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Characterization of a Monoclonal Rat Anti-mouse Interleukin 2 (IL-2) Receptor Antibody and Its Use in the Biochemical Characterization of the Murine IL-2 Receptor

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Anti-murine interleukin 2 (IL-2) receptor monoclonal antibodies (mAb) were made from rats immunized with murine cytotoxic lymphocytes. One mAb, designated M7/20, strongly inhibited the proliferation of both IL-2 dependent CTLL-2 cells and concanavalin A (Con A)-induced T-cell blasts. Inhibition was linearly dependent on the concentrations of both M7/20 and IL-2. Utilizing FACS analysis, M7/20 was shown to bind selectively to mitogen-activated T lymphocytes and, to a lesser degree, to activated B lymphocytes. ^{125}I-Labeled M7/20 binding assays indicated that 48-hr Con A-induced T-cell blasts possessed 89,000 binding sites/cell with a K_d of 1.2×10^{-9} M. Competitive binding analyses indicated that M7/20 and IL-2 occupy the same or overlapping cell surface sites. Preliminary biochemical characterization of M7/20 immunoprecipitates of detergent extracts from both surface-iodinated and internally D-[^{3}H]glucosamine-labeled T lymphoblasts indicated that the murine IL-2 receptor is an *N*-glycosylated 58,000-Da glycoprotein. Together these results suggest that mAb M7/20 binds at or near the IL-2-binding epitope on the murine IL-2 receptor and, thus, upon manipulation may act as an IL-2 agonist.

INTRODUCTION

The acquisition of membrane receptors for interleukin 2 (IL-2),[5] a lymphocyte specific growth factor, marks a critical event in the course of T-cell activation (1–4). The induction of IL-2 receptors on T cells is dependent upon activation with either specific antigen or mitogen (5, 6) and interleukin 1 (7); a near 200-fold amplification of IL-2 receptors is detectable within 24 hr poststimulation (6). Interaction of IL-2 with receptor-bearing cells initiates a cellular program that is

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[5] Abbreviations used: IL-2, interleukin-2; mAb, monoclonal antibody; FBS, fetal bovine sera; BSA, bovine serum albumin; PBS, 0.01 *M* phosphate-buffered saline; Con A, concanavalin A; LPS, lipopolysaccharide; FACS, fluorescence-activated cell sorter; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide get electrophoresis.

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prerequisite for clonal expansion and continued viability of most, if not all, activated T cells (1–14). Other than the observation that IL-2 ligand–receptor complexes are internalized and degraded in lysozomes, the precise molecular and biochemical mechanisms of IL-2 growth promotion remain undefined.

Recently several laboratories have reported on the construction and preliminary characterization of monoclonal antibodies to the murine and rat IL-2 receptor (15–17). We report here on the characterization of a rat anti-mouse IL-2 receptor, mAb (M7/20), which both blocks IL-2-mediated growth and completely inhibits the binding of IL-2 to its cellular receptor. This activity is reminiscent of the properties of anti-TAC, an anti-human IL-2 receptor mAb (18, 19), and the anti-murine IL-2 receptor antibody 37C (20). We also utilize mAb M7/20 in the preliminary biochemical characterization of the murine IL-2 receptor. The construction of anti-murine IL-2 receptor antibodies has particular clinical relevance to the establishment of model systems for studying the management of graft rejection, leukemias, and autoimmunity by the selective eradication of activated or proliferating lymphocytes.

MATERIALS AND METHODS

Media and cell lines. All tissue culture was performed in RPMI 1640 (K.C. Biological, Lenexa, Kans.) supplemented with L-glutamine (2 m*M*), penicillin (100 U/ml), streptomycin (100 U/ml), 2-mercaptoethanol (5×10^{-5} *M*), and 10% fetal bovine sera (FBS) unless indicated otherwise. The IL-2-dependent line CTLL-2 was obtained from Dr. K. Smith (Dartmouth Medical School, Hanover, N.H.). mAb HO 13.4, a mouse IgM anti-Thy 1.2, was obtained from Dr. M. Gefter (MIT, Cambridge, Mass.). mAb Bet 2, a rat IgG_1 anti-mouse IgM, and K25, a mouse IgG_2 anti-rat IgG, were obtained from Dr. J. Kung (NIH, Bethesda, Md.).

