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Immunology

Monoclonal antibodies specific for a murine cytotoxic T-lymphocyte clone

(antigen receptor/interferon/immunoprecipitation)

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ABSTRACT **Two antibody-secreting murine hybridomas, F1G3.1 and F2A11.5, have been established from B10.D2 mice immunized with cells from the murine cytotoxic T-lymphocyte clone G4. The two clones used, G4 and B10, were derived from BALB.B (*H-2*^{b}) mice and the target antigen specificity of both maps to the D^d region of the murine H-2 complex. However, B10 has a lower affinity for the target cells, as shown by its lower specific killing of blasts and its higher susceptibility to blocking by anti-Lyt-2 monoclonal antibody 53-6.75. The monoclonal antibodies, F1G3.1 and F2A11.5, react only with cells from clone G4. Similarly, they block only the specific cytolysis mediated by G4; no effect on cytotoxicity mediated by B10 or by heterogeneous populations of cytotoxic T lymphocytes was found. F1G3.1, especially, is very active in stimulating G4 to secrete immune interferon; B10 in contrast did not show any induction on treatment with these monoclonal antibodies. The structure of the surface antigen on G4 cells recognized by these monoclonal antibodies was revealed by immunoprecipitation studies of radioiodinated cell surface proteins. A protein dimer could be identified with an apparent molecular size of 80,000 daltons consisting of monomers migrating as 42,000-dalton proteins on reduction. So far, electrophoresis in the presence of NaDodSO$_4$ does not indicate any heterogeneity in the size of the monomers. This molecule can be distinguished from the Lyt-2 complex.**

Recent reports from a number of laboratories indicate a consensus of opinion on a candidate for the T-cell antigen receptor. These reports describe similar approaches using monoclonal antibodies generated against cloned T-cell lines. The derived antibodies react only with the T cells used as immunogen. Allison *et al.* (1) reported a monoclonal antibody specific for a murine radiation-induced T-cell lymphoma of unknown antigen specificity and function. Meuer *et al.* (2) immunized mice with a human cytotoxic T-lymphocyte (CTL) clone and generated monoclonal antibodies that inhibit the specific lytic activity of the CTLs. Haskins *et al.* (3) described a monoclonal antibody specific for an Ia (class II)-restricted antigen-specific murine T-cell hybridoma, probably belonging to the helper T-cell lineage. In this case, the monoclonal antibody inhibited the antigen-induced secretion of interleukin 2 (IL-2). In all reports cited, the clone-specific monoclonal antibodies precipitate a surface protein of ≈80,000 daltons that separates into two chains of ≈40,000 daltons on reduction.

In addition to the apparent clone-specific nature of the determinants and the ability of the antibodies to block antigen-specific function, there are other reasons to consider that the determinants recognized are idiotypes on the T-cell antigen receptor. For example, there are some comparative data suggesting microheterogeneity among the 40,000-dalton protein chains derived from different T-cell clones (4). Furthermore, it has been shown that the putative idiotypic determinant is intimately associated with the fine specificity of T cells for foreign antigen and self Ia (5).

In this report we describe two monoclonal antibodies, F1G3.1 (F1) and F2A11.5 (F2), raised against a murine alloreactive CTL clone. These monoclonal antibodies inhibit the lytic activity of the CTL used as immunogen. However, they show no effect on another CTL clone or on heterogeneous CTL populations specific for the same alloantigens. Acting in a clone-specific fashion, these anti-idiotypic antibodies are also able to both induce the secretion of immune interferon and precipitate a dimeric protein of about 80,000 daltons that reduces to monomers of ≈42,000 daltons.

MATERIALS AND METHODS

Mice. BALB.B, BALB/cByJ, B10.A(5R)/SgSn, B10.D2/nSnJ, C3H/ST, C57BL/10SnJ, DBA/2J, D2.GD, and (C57BL/6J × DBA/2J)F$_1$ mice were obtained from the breeding colony at Scripps Clinic and Research Foundation.

