BOTH A MONOCLONAL ANTIBODY AND ANTISERA SPECIFIC FOR DETERMINANTS UNIQUE TO INDIVIDUAL CLONED HELPER T CELL LINES CAN SUBSTITUTE FOR ANTIGEN AND ANTIGEN-PRESENTING CELLS IN THE ACTIVATION OF T CELLS*

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The proliferation of helper T cells and the T-dependent induction of B cell activation are events that are initiated by T cell recognition of antigen in association with Ia glycoproteins. However, the molecular basis of helper T cell antigen recognition remains unknown. Like several other laboratories, we have approached this problem by raising antibodies that are individually specific for functionally characterized cloned T cell lines. Clone-specific antibodies raised against alloreactive Ia-recognizing T cell clones (1), antigen-Ia-specific helper T cell hybrids (2), a human cytotoxic HLA-A recognizing T cell clone (3), and a murine H-2 recognizing cytotoxic T cell clone (4) have already been described. These clone-specific antibodies are either stimulatory themselves, as is the case of antisera raised to alloreactive T cell clones, or inhibit antigen-driven T cell activation, as are antibodies raised against helper T cell hybrids. However, stimulatory antibodies specific for major histocompatibility complex (MHC)[1] restricted, antigen-specific cloned helper T cell lines have not been described. In addition, the question of whether a monoclonal antibody of this specificity can activate a T cell has never been addressed.

In this paper, we describe antisera that both activate the T cells themselves and induce T cell-dependent B cell activation. In addition, we have produced a monoclonal antibody specific for a single cloned helper cell line that can also activate the T cells of this line. The anti-clone antibodies we have produced appear to completely replace the initial antigen-Ia recognition event by the T

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Con A, concanavalin A; FCS, fetal calf serum; IL-1, interleukin 1; IL-2, interleukin 2; LPS, lipopolysaccharide; MHC, major histocompatibility complex; OVA, ovalbumin; PBS, phosphate-buffered saline; RaMBr, rabbit anti-mouse brain serum; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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cell, while apparently not affecting the growth factor requirements of the ensuing pathway of activation. These findings thus strongly support the supposition that clone-specific antibodies react with antigen and/or Ia recognition sites.

The experiments presented here utilize antisera raised against two cloned helper T cell lines that have identical functional properties and genotype, but differ in nominal antigen specificity and in recognition of non-self Ia molecules. These antisera have high titer in biological assays and are absolutely specific for the immunizing clone. The ability of these sera, and the monoclonal antibody, to activate the appropriate T cell is dependent on a second signal from an accessory cell. The requirement for accessory cells, however, can be eliminated by the addition of interleukin-1 (IL-1) or interleukin-2 (IL-2). Thus, this system allows the analysis of the activation of a homogeneous T cell population in the complete absence of other cell types. Although similar to mitogen stimulation, the specificity of the interaction of T cells with anti-clone antibodies should make this system more analogous to an antigen-driven reaction. The effect of clone specific anti-T cell sera on T-dependent B cell activation, the major function of T helper cells, has also not previously been studied. The clone-specific antibodies we describe allow T cells to interact with B cells to induce B cell proliferation and immunoglobulin secretion in the absence of antigen recognition or MHC restriction. Thus, these antibodies mimic many facets of antigen-specific T cell interactions with Ia-bearing cells, and should allow a functional and ultimately molecular dissection of helper T cell "behavior."

Materials and Methods

Animals.

Animals were either purchased from the Jackson Laboratory, Bar Harbor, ME, or raised in our colony at Yale University. All animals were between 2 and 6 mo of age at the time they were used.

Production of T Cell Clones D8 and D10.

To produce cloned helper T cell lines, the procedure of Sredni et al. (5) as modified by our laboratory (6) was followed. In this case, two AKR/J mice were primed, each with 50 μg 5×-recrystallized hens egg albumin (OVA) (United States Biochemical Co., Cleveland, OH) in complete Freund's adjuvant containing *M. tuberculosis* strain H37Ra (Difco Laboratories Inc., Detroit, MI). Mice were immunized in the hind footpads and base of the tail. 6 d later, the draining popliteal and inguinal lymph nodes were removed, the T cells purified by passage over Ig-anti-Ig (7) columns and cultured in Click's EHAA medium containing 200 μg/ml OVA and 5% fetal calf serum (FCS) selected for low mitogenicity. 20×10^6 lymph node T cells were cultured with 5×10^6 mitomycin C-inactivated syngeneic spleen cells in 25-cm^2 flasks for 3 d. The cells were then pelleted and resuspended in Click's medium containing 20% FCS, 200 μg/ml OVA, and 0.33% agar (Difco Bacto Agar). This mixture was layered into a 60-mm petri dish containing 2 ml of 0.5% agar in Click's medium with 20% FCS and 200 μg/ml OVA. After several days, numerous clusters or colonies of cells appeared. These were plucked with a pasteur pipette and transferred to a 96-well microtiter plate containing Click's medium, 200 μg/ml OVA, 10% of a growth supporting FCS, 20% rat Con A supernatant as a source of growth factors and 2×10^5 mitomycin C-treated syngeneic spleen cells/well. Most colonies gave rise to vigorous growth. 20 lines were expanded to 1-ml cultures in multiwell plates and tested for proliferative responses to OVA in the context of syngeneic mitomycin C