Production of monoclonal antibodies. Monoclonal antibody production has been described previously (21, 22). Briefly, 14-month-old (Lewis × Brown Norway)F_1 rats were immunized with a cell population enriched in C57BL/6 mouse anti-Brown Norway lymphoma cytotoxic T lymphocytes. Spleen cells from immunized rats were fused with non-Ig-secreting NSI mouse tumor cells. Hybridomas were cloned and subcloned before functional assays were carried out. Supernatants were prescreened for mAb which preferentially bound to activated T, as compared to B, lymphocytes using a previously described ^{125}I-labeled anti-rat IgG indirect binding assay (22).

Preparation of cells. Cells used throughout were obtained from the spleens of 6- to 8-week-old female C57BL/6 mice (The Jackson Laboratory, Bar Harbour, Maine) according to standard procedures. T cells were purified by depletion of Ig^+ cells on anti-Ig-coated plates followed by passage over nylon wool (23). T-Cell blasts were generated by incubation of purified T cells with 5.0 μg/ml Con A (Sigma, St. Louis, Mo.) for 24–48 hr at 37°C. B cells were purified by harvesting the adherent fraction from anti-Ig-coated plates followed by treatment with 2.0 μg/ml of anti-Thy 1.2 mAb HO 13.4 (24) plus rabbit complement (Pel-Freeze, Rogers, Ark.). B-Cell blasts were obtained by incubation with 10 μg/ml lipopolysaccharide B (LPS; Difco, Detroit, Mich.).

Fluorescence analysis. For labeling with fluorescent probes, 10^6 cells in 50 μl

of Hanks' balanced salt solution with 2% FBS and 0.1% NaN_3 were mixed with 50 μl of purified mAb (10 μg) or 100 μl of control NSI supernatant for 45 min at 4°C, then washed thoroughly, and stained with an excess of purified fluorescein-conjugated rabbit $F(ab')_2$ anti-rat IgG (25). Analysis of 50,000 cells/sample was on a Coulter EPICS V fluorescence-activated cell sorter (FACS).

Assay of IL-2-driven proliferation. Assays of IL-2-driven DNA synthesis were carried out in 200-μl microtiter wells by measuring the [^{3}H]thymidine incorporation of 5×10^3 CTLL-2 cells in the presence of varying amounts of G-100 fractionated IL-2 and purified mAb as described by Gillis *et al.* (26). Cultures were incubated at 37°C in humidified/5% CO_2 for 24 hr and then pulsed with 1 μCi of [^{3}H]thymidine (New England Nuclear Corp. (NEN), Boston, Mass.) for an additional 4 hr. Cultures were performed in triplicate, harvested onto glass-fiber filters, and analyzed in a liquid scintillation system. Data were calculated as the geometric mean of each group ± the standard deviation (± SD).

Purification of antibodies. The mAb M7/20, a rat μ,κ Ig, was purified from the culture supernatants of cells grown in serum-free medium (Hana Labs, Berkeley, Calif.). Supernatants were first ammonium sulfate cut between 40 and 50% saturation. The dialysed precipitate was then passed over DEAE Affi-Gel Blue (Bio-Rad, Richmond, Calif.) in 20 m*M* Tris (pH 7.2), 100 m*M* NaCl, and the eluate was next fractionated on Sephadex G-200 (Pharmacia, Piscataway, N.J.), run in 20 m*M* Tris (pH 7.2), 250 m*M* NaCl, 0.5% *n*-butanol. mAb HO 13.4 was purified by affinity chromatography on Bet 2–Sepharose. mAb Bet 2 was purified by affinity chromatography on K2530–Sepharose as described by Kung *et al.* (34). Fluorescein-conjugated rabbit $F(ab')_2$ anti-rat Ig (Cappel, West Chester, Pa.) was purified on rat IgG–Sepharose and absorbed with mouse IgG–Sepharose prior to use.

Preparation and purification of IL-2 and [^{35}S]IL-2. Crude IL-2 was prepared from Lewis rat spleen cells by incubation of 2×10^6 cell/ml in RPMI supplemented with 5% FBS and 5 μg/ml Con A for 24 hr. IL-2 was ammonium sulfate cut at 40–70% saturation and fractionated on Sephadex G-100 prior to use (25). [^{35}S]IL-2 was prepared from EL-4 thymoma cells grown in the presence of 10 ng/ml PMA as described by Farrar *et al.* (27). Cells were labeled with [^{35}S]methionine, 756 Ci/mmole (NEN), at 250 μCi/ml for 24 hr in methionine-free RPMI (K.C. Biological) with 5% FBS. Purification was achieved by sequential ion-exchange, molecular-sieve, and hydrophobic chromatography followed by flatbed isoelectric focusing, p*I* 4.5–4.6 cut (27–29). Labeled IL-2 (63,166 cpm/pmole) was judged to be free of interleukin 1, colony-stimulating factor, gamma interferon and B-cell growth factor by standard bioassays (27–30); it migrated as a single spot on 2-D polyacrylamide gels. One unit of IL-2 was defined as the amount which stimulated 50% maximal CTLL-2 DNA synthesis.