Cell Lines. The cloned CTL lines B10 and G4 were derived from BALB.B (*H-2*^{b}) mice that had been immunized *in vivo* and *in vitro* with BALB/c (*H-2*^{d}) cells. The clones were isolated by routine technology of limiting dilution and have been maintained by weekly subculture in medium containing irradiated stimulator cells (DBA/2, *H-2*^{d}, spleen cells) and a source of IL-2. The hybridoma line 53-6.75 secreting antibody specific for Lyt-2 (6) was kindly provided by Ian Trowbridge.

Antibodies and Antisera. Monoclonal rat antibody 53-6.75 (IgG2a) was used as culture supernatant as were murine monoclonal antibodies F1 and F2, both IgG1, κ subclass, which were also used as an ascites preparation in pristane-primed BALB/c mice. Rabbit antiserum against rat Ig2a (RARIG) was a gift from R. Wolfert. Antiserum against murine IgG was produced in rabbits (RAMIG).

Immunization. B10.D2/nSnJ, BALB/cByJ, and (C57BL/6J × DBA/2J)F$_1$ mice were immunized with about 10^7 G4 cells suspended in balanced salt solution (i.p.) or emulsified in Freund's adjuvant (s.c.). Injections were repeated every 10 days. Animals selected for fusion were allowed to rest for 4 weeks before receiving a final immunization of 2×10^7 G4 cells i.v. 3 days prior to fusion.

Fusion Protocol. Three days after the final booster immunization, mice were sacrificed and spleen cell suspensions were prepared by standard procedures. After lysis of erythrocytes, spleen cells were mixed with P3-X63 Ag8.653 my-

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Abbreviations: CTL, cytotoxic T lymphocyte; F1, F1G3.1; F2, F2A11.5; IFN, interferon; IL-2, interleukin 2; MAF, macrophage activation factor; RAMIG, rabbit antiserum against murine IgG; RARIG, rabbit antiserum against rat IgG2a; IU, international unit(s).

eloma cells in a ratio of about 10:1. After centrifugation, the mixed pellet was suspended in 0.5 ml of polyethylene glycol (33% in RPMI 1640 medium) and incubated for 2 min at 37°C. Then, 4 ml of warm RPMI 1640 medium was added, and the mixture was transferred to Petri dishes containing 5 ml of RPMI 1640 medium/36% fetal calf serum and incubated for 24 hr at 37°C. Thereafter, the fused cells derived from one spleen were distributed to ≈960 flat-bottomed wells of microtiter plates (Costar, Cambridge, MA) containing HAT medium [RPMI 1640/20% fetal calf serum supplemented with 2 mM glutamine, nonessential amino acids, 1 mM hydroxypyruvate, penicillin at 100 international units (IU)/ml, streptomycin at 100 μg/ml, gentamycin at 50 μg/ml, 0.1 mM hypoxanthine, 0.16 mM thymidine, 4 μM aminopterin, and 50 μM 2-mercaptoethanol]. Cell clones derived from two different fusions of immunized B10.D2 spleen cells were designated F1G3.1 (F1) and F2A11.5 (F2).

Cell-Mediated Lympholysis. Two assays were used. For characterization of the T-cell clones, effector cells and 1×10^4 ^{51}Cr-labeled target cells (P815 or Con A blasts) suspended in assay medium (RPMI 1640, 5% fetal calf serum/5 mM Hepes containing penicillin at 100 IU/ml, streptomycin at 100 μg/ml, 2 mM glutamine, and gentamycin at 50 μg/ml) were incubated for 4 hr in round-bottomed microtiter plates (total vol, 0.2 ml) (Costar) at effector/target ratios ranging between 60:1 and 0.04:1. For testing culture supernatants or sera for their ability to inhibit specific CTL-mediated killing, the effector cells (3×10^4 or 10×10^4 per well) were exposed to the antibody dilution in round-bottomed microtiter plates (Costar) for 30 min at 4°C prior to the addition of 1×10^4 ^{51}Cr-labeled P815 mastocytoma target cells. After centrifugation (1 min, 200 × *g*) followed by incubation at 37°C for 30 min, EDTA in assay medium was introduced at a final concentration of 10 mM. The total assay volume was 0.2 ml of medium per well. After further incubation for 2.5 hr at 37°C, specific lysis was determined (7).

