell culture supernatant.

other hand, although the stimulatory properties of MAb G7 are unique, it has been known for several years that rabbit antisera raised against mouse brain that show Thy-1 specificity are both mitogenic and able to stimulate IL-2 secretion from murine T cells (26–28). Serological and biochemical studies by Maino et al. (29) strongly suggest that the mitogenic determinant recognized on the surface by rabbit anti-mouse brain antiserum is located on the Thy-1 molecule. In the rabbit anti-mouse brain model, T cell activation has been shown to require accessory cells that bear Fc receptors (26, 27). In contrast, G7-mediated induction of IL-2 secretion from T cell hybridomas is performed in the total absence of any type of accessory cells. Studies to evaluate the accessory cell requirements of G7-induced proliferation of normal T cells are in progress.

There are a number of interesting similarities in the functional properties, tissue distribution, and biochemical characteristics of the antigens identified by MAb G7 and those identified by monoclonal reagents, OKT3, Leu-4, and UCHT-1, to the T3 complex on human T lymphocytes. G7 and the anti-T3 reagents are both potent mitogens for resting T cells and in this respect are unique among MAb reacting with normal T cells. However, the functional properties of these reagents exhibit several differences. Although the anti-T3 reagents at low concentrations induce DNA synthesis, at higher concentrations the antibodies block the proliferative responses to soluble protein antigens and alloantigens (30) as well as both the generation and effector phases of cytolytic T lymphocyte (CTL) function (4, 30). In contrast, G7 in all concentrations thus far tested always stimulates proliferation and preliminary data indicate that G7 does not inhibit the effector phase of CTL function (results not shown). One other functional characteristic of the T3 complex is that it can be easily modulated from the cell surface by culture with anti-T3 reagents at 37°C (31); G7 thus far has failed to modulate its target antigen under a number of culture conditions.

Although the OKT3 MAb was initially reported to react with all peripheral T lymphocytes and only 10% of human thymocytes (1), Leu-4 reacted with 70–90% of human thymocytes (32). In addition, both Leu-4 and UCHT-1 reacted with cerebellar Purkinje's cells of several species (33). Thy-1 is also present on most rodent neuronal cells as reported by Campbell et al. (34). Although there have been several reports of Thy-1-positive human peripheral T lymphocytes (35, 36) and of at least one monoclonal antibody to a human Thy-1-like molecule (37), the human Thy-1 homolog is absent from the majority of human thymocytes. Also, in the murine system, a variant of the Thy-1 molecule has been described that is present only on thymocytes, but not peripheral T lymphocytes (38). Although multiple explanations exist to explain these differences in tissue distribution, one interpretation of these data would be that a basic T3/Thy-1 complex exists on all T cells in men and mice, but that there are subtle differences in carbohydrate or protein structures restricted to various cellular subpopulations, which may account for the differential reactivity of the various antibodies mentioned above.

The similarity between the T3 antigen(s) and the antigens identified by G7 also partially extends to the biochemical properties of the antigens. The main target of the anti-T3 reagents is thought to be borne by a 20 kD glycoprotein.

In addition, glycoproteins of 25–28, 37, and 44 kD were found in anti-T3 immunoprecipitates from surface-labeled cells (39). G7 immunoprecipitates consisted of a 28 kD band and a less prominent 50 kD protein; several other rat monoclonal anti-Thy-1 reagents also identified a p50 component. The p50 band is an attractive candidate for one of the higher molecular weight bands seen by the anti-T3 reagents. Although Thy-1 glycoproteins have not been previously identified in association with other membrane proteins, the possibility remains that Thy-1 and p50 exist as a complex on the T lymphocyte cell surface. Alternatively, the two molecules may share epitopes recognized by a number of rat anti-mouse Thy-1 antibodies. We also cannot rule out the possibility that p50 represents a dimer of the Thy-1 molecule, although such a dimer would have to be resistant to the SDS and reducing conditions used in these experiments.

Recent studies of the T3 antigen on cloned lines of antigen-specific T cells have suggested that the T3 complex is closely associated with the T cell receptor (5, 40). Immunoprecipitation with G7 under a variety of detergent and buffer conditions have not revealed coprecipitation of a heterodimer resembling the T cell antigen receptor (data not shown). In addition, sequential immunoprecipitation studies using a clonotypic monoclonal antibody to the T cell antigen receptor produced by Samelson et al. (41) have shown that the p50 band is not part of the T cell receptor heterodimer (Samelson and Gunter, unpublished observations). It is possible that the noncovalent interactions between putative Thy-1/T cell receptor complexes are weaker in the mouse than in man and that the molecules dissociate upon detergent solubilization, or that the G7 MAb itself may disrupt molecular associations present in the detergent lysate.

Although considerably more experimental analysis will be required to prove the hypothesis that the Thy-1 antigen is part of a molecular complex which resembles the T3 complex on human cells, the G7 MAb should prove to be a very useful reagent for analysis of some of the early events in the T cell activation process. As G7 can stimulate IL-2 production from a homogeneous, cloned population of T hybridoma cells in the absence of accessory cells, it can be postulated that the interaction of G7 with its antigen mimics an antigen- or mitogen-binding event necessary for triggering of IL-2 release. It is possible that when G7 binds to its epitope on the hybridoma cell surface, it may induce membrane conformational charges and/or initiate membrane ionic fluxes necessary for IL-2 production. An analysis of the mechanism of G7-induced activation of normal cells is somewhat more complicated. G7 does not act directly to drive resting T cells to proliferate since IL-2 receptors are not present on resting T cells (20, 21). G7 must first induce IL-2 receptor expression; then IL-2, produced as a result of G7 binding, acts to stimulate proliferation. The induction of IL-2 receptors may require additional cytokines present in the heterogeneous cell culture. One practical use of G7 may be as an inducing agent to produce lymphokine-rich supernatants from T cell hybridomas and tumors. G7 could easily be removed by protein A affinity chromatography after the induction step. This procedure would be far superior to the use of agents such as mitogens or phorbol esters which are very difficult to remove from the lymphokine-containing supernatant.

In any case, the ability of the G7 monoclonal antibody to stimulate IL-2

receptor expression, IL-2 secretion, and T cell proliferation is similar to the responses of T cells to antigens or mitogens. This suggests that the interaction of G7 with its epitope initiates a series of events common to T cell activation mediated by multiple types of ligands. If this is true, then Thy-1 could be thought of as a transducing molecule intimately involved in T cell activation. For example, Thy-1 may be a site for aggregation of T cell antigen receptor molecules or other cell surface proteins involved in antigen recognition. Thus, the Thy-1 molecule may represent a "final common pathway" by which T cell activation mediated by any stimulus may proceed.

Summary

We have identified a single rat monoclonal antibody, G7, that is a potent inducer of interleukin (IL-2) production from all functioning T cell hybridomas as well as from normal T cells. G7 is also mitogenic for normal T cells and is a very effective inducer of IL-2 receptor expression. On fluorescence-activated cell sorter analysis, G7 recognized a pan-T cell antigen. Immunoprecipitation studies demonstrated that G7 recognized a cell surface molecule of 28–32 kD that appeared to be identical to Thy-1 in coprecipitation studies. In addition, G7 precipitated a protein of 50 kD. The possible relationship of the putative molecular complex identified by G7 on murine cells to the molecular complex identified on human T cells with anti-T3 reagents is discussed. In addition, G7 should prove to be a very useful reagent for studying the early events of lymphocyte activation as well as an inducer of lymphokine-rich supernatants.

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