Radiolabeled binding assays. Purified immunoglobulins were labeled with Na^{125}I (NEN; carrier-free) using lactoperoxidase-coupled enzymobeads (31). Labeled antibodies were separated from unbound label by passage over Sephadex G-25 columns prewashed with 2% BSA in PBS and run in PBS. M7/20 and anti-Thy 1.2 were labeled to specific activities of 6142 and 5386 cpm/ng, respectively.

Binding studies were conducted in 50-μl aliquots containing 10^6 cells in PBS,

0.5% BSA, and 0.2% NaN_3. Labeled antibody was added in increasing amounts (0–100 n*M*) to cells and incubated at 4°C for 60 min. The extent of nonspecific binding was determined by quantitating the binding of labeled mAb in the presence of a 100-fold excess of cold mAb. Cells were layered over 250 μl of phthalate oils in conical microfuge tubes and the cells pelleted by centrifugation as described by Dower *et al.* (32). The tips of the tubes were excised and the amount of ^{125}I-labeled mAb was determined by counting in a gamma counter.

Competitive binding assays were performed using 48-hr Con A-induced T blasts suspended in PBS containing 0.5% BSA and 0.2% NaN_3. Incubations (50 μl) were conducted in the presence of either ^{125}I-labeled or unlabeled mAb for 60 min at 4°C, followed by the addition of unlabeled or ^{35}S-labeled IL-2 and incubation for an additional 60 min at 4°C. Cells were harvested through phthalate oils and cell-bound ^{125}I was determined as described previously (32). Levels of cell-bound [^{35}S]IL-2 were determined by solubilization of cells harvested in microfuge tips with 100 μl of Protosol (NEN) followed by the addition of 3 ml of Aquasol II (NEN) and counting in a liquid scintillation system.

Receptor radiolabeling, immunoprecipitation, and SDS–PAGE. Cells were surface labeled with Na^{125} I using a modification of the chloroglycoluril method as described previously (25). Aliquots of cells (3×10^7) were labeled with 1 mCi Na^{125}I (carrier-free) for 10 min at room temperature, washed thoroughly, and then solubilized in 2 ml of 10 m*M* Tris–HCl (pH 7.5), 1% Triton X-100, 1% hemoglobin, 1 m*M* phenylmethylsulfonyl fluoride for 30 min at 4°C. The supernatant was cleared by centrifugation at 100,000*g* for 1 hr and dialyzed to remove unbound label.

T-Cell blasts (10^7 cell/ml) harvested 24 hr following incubation with Con A were extensively washed and then labeled with 250 μCi/ml of D-[1,6-^{3}H(N)]glucosamine HCl, 57.5 Ci/mmole (NEN), for 8 hr at 37°C in RPMI containing 5% FBS and antibiotics. Cells were harvested, washed, and solubilized as above.

Immunoprecipitation of ^{125}I-surface-labeled proteins was accomplished by incubation of equal quantities (200,000 cpm) of labeled aliquots with either 50 μl of M7/20 mAb or NSI culture supernatants followed by indirect immunoprecipitation with saturating amounts of rabbit anti-rat IgG (Cappel) as described previously (25, 33). Immunoprecipitation of [^{3}H]glucosamine-labeled proteins was accomplished by direct immunoprecipitation of solubilized proteins (100,000 cpm) with either 2.5 μg of purified M7/20 mAb coupled to Sepharose beads or 2.5 μg of Bet 2-coupled Sepharose (control). Incubations were conducted at 4°C for 60 min. mAb were coupled to Sepharose CL-4B (Pharmacia) according to Cuatrecasas (35).

All immunoprecipitates were extensively washed in detergent lysis buffer and then in PBS prior to loading on gels. Electrophoresis was conducted on discontinuous 5–10% polyacrylamide gels under reducing conditions as described by Samuel and Joklik (37).