Interferon (IFN) Induction. IFN release from G4 and B10 was stimulated by incubating the cloned cell lines at cell densities of 10^6 cells per ml in induction medium [RPMI 1640/5% fetal calf serum/5 mM Hepes/2 mM glutamine/48 mM methyl α-D-mannopyranoside/10% T-cell hybridoma AOFS supernatant containing IL-2 (8)/penicillin at 100 IU/ml/streptomycin at 100 μg/ml/gentamycin at 50 μg/ml/50 μM 2-mercaptoethanol)] with monoclonal antibody F1G3.1 at various dilutions for 24 hr at 37°C. Methyl α-D-mannopyranoside was omitted when Con A (4.8 μg/ml) was used.

IFN Assay. IFN was measured in a cytopathic-effect inhibition assay of vesicular stomatitis virus on L cells as reported (9).

Macrophage Activation Factor (MAF) Assay. Peritoneal exudate cells (1.2×10^5 per well) from C3H/St mice that had been injected i.p. with protease peptone (10%, 1.5 ml) 3 days earlier were incubated for adherence in half-area flat-bottomed microtiter wells (Costar). After vigorous washing to remove nonadherent cells, diluted test superantants, and ^{51}Cr-labeled P815 target cells (2×10^4 per well) were added in MAF assay medium (RPMI 1640/5% fetal calf serum/2 mM glutamine/1 mM sodium pyruvate/0.0375% sodium bicarbonate/lipopolysaccharide at 15 ng/ml/1 μM indomethacin/penicillin at 50 IU/ml/streptomycin at 50 μg/ml/50 μM 2-mercaptoethanol). After incubation at 37°C for 24 hr, specific lysis was determined (10).

Cell Surface Labeling and Cell Lysis. Cell membrane proteins were radioiodinated by the lactoperoxidase method (11). After centrifugation over Ficoll to remove cell debris, cells were washed and suspended at 10^7/ml in phosphate-buffered saline/5 mM glucose. $Na^{125}I$ (0.5 mCi/ml; 1 Ci = 37 GBq), lactoperoxidase (20 μg/ml), and glucose oxidase (0.1 IU/ml) were added in succession; the mixture was incubated for 20 min at room temperature; the cells were washed in phosphate-buffered saline three times; and the pellet was lysed in immunoprecipitation buffer (0.01 M Tris·HCl/0.12 M NaCl/10 mM EDTA/8 mM *N*-ethylmaleimide/aprotinin at 20 IU/ml/ova trypsin inhibitor at 0.02 mg/ml/1 mM phenylmethylsulfonyl fluoride/0.5% Nonidet P-40, pH 8.0) at 12.5×10^6 cells per ml. The lysate was stored on ice for 1 hr and centrifuged at 20,000 × *g* for 15 min. Aliquots of the supernatant were used for immunoprecipitation studies.

Immunoprecipitation. Samples of the cell lysates were precleared with rabbit antisera (RAMIG and RARIG)-coated protein-A-Sepharose beads (protein A-Sepharose CL-4B; Pharmacia, Uppsala, Sweden). The precleared lysates were incubated with aliquots of supernatants of the cloned hybridoma lines F1, F2, and 53-6.75 for 18 hr at 4°C. The antigen–antibody complexes were precipitated with rabbit antisera-coated protein-A-Sepharose beads and extensively washed with wash buffer (0.01 M Tris·HCl/0.12 M NaCl/0.5% deoxycholic acid/0.5% Nonidet P-40, pH 8). Samples were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis according to the procedure of Laemmli (12).