inactivated spleen cells. 19/20 gave significant responses to OVA. Two of these, D10 and D8, were characterized extensively. D10 was also recloned twice in succession by limiting dilution, first in U-bottom microtiter plates, where growth was obtained at low efficiency at 1 cell/well, and subsequently in V-bottom microtiter plates, where growth was obtained with close to 100% efficiency at 0.5 cells/well. Clone D10.G4.1 was obtained from one of these wells. Cells were expanded in 25 and 75 cm^2 tissue culture flasks fed twice per week with medium and antigen, and weekly with 5–10 × 10^5 mitomycin C-inactivated spleen cells per milliliter of culture. Vigorous growth has been maintained for 18 mo under these conditions with these cloned lines. Recently, we have found that the United States Biochemical Co. lot of OVA we have used contains conalbumin as a contaminant. D10 and its subclones have since been demonstrated to exhibit antigen specificity for this protein, and not ovalbumin, and are currently maintained using commercially purified conalbumin (Sigma Chemical Co., St. Louis, MO) as antigen. D8 has been grown with various batches of ovalbumin, including one commercially prepared lot that we have found to be conalbumin free (Sigma Chemical Co.).

Antibodies.

Anti-D10 and Anti-D8. Anti-T cell antisera were produced by weekly immunizations of BALB.K mice with 3–10 × 10^6 cloned T cells intraperitoneally in phosphate-buffered saline (PBS). Cloned T cells were harvested 7–10 d after the last addition of feeder cells and washed several times in PBS before injection. Mice were bled 1 wk after the last immunization and the sera collected and tested. After four injections with D10, or its subclones, the immune sera had detectable stimulatory activity in a T cell proliferation assay. These mice were subsequently boosted and bled on alternate weeks. Anti-D10 is a pooled serum from these animals, derived from multiple bleeds. Six out of six BALB.K mice have produced anti-D10 activity by this protocol. Two other BALB.K mice also produced anti-D10 activity following a single boost 3 wk after priming. Two out of four (AKR × B6)F1 mice have produced anti-D10 activity after six immunizations. The cloned T cells (D10.G4.1) used to immunize these F1 mice were grown exclusively with AKR/J feeder cells.

Two BALB.K mice were also immunized with D8 cells by the above procedure. One of these mice produced serum with detectable activity after the seventh immunization and was subsequently boosted. Anti-D8 is a pool of serum from this animal, derived from multiple bleeds.

Monoclonal Anti-D10 Antibody (3D3). A BALB.K mouse was immunized with 5 × 10^6 D10.G4.1 intraperitoneally in PBS and boosted 3 wk later with 4 × 10^6 D10.G.4.1 intravenously. 3 d later the immune spleen cells were fused to P3X63-Ag8.653 cells as described previously (8). The resulting hybridoma culture supernatants were screened for the induction of D10.G4.1 proliferation in the absence of accessory cells and the presence of 1–5% of an IL-1 rich P388D1 supernatent. Out of 120 hybridomas screened, 1, 3D3, was positive in this assay. 3D3 was subsequently cloned on agar and by limiting dilution. ~90% of the 3D3 clones tested (39 total) were positive in the above assay. Two agar clones, 3D3.1 and 3D3.2, were used in experiments presented here. 3D3 is an IgG1-secreting hybridoma.

Monoclonal Anti-Fc Receptor. This antibody, 2.4G2, was the generous gift of Dr. I. Mellman (Yale University, New Haven, CT) and has been described previously (9).

Rabbit Anti-Mouse Brain (RaMBr). RaMBr serum was produced as described previously (10).

Assays.