RESULTS

Production of Rat mAb that Inhibit IL-2-Dependent Proliferation

Twenty-seven mAb, which react with T cells, were initially screened for their

capacity to alter the proliferation of the IL-2-dependent line CTLL-2. Eighteen mAb had no effect on this system, including mAb M7/14, subsequently identified as an anti-LFA-1 antibody which inhibits the effector function of murine cytotoxic T lymphocytes (22, 25). The remaining nine mAb had either stimulatory or inhibitory effects of CTLL-2 DNA synthesis. The effects of five of these mAb, which react preferentially with activated T cells, are shown in Table 1.

Only clone M7/20 consistently displayed a significant inhibitory activity (≈80%) on the mitogenic effect of IL-2. This activity was later shown not to be the result of mAb binding to free IL-2 by M7/20 using a Sepharose–M7/20 absorption assay (not shown). mAb M7/20 was subcloned twice in soft agar. Ouchterlony analysis of concentrated culture fluids indicated that M7/20 was a rat IgM, kappa antibody.

Distribution of mAb M7/20 Binding on Lymphoid Cells

The cellular distribution of mAb M7/20 binding was assessed on a variety of lymphoid cells by indirect immunofluorescence using the FACS (Fig. 1). As predicted from our initial screening procedures, CTLL-2 cells bound M7/20 to a high degree (Fig. 1A). In contrast, unfractionated spleen cells and purified splenic T-cell preparations (>95% Ig^-, Thy^+) did not bind M7/20 (Figs. 1B and C). While M7/20 did not bind resting T cells, purified splenic T cells incubated with 5 μg/ml Con A for 24 and 48 hr (Figs. 1D and E) displayed increasing levels of M7/20 binding; by 48 hr >95% of T cells bound M7/20. Binding to 48-hr LPS-induced B-cell blasts (>97% Ig^+, Thy^-) was substantially lower but consistently reproducible (Fig. 1F). Thus, M7/20 defines a surface antigen present on activated lymphocytes.

The Effect of mAb M7/20 on IL-2- and Mitogen-Dependent Proliferation

To further characterize the inhibition of IL-2-induced DNA synthesis by M7/20, quantitative inhibition experiments were performed using purified mAb. The effect of mAb M7/20 on the proliferation of CTLL-2 cells was assessed using increasing concentrations of mAb in the presence of a constant amount of Sephadex G-100-fractionated IL-2 (Fig. 2A) as well as increasing concentrations of G-100-fractionated IL-2 in the presence of a constant amount of M7/20 (Fig. 2B). Inhibition of IL-2-dependent CTLL-2 proliferation by M7/20 was observed in a saturable, linear, dose-dependent manner. Inhibition was not detected in the pres-

TABLE 1

mAb	Ig heavy chain	Number of experiments	% Change in IL-2-dependent DNA synthesis (±SD)
M5/114	γ2b	3	+101 ± 15
M5/106	μ	3	+33 ± 9
M7/8	μ	5	+43 ± 14
M7/85	μ	3	+14 ± 8
M7/20	μ	18	−82 ± 18

Note. Five hybridoma supernatants containing mAb that bound specifically to activated T cells were tested at a 1:20 dilution for the capacity to alter the IL-2-dependent proliferation of CTLL-2 cells in the presence of a 1:5 dilution of a crude rat IL-2 supernatant.