RESULTS

Characterization of the CTL Clones. Both of the CTL clones used in this study, G4 and B10, were derived from BALB.B (*H-2^b*) mice that had been immunized *in vivo* and *in vitro* with H-2^d cells. After cloning by limiting dilution, the lines were maintained *in vitro* by weekly subcultures with irradiated stimulator cells and a source of IL-2. The two CTL clones kill P815 (DBA/2 mastocytoma, H-2^d) target cells with approximately equal efficiency (Fig. 1). CTL clone G4 is also able to lyse antigen-bearing Con A-induced blasts extremely efficiently. Fig. 1*A* shows that B10.A(5R) Con A blast target cells that express K^b D^d are sensitive to lysis while D2.GD target cells (K^d D^b) are not lysed. Thus, the target antigen for CTL clone G4 maps to the D^d region. CTL clone B10 actually has the same pattern of lysis on these target cells (Fig. 1*B*) although in this case lysis of Con A blast targets is extremely inefficient. However, the weak positive lysis of Con A blasts as target cells that express H-2D^d is a reproducible finding. This difference in the ability of G4 and B10 CTL clones to lyse Con A blast target cells in conjunction with data on their susceptibility to the blocking activity of anti-Lyt-2 antibody (see below) suggests that G4 may have a higher affinity for the specific antigen than clone B10.

Production of Blocking Antisera. Groups of BALB/c (*H-2^d*), B10.D2 (*H-2^d*), and (C57BL/6 × DBA/2)F$_1$ (*H-2^b* × *H-*

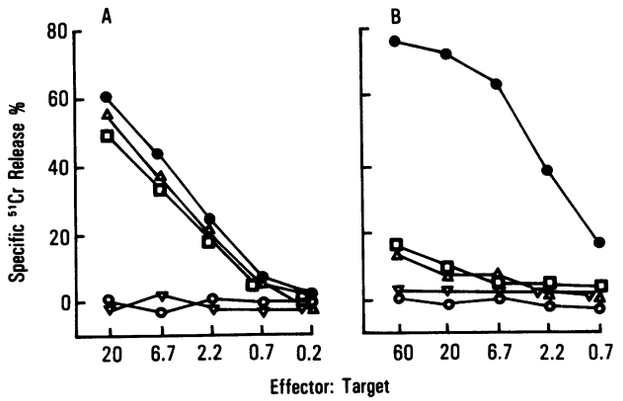

FIG. 1. Characterization of the target specificity of CTL clones G4 and B10. G4 (*A*) and B10 (*B*) effector cells at various effector/target ratios were tested for their specific lytic activity on a panel of target cells including P815 mastocytoma cells (DBA/2, H-2 K^d D^d) (●) and Con A-induced blasts—BALB/c (H-2 K^d D^d) (△), C57BL/10 (H-2 K^b D^b) (○), B10.A(5R) (H-2 K^b D^d) (□), and D2.GD (H-2 K^d D^b) (▽). Results of a representative experiment are shown.

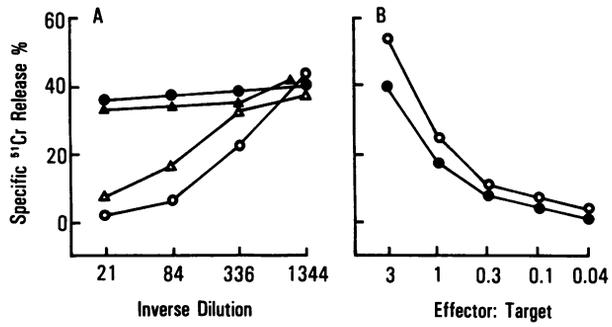

FIG. 2. Blocking activity of whole alloantisera on CTL-mediated lysis. (*A*) Blocking activity of two sera ○/●, △/▲) derived from B10.D2 mice immunized with viable G4 assayed on G4 (○ and △) and B10 (● and ▲). An effector/target ratio of 3:1 and serum dilutions as indicated were used. (*B*) Lytic activity of noninhibited G4 (○) and B10 (●) is plotted against effector/target ratio. Target cells used in both experiments were ^{51}Cr-labeled P815.