All assays, except the T-dependent induction of B cell immunoglobulin secretion, were performed in Click's medium containing 5% FCS. The induction of polyclonal immunoglobulin secretion was performed in RPMI-1640 containing 10% FCS and 60 μM 2-mercaptoethanol. All proliferation assays were harvested with a PHD Cell Harvesting System (Cambridge Technology, Inc., Cambridge, MA). Other methods were as follows:

T Cell Proliferation. 2 × 10^4 cloned T cells were cultured in 0.2 ml containing feeder

cells inactivated with mitomycin C (Sigma Chemical Co.) and various additions. Feeder cells were either whole spleen cells (2×10^5 cells/culture) or splenic adherent cells (2×10^4 cells/culture). Splenic adherent cells were prepared by a modification of the method of Steinman et al. (11). Briefly, whole spleen cells (1×10^7 cells/ml in Dulbecco's Modified Eagle Medium containing 10% FCS) were allowed to adhere to plastic petric dishes (100 mm; Costar, Cambridge MA). After 2 h at 37°C, nonadherent cells were removed by gentle washing with warmed medium in the absence of FCS. Medium containing FCS was replaced and the incubation continued overnight at 37°C. At the end of this incubation period, adherent cells were harvested by vigorous pipetting with warmed medium. Cell yields range from 2–8% of the input cell number. In some experiments soluble factors were added in place of feeder cells. Cultures were harvested after 72 h following a 3–4 h (70 Ci/mmol) or 16 h (6.7 Ci/mmol) ^{3}H-TdR (New England Nuclear, Boston, MA) pulse of 1 μCi/culture.

T-Dependent B Cell Proliferation and Immunoglobulin Secretion. Cloned T cells were inactivated with mitomycin C and added to B cells as previously described (12). B cells were prepared by treatment of whole spleen cells with a rat monoclonal antibody that reacts with a nonpolymorphic determinant on the Thy-1 molecule followed by rabbit complement. Proliferation was measured by a 4 h ^{3}H-TdR pulse of 1 μCi/culture following a 48-h culture period. B cell immunoglobulin secretion was assayed in a plaque-forming cell assay as previously described (13), culturing 3×10^4 cloned T cells and 4×10^5 B cells in 0.2-ml cultures. Triplicate cultures were pooled and assayed by this method after 3, 4, or 5 d in culture.

IL-2 Assay. The presence of IL-2 was determined by the ability to support the growth of the IL-2-dependent cell line HT-2, by a modification of the method of Gillis (14). 1×10^4 HT-2 cells were cultured in 0.1 ml containing various amounts of supernatants to be assayed. Cultures were pulsed for the final 4 h of a 24-h culture period with 1 μCi ^{3}H-TdR/culture. HT-2 cells were kindly provided by Dr. P. Marrack (National Jewish Hospital and Research Center, Denver, CO).

Supernatant Factors.

Con A-Induced Spleen Cell Supernatant. The production of conconavalin A (Con A) supernatants from rat spleen cells has been described previously (6). Residual Con A was inactivated by the addition of α-methyl-D-mannoside (20 mg/ml).

AOFS Supernatants. AOFS 21.10.9 cells were the kind gift of Dr. P. Marrack. IL-2 containing supernatants were produced by incubating AOFS 21.10.9 cells at an initial concentration of 5×10^5/ml with 2 μg/ml Con A for 24 h at 37°C in medium lacking FCS. Supernatants were harvested and α-methyl-D-mannoside (20 mg/ml) was added before use.

IL-1-Rich P388D1 Supernatant. A partially purified IL-1-rich supernatant from lipopolysaccharide (LPS)-induced P388D1 cells was the generous gift of Dr. S. Durum (Yale University New Haven, CT). The production and purification of this supernatant has been described previously (15). Briefly, cultures of P388D1 cells were stimulated 4 d with LPS at a concentration of 20 μg/ml. Supernatants were partially purified by a 65% saturated ammonium sulfate precipitation followed by Sephadex G-75 chromatography. Biologic activity (thymocyte proliferation assay) was contained in the 15,000–20,000-dalton fractions. This source of IL-1 contains no detectable IL-2 (see Table V).

Absorption of Anti-D10 Mitogenic Activity. 1 ml of 1:1,000 anti-D10 diluted in Click's medium containing 5% FCS was absorbed with 250 μl of protein A-coupled Sepharose (Pharmacia Fine Chemicals Co., Sweden) or bovine serum albumin (BSA)-coupled Sepharose 4B (BSA coupled to CNBr-activated Sepharose at 1 mg protein/ml of Sepharose). After 1 h at 4°C with frequent mixing, the Sepharose was pelleted and the supernatant assayed for the ability to induce D10.G4.1 proliferation. The Sepharose beads used for absorption were extensively washed in PBS and eluted with 1 ml of 0.1 M sodium citrate pH 3.5 for 15 min at 4°C. This eluate was dialyzed against medium and also assayed for activity.