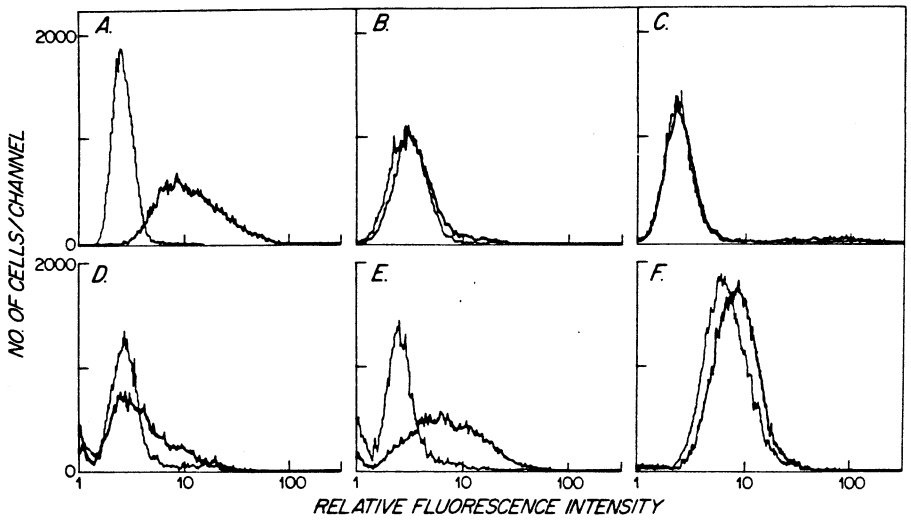

FIG. 1. Cell distribution of mAb M7/20 binding. Various cell populations were incubated with either control NSI culture supernatant (---) or M7/20 culture supernatant (—) and then stained with an excess of fluoroscein-conjugated rabbit anti-rat IgG. (A) CTLL-2 cells. (B) C57BL/6 spleen cells. (C) C57BL/6 purified splenic T cells. (D) C57BL/6 24-hr Con A-induced T-cell blasts. (E) C57BL/6 48-hr Con A-induced T-cell blasts. (F) C57BL/6 48-hr LPS-induced purified B-cell blasts.

ence of an irrelevant mAb (anti-Thy 1.2), which bound to the surface of CTLL-2 cells, nor in the presence of NSI control supernatants (not shown). Cell viability as determined by dye exclusion exceeded 90% in all test samples. As shown in Fig. 3, M7/20 also blocked the proliferation of splenic T cells which were incu-

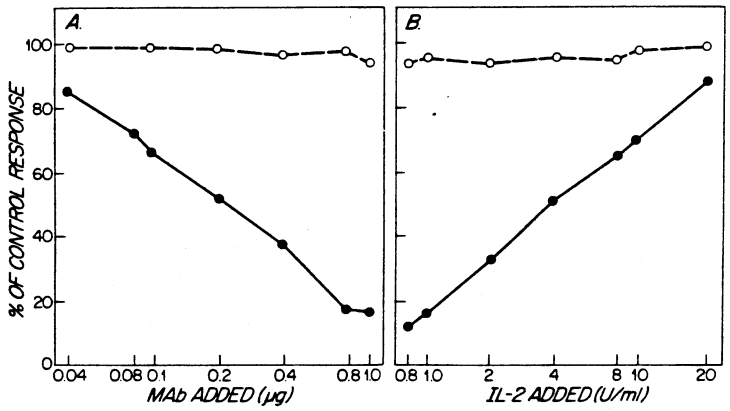

FIG. 2. Inhibition of the proliferative response of CTLL-2 cells to IL-2 by M7/20. CTLL-2 cells (5×10^3 in 200 μl) were cultured with (A) increasing amounts of either purified mAb M7/20 (●) or anti-Thy (○) in the presence of 1 U/ml IL-2; or (B) increasing amounts of rat IL-2 in the presence of 1 μg of either M7/20 (●) or anti-Thy (○). The cells were pulsed for the final 4 hr of a 24-hr incubation using 1 μCi [^{3}H]thymidine/well. Results are presented as the percentage of [^{3}H]thymidine incorporation in cultures which contained mAb relative to control cultures conducted in the absence of mAb. All points represent the means of triplicate cultures, with a standard deviation <4% for all points. One hundred percent activity corresponded to 24,320, 10,061, and 2554 cpm for cultures containing 20, 4, or 1 U/ml of IL-2, respectively.

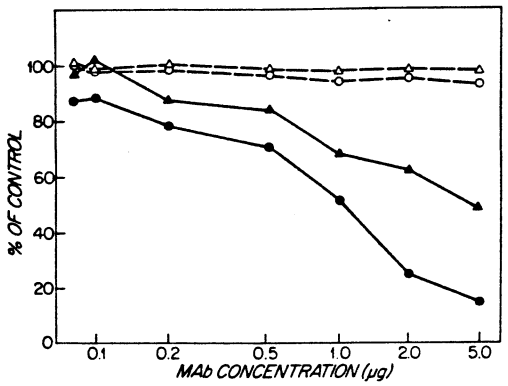

FIG. 3. Inhibition of the proliferative response of splenic T cells to Con A by M7/20. Purified splenic T cells (2×10^4 in 200 μl) were incubated with 5.0 μg/ml Con A in the presence of increasing concentrations of mAb M7/20 (●,▲) or anti-Thy (○,△) and incubated for either 24 (○,●) or 48 hr (△,▲) prior to a 4-hr pulse with 1 μCi [^{3}H]thymidine. All experiments were performed in triplicate, with a standard deviation <5%. One hundred percent activity corresponded to 16,432 and 25,413 cpm/culture at 24 and 48 hr, respectively.

bated with Con A for 24 or 48 hr. Similar results were seen when isolated T blasts were incubated with exogenous IL-2 in the presence of M7/20 (not shown). Thus, the mechanism of DNA synthesis inhibition by M7/20 is consistent with a direct effect on the binding of IL-2 to its cellular receptor.