2^d) mice were immunized with G4 cells either suspended in balanced salt solution or emulsified in Freund's adjuvant. After three immunizations in saline or two immunizations with adjuvant, the mice were bled and individual sera were tested for their ability to block the G4-mediated lysis of labeled P815 target cells. Only the groups of B10.D2 and BALB/c mice that had been immunized with viable G4 cells in saline responded with sera capable of blocking specific lysis. We routinely find that >80% of B10.D2 and BALB/c mice immunized against viable G4 cells can make a strong blocking response to this clone, whereas immunization of (C57BL/6 × DBA/2)F_1 mice with viable G4 cells does not induce antibodies with comparable properties. It may be that the H-2 difference between the responding mice and the BALB.B-derived CTL clone acts as a carrier antigen for this response (13). Examples of the blocking activity of whole sera from two immunized B10.D2 mice are shown in Fig. 2. Quite profound inhibition of the cytolytic activity of G4 CTL is seen even at serum dilutions of 1:84 for both sera, whereas no effect was found on the lytic activity of clone B10. Since the antisera against G4 (BALB.B, β_2-microglobulin phenotype A) were raised in B10.D2 mice (β_2-microglobulin phenotype B) it could be argued that antibodies against β_2-microglobulin phenotype A were produced (14). These antibodies (anti-β_2-microglobulin A) could mask the target antigen on P815 (DBA/2, β_2-microglobulin phenotype A) prohibiting its recognition by the effector cell. No such reactivity could be detected however (data not shown).

Production of Monoclonal Antibodies. After they had been allowed to rest for 4 weeks, hyperimmunized B10.D2 mice with high serum titers of blocking antibodies were given a final intravenous immunization with viable G4 cells in saline, and spleen cells from these animals were used for fusion 3 days later as described above.

In separate fusions of spleen cells obtained from different mice, two hybridoma clones were derived that secrete antibodies capable of blocking G4-mediated lysis. In each case, these were the only positive clones from approximately 1000 hybrid clones. As the supernatant of each hybridoma well was screened for its ability to block G4-mediated lysis, only antibodies interfering with the function of specific killing could be detected. In this procedure, EDTA was used in the assay to increase its sensitivity by not permitting second attacks of killer cells after they had recognized and lysed a target cell or had removed the blocking agent from their surface (7). Positive wells were retested and subcloned and positive subclones were selected. The blocking ability of F1 and F2 tissue culture supernatants and of supernatants from the rat anti-Lyt-2 hybridoma cell line 53-6.75 tested against CTL clones G4 and B10 and against cells from a 6-day primary BALB.B anti-H-2^d mixed lymphocyte culture was determined (Fig. 3). The lytic activity on P815 target cells of all three effector populations was effectively inhibited by the monoclonal anti-Lyt-2 antibody. The activity of clone B10 seems to be more easily inhibited by this antibody, reinforcing the suggestion that B10 has a lower affinity for the target antigen (15). Monoclonal antibodies F1 and F2, however, inhibit the cytotoxic activity only of G4, the CTL clone used as immunogen. We have also produced ascites fluids from pristane-primed hybridoma-bearing BALB/c mice that show the same specific blocking of G4 but with ≈200-fold higher titers.

In indirect fluorescence tests using fluorescein-labeled rabbit anti-mouse immunoglobulin sera as second antibodies, we found that the monoclonal antibodies bind to the surface of viable G4 cells but not to B10 cells or to a detectable fraction (<5%) of BALB.B thymocytes (data not shown).

Stimulation of Immune IFN Secretion by Monoclonal Antibodies. A second effector function of all CTL clones so far described appears to be the release of immune IFN induced by the recognition of antigen or exposure to a T-cell mitogen (9, 10, 16). A preliminary experiment using whole sera from G4-immunized B10.D2 mice suggested that the sera induced the release of IFN specifically from G4 CTLs. The data in Table 1 show that CTL clones G4 and B10 can release immune IFN after culture with IL-2-containing medium plus Con A. Even high dilutions of ascites fluid containing monoclonal antibody F1 were also able to induce the release of immune IFN but in this case only from clone G4. Supporting results were obtained using an assay for MAF activity (Fig. 4). Thus, the reaction of G4 CTLs with the clone-specific monoclonal antibody mimics that of antigen binding or mitogen stimulation.