Results

D10 and D8 are Helper T Cell Clones.

The cloned helper T cell lines used in these studies have not been described previously; their production, recloning, and analysis are as follows: D10 and D8 are T cell colonies produced from the pooled lymph node T cells of two OVA-primed AKR/J mice by the method of Sredni et al. (5), as modified by our laboratory (6). The D10 clone D10.G4, and its subclone D10.G4.1, were produced by the method of limiting dilution. Analysis by SDS-PAGE has recently revealed that the commercially obtained batch of 5× crystallized OVA (USBC) used for in vivo priming and in vitro culturing of these clones was, in fact, a mixture of chicken egg white proteins, including conalbumin and OVA. Although we originally interpreted our data as demonstrating that D10 showed antigen specificity for OVA (16), we have since, using purified egg white proteins, established that the true antigen is conalbumin. All subsequent references to OVA in this paper will refer to conalbumin-free OVA. Table I shows the antigen reactivity patterns of D8 and of D10 and its subclones. D8 proliferates to OVA but not conalbumin in the presence of H-2^k feeder cells. Conversely, D10 and its subclones proliferate to conalbumin, but not OVA, in an H-2-restricted manner. We have not observed alloreactivity with D8 when tested against eight independent haplotypes. D10, however, was initially found to be alloreactive to H-$2^{b,p, \text{ and } q}$. Upon further cloning to yield D10.G4 and D10.G4.1, only reactivity to H-2^b has been maintained.

Functionally, in vitro, D10 and D8 induce hapten-specific plaque-forming cells in the presence of low doses of haptenated antigen (reference 16, and K. Bottomly, personal communication). At high antigen doses, D10 and D8 induce polyclonal B cell proliferation and immunoglobulin secretion. As expected (12, 16), all of these helper cell functions are antigen specific and H-2 restricted.

Anti-clone Sera Are Mitogenic and Specific For the Immunizing Clone. In an attempt to identify cell surface molecules involved in helper cell functions, we have raised antisera against D10 and, more recently, against D8. The choice of BALB.K mice as recipients for these immunizations eliminated the potential production of anti-H-2 antibodies that might have influenced screening assays, while favoring the production of antibodies directed against other alloantigens. Individual mice received 3–10 × 10^6 cloned T cells intraperitoneally in PBS on a weekly basis. Mice were bled 1 wk after the last immunization, and the sera collected and assayed for the ability to block or stimulate the proliferative response of the appropriate T cell clone. The antisera produced after four immunizations with D10 or its subclones was found to be mitogenic for the inducing clone. These mice were subsequently boosted and bled on alternate weeks. We have observed no qualitative differences in the activity of sera between individual mice immunized with a single D10 subclone, or between mice immunized with different D10 subclones. Anti-D10 refers to a pool of such sera that was used for all the experiments presented here. Anti-D8 was produced similarly, with the exception that no activity was detected until after the seventh immunization. We have not observed inhibition of antigen-induced T cell proliferation with any of our sera.

Fig. 1 summarizes an individual experiment measuring the proliferative re-

TABLE I

T Cell Clones D10 and D8 are H-2 Restricted and Antigen Specific: T Cell Proliferation

A. D10 and D8 proliferate to antigen in an H-2-restricted manner.

H-2I* A, E	Feeders[‡]	D8[‖] Antigen[§]		D10 Antigen		D10.G4 Antigen		D10.G4.1 Antigen	
		−	+	−	+	−	+	−	+
k,k	AKR	1,290	5,428[¶]	457	15,232			1,122	10,993
k,k	B10.BR	1,250	7,826	333	9,163				
k,k	B10.A					1,611	12,320		
k,k	B10.A(2R)					738	13,094		
b,b	B10	215	233	7,873	7,419				
b,b	C57Bl/6					32,300	26,809	8,272	5,835
b,k	B10.A(5R)	174	111	10,294	10,808	18,078	15,160		
d,d	BALB/C	299	156	529	1,321	2,859	2,046	558	659
d,d	B10.D2	1,911	639	322	1,285				
(dxb)	CB6F1	1,226	251	12,968	12,635				
s,s	B10.S	176	356	1,064	3,414				
s,k	B10.S(9R)					2,621	1,846		
p,p	B10.F(13R)	1,045	1,843	7,185	9,310	3,304	2,906		
q,q	B10.G	167	318	9,152	8,711	4,709	3,925		
r,r	B10.RIII	76	118	544	2,072	2,389	2,740		
f,f	B10.M	436	1,048	765	1,831	2,011	2,030		

B. D10 and D8 are antigen specific.

T Clone[‖]	Antigen[§]	Response**
D10.G4.1	—	236
D10.G4.1	Conalbumin	14,237
D10.G4.1	OVA	338
D8	—	45
D8	Conalbumin	64
D8	OVA	10,925

* Haplotype of origin of the I-A and I-E subregions of various stimulator cells. Strains with I-E[b,s,f,q] express no I-E molecules on the cell surface.