Kinetics of M7/20 Binding and Inhibition on T Lymphoblasts

To determine the number and affinity of M7/20 binding sites on activated T cells, quantitative binding studies were conducted using ^{125}I-labeled mAb (Fig. 4A) and data were analyzed by the method of Scatchard (36) (Fig. 4B). These

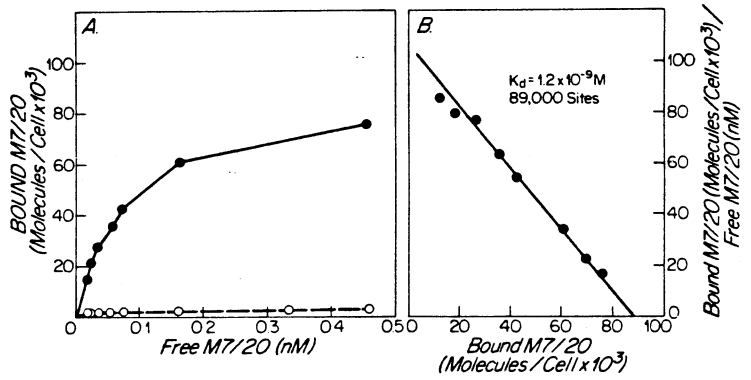

FIG. 4. Kinetics of binding of M7/20 to mouse T lymphoblasts. (A) Purified mouse splenic T cells were incubated with (●) or without (○) Con A for 48 hr, then extensively washed, and 1×10^6 cells were incubated with varying amounts of ^{125}I-M7/20 and the number of ^{125}I-M7/20 molecules specifically bound on cells was determined as described under Materials and Methods. The number of ^{125}I-M7/20 molecules bound/cell was plotted as a function of free ^{125}I-M7/20 by subtracting the amounts of bound from total ^{125}I-M7/20 added. (B) Scatchard plot of the data presented in A.

studies indicated that treatment of splenic T cells with mitogen (Con A, 5 μg/ml for 48 hr) induced the appearance of 8.9×10^4 M7/20 receptor sites/cell. Less than 2% of this value was detected on unactivated T cells. Binding to T blasts was saturable and of high affinity, $K_d = 1.2 \times 10^{-9}$ *M*.

To directly assess whether the inhibition of IL-2-mediated proliferative responses by M7/20 resulted from displacement of IL-2 binding to its specific cell surface receptor, radiolabeled competition assays were performed. Initially, we measured the ability of mAb to compete with radiolabeled IL-2 binding (Fig. 5A). ^{35}S-Labeled IL-2 was prepared from PMA-stimulated EL-4 thymoma cells (24) pulsed with [^{35}S]methionine using a combination of chromatographic and electrophoretic techniques as described (25–27). Because of the higher affinity of IL-2 for its receptor, $K_d \simeq 10^{-12}$ *M* (6), than that of M7/20, T blasts were first incubated with increasing amounts of mAb, a fixed amount of ^{35}S-labeled IL-2 was then added, and the incubation continued for an additional 60 min. As shown in Fig. 5A, M7/20 effectively displaced IL-2 binding to T blasts by 87% at maximal concentrations while anti-Thy 1.2 had no effect.

The possibility that IL-2 and mAb M7/20 compete for the same binding site was further tested by measuring the ability of unlabeled IL-2 to displace radiolabeled mAb from binding to T lymphoblasts. As shown in Fig. 5B, at concentrations of >50 ng/10^6 cells IL-2 blocks 90% of ^{125}I-M7/20 binding, but <2% of ^{125}I-anti-Thy 1.2 binding. These data support the hypothesis that M7/20 recognizes the IL-2 receptor by direct interaction at the IL-2 binding domain.