Biochemical Characterization of the Surface Proteins Recognized by Clone-Specific Monoclonal Antibodies. In confirmation of the recent results of other groups, we have found that the clone-specific monoclonal antibodies raised against murine CTL clone G4 precipitate a surface-labeled protein

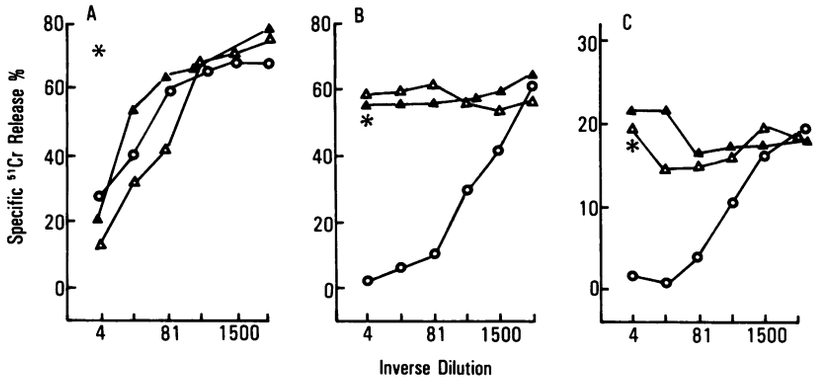

FIG. 3. Blocking activity of monoclonal antibodies F1, F2, and 53-6.75 on lytic activity. G4 (*A*), B10 (*B*), and BALB.B anti-BALB/c MLC cells (*C*) were incubated with F1 (△), F2 (▲), and 53-6.75 (○) culture supernatants at various dilutions. CTL activity against ^{51}Cr-labeled P815 target cells was assayed using an effector/target ratio of 3:1 (*A* and *B*) or 10:1 (*C*). *, Noninhibited lysis under the same conditions.

Table 1. IFN in supernatants from G4 and B10 cells stimulated with monoclonal antibody F1

Antibody dilution*	IFN titer after stimulation of CTL	
	G4	B10
None	<6	<6
1:10	<6	<6
1:31	6	<6
1:94	24	<6
1:283	96	12
1:850	96	<6
1:2550	>192	<6
Con A†	96	96

Expressed as highest dilution of supernatant that protects >50% of triplicate L-cell microcultures from challenge with vesicular stomatitis virus at 200 times the tissue culture ID_{50}.
*Final dilution of F1-containing ascites in the stimulation medium.
†Final concentration of Con A in the stimulation medium was 4.8 μg/ml.

with comparable properties. The results of experiments in which Nonidet P-40 lysates of ^{125}I surface-radioiodinated G4 cells were allowed to react with monoclonal antibodies F1 and F2, and then the antigen–antibody complexes were precipitated with protein A beads coated with RAMIG are shown in Fig. 5. In unreduced 8.5% polyacrylamide gels, the band specifically precipitated runs with an apparent molecular size of ≈80,000 daltons. The specific precipitates and the control precipitates contain a number of background bands, including the T200 surface glycoprotein with an approximate molecular size of 200,000 daltons and a band at about 45,000 daltons, possibly actin. The precipitates found after reduction with 2-mercaptoethanol and electrophoresis in 10% acrylamide gels show a specifically precipitated band with an approximate molecular size of 42,000 daltons. The arc bands above the specific molecule are caused by displacement of radioactive material by the heavy chains of rabbit and murine immunoglobulins. In the reduced and unreduced gels the specifically precipitated bands are rather diffuse but in no case so far did we find evidence that two bands of different molecular size were precipitated.

In some respects the molecule precipitated by our clone-specific monoclonal antibodies markedly resembles the Lyt-2 complex on murine CTLs. Antibodies against either inhibit