‡ 2×10^5 mitomycin C treated whole spleen cells/0.2 ml culture.

§ 200 μg/ml of OVA (D8) or conalbumin (D10) added (+) or with no antigen (−).

‖ 2×10^4 cloned T cells/0.2 ml culture.

¶ Mean cpm ^{3}H-TdR incorporation of triplicate cultures, pulsed for the final 3 h of a 72-h culture period; significant stimulation underlined.

** Mean cpm ^{3}H-TdR incorporation in the presence of 2×10^5 mitomycin C-treated whole spleen cells as feeders.

sponse of D10.G4.1 and D8 to anti-D10 and anti-D8. Anti-D10 is a potent mitogen for D10.G4.1 with a titer greater than 1:1,000. However, this serum has no effect even at high doses on D8 cells, despite the fact that in this particular experiment D8 gave a more vigorous antigen response than D10.G4.1. Conversely, anti-D8 stimulates D8 to proliferate while exhibiting no apparent effect on D10.G4.1. The lower titer of anti-D8 may reflect a lower immunogenicity of these cells, consistent with the finding that a greater number of immunizations are required to induce the activity. Indeed, in recent experiments we have found that anti-D10 can be induced following a single boost 3 wk after priming.

To further examine the specificity of anti-D10, we have assayed normal AKR

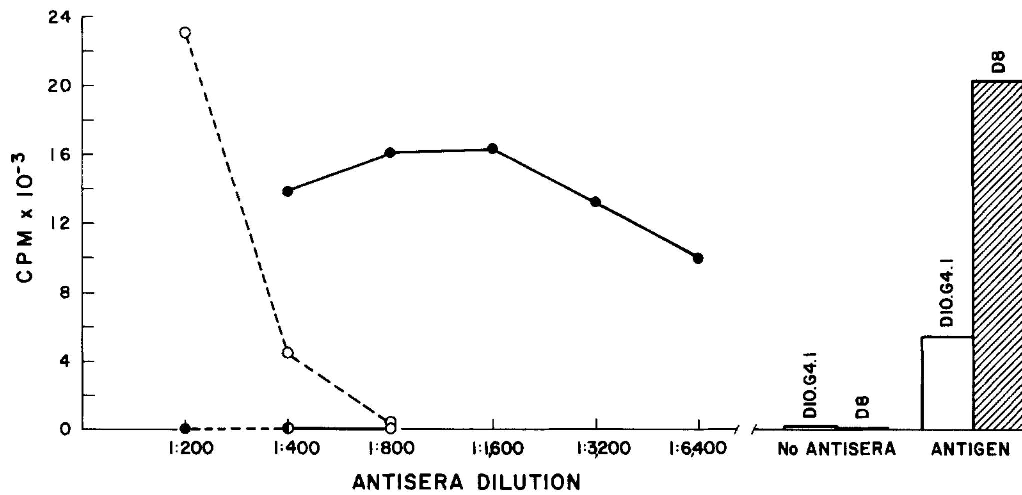

FIGURE 1. Clone-specific antisera induce T cell proliferation. D10.G4.1 (●) and D8 (○) proliferation measured in a 72-h assay containing 2×10^4 cloned T cells and 2×10^5 mitomycin C-treated BALB.K whole spleen cells per 0.2 ml culture in the presence of various concentrations of anti-D10 (——) or anti-D8 (-----). D10.G4.1 and D8 antigen responses are assayed similarly, with the addition of conalbumin (D10.G4.1) or OVA (D8) at 200 μg/ml (histograms). Data are expressed as mean cpm ^{3}H-TdR incorporation of triplicate cultures following a 16-h pulse.

spleen cells, lymph node cells, and Con A blasts in a proliferative assay. In no case did anti-D10 induce a response from these cells. In addition, absorption with AKR spleen cells, Con A blasts, or the AKR thymoma BW5147 does not remove any of the mitogenic activity directed at D10.G4.1 (data not shown). We have seen significant absorption of anti-D10 activity using D10 cells, and no absorption with D8 cells, but the technical difficulty in obtaining large numbers of cloned cells has made these experiments inconclusive. These studies will be repeated with D10- and D8-derived T cell hybridomas currently being generated.