Biochemical Characterization of the Murine IL-2 Receptor by Immunoprecipitation

The putative cell surface receptor for IL-2 on T lymphoblasts was physically characterized by SDS–PAGE analysis of the immunoprecipitates of lympho-

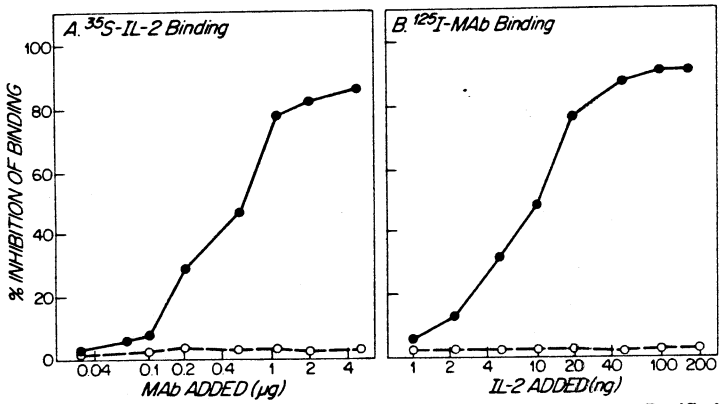

FIG. 5. Competitive binding analysis of M7/20 and IL-2 on T lymphoblasts. Purified splenic T-cell blasts (1×10^6) were incubated with either (A) increasing concentrations of mAb M7/20 (●) or anti-Thy (○) in the presence of a constant amount (500 fmol) of ^{35}S-labeled IL-2; or (B) increasing concentrations of IL-2 in the presence of a constant amount (20 fmol) of ^{125}I-labeled M7/20 (●) or anti-Thy (○). Incubations were conducted for a total of 90 min at 4°C and then bound label was detected as described under Materials and Methods. Data are presented as the percentage inhibition of binding of label versus competitor concentration. One hundred percent binding represented 80,153, 67,056 and 10,527 cpm bound/10^6 cells for ^{125}I-M7/20, ^{125}I-anti-Thy, and [^{35}S]IL-2, respectively.

blasts, both ^{125}I-labeled on the surface, and [^{3}H]glucosamine-labeled internally. Immunoprecipitation of ^{125}I-labeled T blasts (Fig. 6) conducted in the presence of M7/20 or control NSI supernatants identified a single unique protein of M_r 58,000 under reduced and nonreduced (not shown) conditions. Immunoprecipitates conducted in a similar manner on LPS-induced B blasts did not contain any prominent unique bands. Immunoprecipitates of [^{3}H]glucosamine-labeled T blasts (Fig. 7) also contained a prominent band at 58,000 Da, indicating that the IL-2 receptor is a surface glycoprotein.

DISCUSSION

mAb M7/20, raised in rats primed with murine cytotoxic T lymphocytes, appears to recognize the murine cell surface receptor for IL-2. M7/20 was shown by FACS analysis to selectively bind to the surface of activated lymphocytes. Previous studies have documented a near 200-fold increase in IL-2 receptors on T cells following mitogenic activation (6). mAb M7/20 was also shown to block the IL-2-dependent proliferation of both the CTLL-2 line and Con A-activated splenic T blasts. This inhibitory effect was linear and dose-dependent, as determined by the use of varying concentrations of both M7/20 and IL-2. Collectively these data suggest that M7/20 interacts directly with the IL-2 receptor. The failure

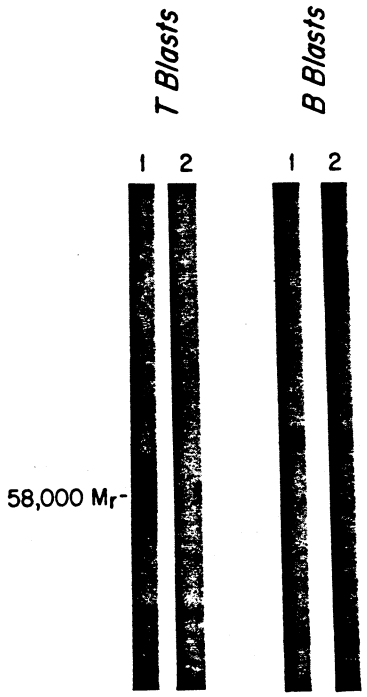

FIG. 6. SDS–PAGE of ^{125}I-labeled M7/20 immunoprecipitates. C57B/6 Con A- or LPS-induced blasts (3×10^7) were labeled with ^{125}I using Iodo-Gen and extracted with a 10 m*M* Tris (pH 7.5) buffer containing 1% Triton X-100. Supernatants were cleared of debris and then 200,000 cpm were mixed with 50 μl of either M7/20 (lane 1) or NSI (lane 2) culture supernatants and immunoprecipitated with rabbit anti-rat IgG. Immunoprecipitates were run under reduced conditions on a discontinuous 5–10% SDS–PAGE and exposed to Kodak XOMAT AR film.