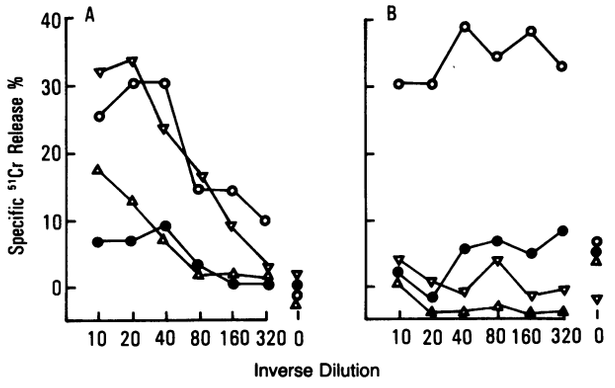

FIG. 4. Induction of MAF activity by the clonotypic monoclonal antibody F1. Supernatants derived from G4 (*A*) and B10 (*B*) cells incubated for 24 hr with an F1 ascites preparation at final dilutions of 1:94 (△) and 1:2550 (▽) were tested for MAF activity as measured by the peritoneal exudate cell-mediated lysis of ^{51}Cr-labeled P815 cells. The activity of 24-hr supernatants of Con A-stimulated (○) or nonstimulated (●) CTLs was determined as positive or negative control, respectively.

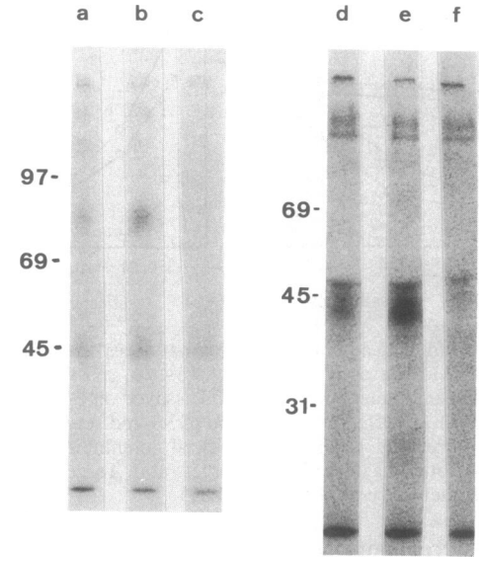

FIG. 5. Immunoprecipitation with clone-specific monoclonal antibodies F1 and F2. Antigens from surface radioiodinated G4 cells were immunoprecipitated with F1 (lanes a and d) and F2 (lanes b and e) culture supernatants and by medium (lanes c and f). Precipitates were analyzed by $NaDodSO_4$/PAGE under nonreducing (lanes a–c, 8.5% polyacrylamide gel) and reducing (lanes d–f, 10% polyacrylamide gel) conditions.

the lytic function of CTLs and in both cases the molecules are at least disulfide-bonded dimers. However, as shown in Fig. 6, the molecule precipitated by anti-Lyt-2 antibody can be distinguished from that precipitated by monoclonal antibody F2 on the basis of mobility on $NaDodSO_4$ gel electrophoresis. The Lyt-2 complex precipitated from G4 or B10 CTL clones migrates in the unreduced condition with an approximate molecular size of 70,000. The molecule precipitated by the clone-specific antibody from G4 has a molecular size of 80,000 daltons, and the clone-specific monoclonal antibody precipitates no surface-labeled band from CTL clone B10. In contrast to the Lyt-2 complex precipitated from murine thymocytes, neither G4 nor B10 shows two distinct specific bands on reduction but only one band of ≈38,000 daltons (Fig. 6).

DISCUSSION

In the present report, we describe two monoclonal antibodies that react with clonally unique surface determinants on a

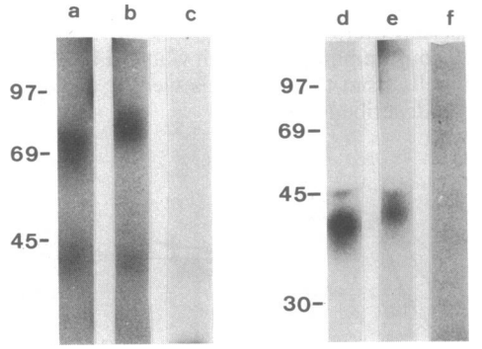

FIG. 6. Immunoprecipitation with monoclonal antibodies F2 and 53-6.75. Radioiodinated surface proteins from G4 (lanes a, b, d, and e) and B10 (lanes c and f) cells were immunoprecipitated with F2 (lanes b, c, e, and f) and 53-6.75 (lanes a and d) and analyzed by $NaDodSO_4$/PAGE under nonreducing (lanes a–c, 8.5% polyacrylamide gel) and reducing (lanes d–f, 10% polyacrylamide gel) conditions.