Anti-D10 Mitogenic Activity Is Due to Antibody.

Although nonimmune BALB.K sera were not mitogenic for D10, we wanted to verify that antibody was responsible for the observed activity. Anti-D10 was diluted to a break point in activity and absorbed with protein A-coupled Sepharose or, as a control, BSA-coupled Sepharose. Only the protein A-Sepharose was able to remove the mitogenic activity for D10.G4.1 (Fig. 2*A*). In addition, activity was recovered in the acid eluate of the protein A-Sepharose (Fig. 2*B*). Therefore, the ability of this serum to activate D10.G4.1 is the property of antibodies directed against the cloned T cell.

Anti-clone Mitogenic Activity Requires Accessory Cells.

Recent evidence suggests that the induction of T cell proliferation by antigen or mitogen is a multistep process (17, 18). Thus, we asked whether anti-D10 alone was sufficient to induce proliferation or whether a second signal from an accessory cell was also involved. Anti-D10 in the absence of accessory cells has almost no mitogenic activity for D10 or its subclones (Table II*A*). The slight

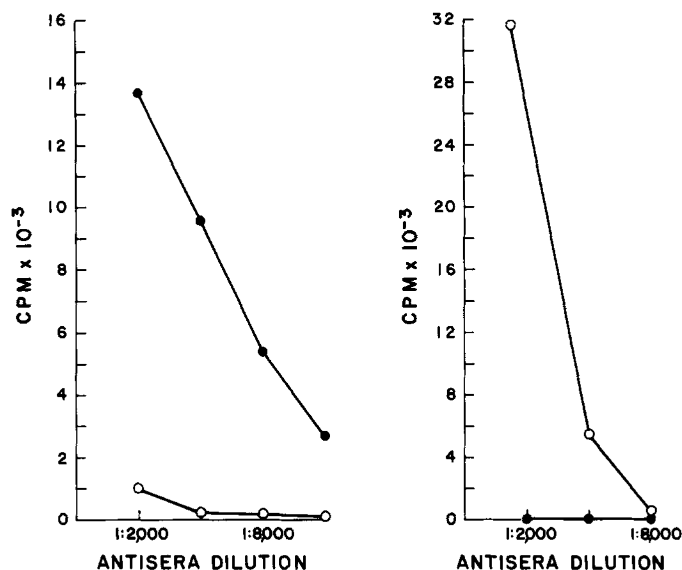

FIGURE 2. Anti-D10 mitogenic activity is specifically removed by protein A-Sepharose. *Left*: 1 ml of 1:1,000 anti-D10 in medium was absorbed with 250 μl of protein A-Sepharose (○) or BSA-Sepharose (●). After 1 h on ice the mixture was centrifuged and the supernatant assayed for the ability to induce D10.G4.1 proliferation in a 72-h assay (see Fig. 1). *Right*: The protein A-Sepharose (○) or BSA-Sepharose (●) beads used for absorption were extensively washed and eluted with 1 ml of 0.1 M citrate pH 3.5. After dialysis against medium, the eluate was assayed as above. The data are expressed as the mean cpm ^{3}H-TdR incorporation of triplicate cultures.

residual activity seen is not a consistent finding and may represent minor feeder cell contamination of the T cell clone, or direct accessory cell-independent activation. Anti-D8 also requires accessory cells to induce proliferation (for example, see Table IV). In order to further characterize the cell involved in the anti-D10 response we have used spleen cells enriched by adherence to plastic (splenic adherent cells) as accessory cells. This preparation contains a variety of cell types. 10-fold fewer splenic adherent cells than spleen cells support an equal or better antigen response, consistent with the finding that such adherent cells are enriched for antigen-presenting cells (19). When tested in the presence of anti-D10, these same cells were also found to be enriched for cells that support D10.G4.1 proliferation (Table II*B*).