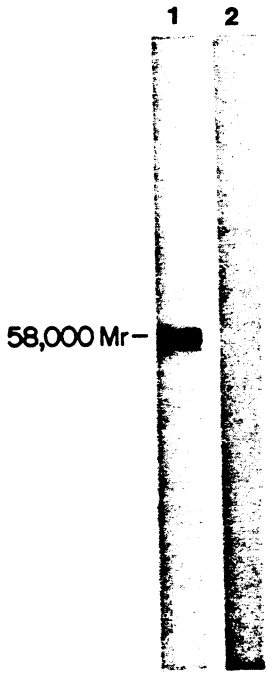

FIG. 7. SDS–PAGE of [^{3}H]glucosaime-labeled M7/20 immunoprecipitates. Twenty-four-hour Con A-induced T-cell blasts (5×10^7) were labeled with D-[1,6-^{3}H(N)]glucosamine for 8 hr at 37°C in RPMI containing 5% FCS. Washed cells were solubilized in Tris-buffered 1% Triton X-100 and cleared as described in the legend to Fig. 6. Extracts containing 100,000 cpm were immunoprecipitated by the addition of either M7/20-coupled Sepharose beads (lane 1) or Bet 2-coupled Sepharose beads (lane 2) for 60 min at 4°C. Immunoprecipitates were run under reduced conditions on discontinuous 5–10% SDS–PAGE and exposed using fluorography to Kodak XOMAT AR film.

of control mAb (anti-Thy 1.2) to block proliferation indicated that the action of M7/20 is not simply a nonspecific consequence of membrane perturbation.

Confirmation of the direct effect of M7/20 on altered IL-2 binding was achieved by the demonstration that M7/20 competitively inhibited the binding of ^{35}S-labeled IL-2, and conversely that IL-2 inhibited the binding of ^{125}I-labeled M7/20. Thus, M7/20 is distinct from both of the recently described anti-murine IL-2 receptor mAb (7D4 (15) and AMT-13 (16)) and from anti-rat IL-2 receptor mAb (ART-18 (17)), and more closely resembles the anti-human IL-2 receptor mAb anti-TAC (18, 19) and the anti-murine mAb 37C (20) in that the epitope recognized by the mAb also mediates IL-2 binding. Studies are currently underway which utilize mAb M7/20 as a probe to elucidate the mechanism of IL-2 receptor signalling.

Immunoprecipitation studies indicated that M7/20 binds to a 58,000-Da surface protein that is *N*-glycosylated. Again, this result is consistent with those reported in the murine, rat, and human systems. The most simplistic explanation of these results is that M7/20 binds to the murine IL-2 receptor at, or very near to, the IL-2 binding site and blocks IL-2 by spatial constraints. It is possible, however, that IL-2 and M7/20 do not compete for occupancy at the same site(s); for ex-

ample, M7/20 may sterically hinder IL-2 binding, while IL-2 binding may induce conformational receptor changes which affect M7/20 binding.

Binding studies revealed that activated T blasts possess 89,000 sites/cell which is 9–10 times in excess of values predicted from radiolabeled IL-2 binding (6). The ability of labeled IL-2 to block 90% of M7/20 binding indicates that previous estimates of IL-2 receptor number were significant underestimates. The most likely explanation, as advanced by Greene and colleagues (38, 19), is that there are two classes of receptors which bind IL-2 with greatly different affinities and that the low-affinity receptor was not detected in initial studies.

Our results also reinforce the growing body of data which indicate that activated B cells also possess IL-2 receptors (13–15), which may in part regulate B-cell proliferation (39). Both FACS and radiolabeled M7/20 binding data (not shown) indicated that activated B cells possess 10-fold lower levels of IL-2 receptors than those seen in activated T cells. As yet it has not been possible to demonstrate the immunoprecipitation of IL-2 receptors from B-cell blasts although IL-2 receptors have been isolated from hairy cell leukemia cells (40). More detailed studies are now in progress to more fully characterize the murine T-cell IL-2 receptor and its relationship to IL-2 receptors on B cells.

Antibodies to the IL-2 receptor hold great promise for the clinical management of responses governed by proliferating (IL-2 receptor-bearing) T and perhaps B lymphocytes. Examples include the control of graft rejections, of leukemia growth, and of the extent of autoimmune diseases. Studies are currently underway to evaluate the effects of free and toxin-coupled M7/20 in murine models of each of these systems. Antibodies directed at the ligand-binding site of receptors also hold particular interest as potential receptor agonists (41). In support of this, preliminary studies indicate that M7/20 acts as a partial IL-2 agonist in suboptimal mixed lymphocyte reactions (D. Eardley, unpublished observations).

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