cloned CTL line, G4, but not with another cloned CTL line, B10, nor with a detectable fraction of normal T cells. We used two protocols and three mouse strains to raise antisera against G4. Our results indicate that injection of viable G4 cells into H-2-different responders stimulates production of anti-idiotypic antibodies, suggesting that this difference acts as a carrier for the response. The two monoclonal antibodies, F1 and F2, were derived by fusion of B10.D2 spleen cells immunized against G4 with myeloma P3-X63 Ag8.653. Using our immunization protocol, we never found antisera or monoclonal antibodies that blocked the lytic activity of both G4 and B10, suggesting reactivity with a common (presumably a constant) part of the molecule responsible for this function. In the strain combinations we used, it might be impossible to raise such alloantibodies against an antigenic site common for all CTL cells; on the other hand, our screening procedure—i.e., looking for blocking activity—may bias against detection of antibodies reacting against areas of the antigen receptor complex that are not directly involved in lytic function.

Both CTL clones were derived from BALB.B mice immunized against H-2^d cells but showing different affinity toward the target antigen, H-$2D^d$. B10 lyses Con A-induced blasts as target cells less efficiently than G4, and its killing activity is more easily blocked by rat anti-Lyt-2 monoclonal antibody 53-6.75. These findings suggest a weaker affinity of B10 (15). However, both monoclonal antibodies, F1 and F2, did not interfere with the lytic activity of B10 as they did with the cytotoxic function of G4. Similarly, no effect could be detected on the specific killing efficiency of a primary MLC in which BALB.B cells had been stimulated *in vitro* with irradiated BALB/c cells. On increasing the concentration of specific antibody by using ascites as source for anti-idiotypic antibodies F1 and F2, we obtained similar results, the ascites showing 200 times higher ability to block specific lysis mediated by G4.

Another intriguing finding was also made—i.e., the specific induction of immune IFN (also MAF activity) release from G4 but not from B10 by the anti-idiotypic monoclonal antibodies. This response of CTL clone G4 could be induced by antibody concentrations about 0.1 times the most effective concentration used for the blocking of the lytic activity. Monoclonal antibodies F1 and F2 influence both of the major functions of a CTL clone, blocking of specific cytotoxicity and induction of IFN in an idiotypic fashion. These two findings support the conclusion that monoclonal antibodies F1 and F2 detect the cell surface structure responsible for antigen recognition—i.e., the T-cell antigen receptor.

Preliminary biochemical characterization of the molecule precipitated from G4 provides results similar to those of groups examining T-cell lymphoma, T-helper hybridomas, and human CTLs (1–3, 5, 17). We could precipitate a dimeric protein molecule having an apparent molecular size of 80,000 daltons in the unreduced state, which separates into two chains of ≈42,000 daltons. So far, we could not find two heterologous chains precipitated from our murine CTL clone, G4, as found in other models. The limited information at hand suggests that a species difference may exist with respect to an easily demonstrable size difference in the subunits—i.e., the human clonotypic structure is easily resolved into bands of ≈40,000 and 47,000 daltons (refs. 2 and 17; P. Marrack, personal communication) while the subunits of the murine clonotypic molecule are not easily resolved (refs. 1 and 3; this work). Up to now we have no information whether the same molecular species is found in both CTLs and helper T cells as antigen-specific receptor. At least, the preliminary results show homology in terms of the subunit structure.

The exact function of the Lyt-2 complex in the cytolytic response of T lymphocytes is not known, although some evidence suggests a requirement to stabilize the binding between CTLs and the target cell (15). We have shown that the Lyt-2 complex is not an integral part of the T-cell antigen receptor. However, it cannot be concluded that the Lyt-2 complex as a distinct molecule is not associated with the receptor molecule, although we were not able to coprecipitate this molecule with the anti-idiotypic antibodies under the conditions used. Another observation should be mentioned, that the Lyt-2 complex of fully differentiated CTLs shows different properties than those of thymocytes—i.e., in the reduced state only one diffuse chain of 38,000 daltons could be found for both G4 and B10 (Fig. 6) while thymocytes have subunits of ≈38,000 and 32,000 daltons (18, 19).

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