Although anti-D10 activity could not be absorbed out using spleen cells, and therefore was not likely to interact directly with the accessory cell population, it was possible that antibody aggregated on the T cell surface was interacting with Fc receptors to induce a second signal. Particularly relevant is the finding that aggregated immunoglobulin can induce the production of IL-1 from macrophages (20). The addition of the monoclonal anti-Fc receptor antibody 2.4G2 (9), however, has no effect on the anti-D10 response whether spleen cells or splenic adherent cells are used as accessory cells (Table II*C*). Rabbit anti-mouse brain (RaMBr) is known to be mitogenic for T cells, and RaMBr mitogenic activity requires a B cell as accessory cell, and an intact Fc (10). Note that the proliferative response of D10.G4.1 to RaMBr is almost totally eliminated in the

TABLE II

Anti-D10 Mitogenic Activity Requires Splenic Adherent Accessory Cells

A. Mitogenic activity requires accessory cells.

D10*	H-2^k feeders[‡]	Anti-D10[§]	Response[‖] D10 Exp 1	Response[‖] D10.G4 Exp 2	Response[‖] D10.G4.1 Exp 3
+	+	−	71	116	208
+	+	+	29,269	27,431	7,984
+	−	−	60	44	88
+	−	+	786	946	1,150

B. Splenic adherent cells are enriched for cells that support anti-D10–induced mitogenesis and present antigen.

D10.G4.1*	Addition[§]	Response[‖] 2×10^5 Spl[‡]	Response[‖] 2×10^4 SAC[¶]
+	—	474	146
+	Conalbumin	7,750	14,727
+	NMS	165	177
+	Anti-D10	9,749	12,579

C. Anti-D10 does not require interaction with Fc receptors to induce D10 proliferation.

D10.G4.1*	Antisera[§]	Anti-FcR**	Response[‖] 2×10^5 Spl[‡]	Response[‖] 2×10^4 SAC[¶]
+	—	−	42	92
+	Anti-D10	−	14,377	13,931
+	RaMBr	−	13,837	2,916
+	—	+	32	46
+	Anti-D10	+	11,771	16,160
+	RaMBr	+	71	273

* 2×10^4 cloned T cells/0.2 ml culture.

‡ 2×10^5 mitomycin C-treated AKR/J or BALB.K whole spleen cells/0.2 ml culture.

§ NMS and Anti-D10 1:200–1:800 (concentration of NMS and anti-D10 are identical in an individual experiment); RaMBr 1:150; conalbumin 200 μg/ml.

‖ Mean cpm ^{3}H-TdR incorporation of triplicate cultures, pulsed for the final 16 h of a 72-h culture period.

¶ 2×10^4 AKR/J or BALB.K splenic adherent cells/0.2 ml culture prepared as described in methods section (both spleen cells and adherent cells are derived from the same mouse strain in an individual experiment).

** Monoclonal antibody 2.4G2, 1 μg/ml.

presence of 2.4G2. In addition, splenic adherent cells are not enriched for accessory cells for the RaMBr response. Although 2.4G2 is directed against the IgG2b/IgG1 receptor (9), it also binds the IgG2a receptor via its own Fc (I. Mellman, personal communication). It remains possible, however, the anti-D10 interacts with an Fc receptor not blocked by this monoclonal antibody. Finally, we asked whether the interaction with an accessory cell in the anti-D10 and anti-D8 response was H-2 restricted. The experiments in Table III demonstrate that while the antigen responses of D10.G4.1 and D8 are H-2 restricted, there is no apparent restriction in the anti-D10 and anti-D8 responses.

TABLE III
Anti-clone-Induced Proliferation Does Not Require H-2 Matched Accessory Cells

Exp.	T Clone*	Feeders‡	Antigen§	Antisera‖	Response¶
I.	D10.G4.1	BALB.K	—	—	330
	D10.G4.1	BALB.K	Conalbumin	—	6,552
	D10.G4.1	BALB.K	—	Anti-D10	10,072
	D10.G4.1	BALB.K	—	Anti-D8	204
	D10.G4.1	BALB/c	—	—	118
	D10.G4.1	BALB/c	Conalbumin	—	133
	D10.G4.1	BALB/c	—	Anti-D10	11,725
II.	D8	BALB.K	—	—	45
	D8	BALB.K	OVA	—	10,925
	D8	BALB.K	—	Anti-D8	15,467
	D8	BALB.K	—	Anti-D10	36
	D8	BALB/c	—	—	85
	D8	BALB/c	OVA	—	154
	D8	BALB/c	—	Anti-D8	9,330

* 2×10^4 cloned T cells/0.2 ml culture.
‡ Mitomycin C-treated whole spleen cells, 2×10^5/0.2 ml culture.
§ Conalbumin or OVA 200 μg/ml or no antigen added (—).
‖ Anti-D10 1:800; anti-D8 1:200.
¶ Mean cpm ^{3}HTdR incorporation of triplicate cultures, pulsed for the final 16 h of a 72-h culture period.

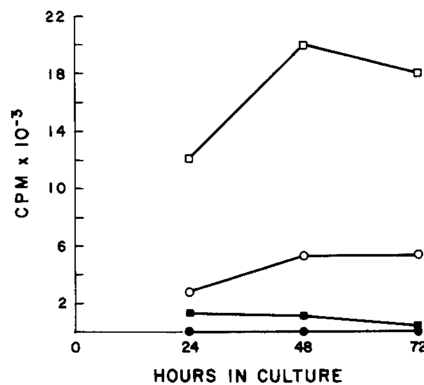

FIGURE 3. Anti-D10 and a Con A supernatant synergize to induce D10.G4.1 proliferation in the absence of accessory cells. D10.G4.1 proliferation was assayed in 0.2-ml cultures containing 2×10^4 cloned T cells and: no addition (●), 15% Con A supernatant (○), anti-D10 1:500 (■), or anti-D10 1:500 and 15% Con A supernatant (□). Data are expressed as the mean cpm ^{3}H-TdR incorporation of triplicate cultures pulsed for the final 4 h of a 24-h, 48-h, or 72-h culture period.

IL-1- or IL-2-Rich Supernatants Can Replace Accessory Cells in the Response to Anti-clone Antibodies.

The lack of genetic restriction between D10 and the accessory cells in the anti-D10 response suggested to us that a lymphokine might be involved in the activation of the T cell clone. We therefore tested whether a Con A-induced rat spleen cell supernatant would be sufficient to support an anti-D10 response. Fig. 3 shows the kinetics of such an assay in the absence of accessory cells. Clearly, anti-D10 and a Con A supernatant will synergize to induce D10.G4.1 prolifera-

TABLE IV
IL-1-Rich Supernatant Supports Anti-clone–dependent T Cell Proliferation in the Absence of Accessory Cells

Exp.	T Clone*	IL-1-Rich superna-tant‡	—	Response§		
				Anti-D10‖	Anti-D8‖	Anti-gen‖
I	D10.G4.1	−	37	878	ND**	ND
	D10.G4.1	+	159	49,929	ND	ND
	D10.G4.1	− +LPS¶	ND	1,256	ND	ND
II	D10.G4.1	−	491	329	ND	133
	D10.G4.1	+	33	33,194	ND	32
III	D10.G4.1	−	113	1,427	ND	92
	D10.G4.1	+	87	161,144	120	190
IV	D8	−	43	ND	104	ND
	D8	+	49	ND	31,772	ND
V	D8	−	43	ND	168	53
	D8	+	48	ND	31,422	33

* 2×10^4 cloned T cells/0.2 ml culture.
‡ Presence (+) or absence (−) of 1% IL-1 rich P388D1 supernatant.
§ Mean cpm ^{3}H-TdR incorporation of triplicate cultures, pulsed for the final 16 h of a 72-h culture period.
‖ Conalbumin (D10.G4.1) or OVA (D8), 200 μg/ml; anti-D10 1:800; anti-D8 1:200.
¶ LPS, 50 μg/ml.
** Not done.

tion. Con A-induced supernatants contain many soluble mediators including IL-2 and IL-1. The role of these proteins in the activation of T cells has been extensively studied. It is generally accepted that antigen and Ia glycoproteins trigger a T cell to become receptive to IL-1 released by activated antigen-presenting cells. IL-1, in turn, induces the T cell production of the actual mitogenic signal IL-2 (reviewed in reference 21). It is not known at this time whether the induction of receptivity to IL-2 is also IL-1 dependent. It was of interest to compare this proposed mechanism of activation to that observed with anti-clone antisera. If such antisera trigger the T cell clone in a manner analogous to antigen and Ia, one might expect that anti-clone sera in the presence of IL-1 would be sufficient to trigger proliferation. This is indeed the case. The experiments in Table IV demonstrate that in the absence of accessory cells, a partially purified IL-1-rich P388D1 supernatant will induce D10.G4.1 or D8 to proliferate only in the presence of the appropriate anti-clone sera. LPS, the inducing agent for the production of IL-1 from P388D1 cells, and a possible contaminant of the preparation used, has no effect. We are currently testing whether this IL-1-induced proliferation is mediated by endogenously produced IL-2.

Although IL-1 can replace accessory cells in an anti-clone response, it can not replace antigen-presenting cells in an antigen-driven response (Table IV). This is consistent with the work of Mizel and Ben-Zvi (22) who have demonstrated that, while IL-1 can enhance the proliferation of primed lymph node cells under conditions where antigen-presenting cells are limiting, IL-1 can not support a response in the total absence of antigen-presenting cells. In fact, our data strongly suggest that the addition of soluble antigen and IL-1 to a helper T cell clone is

TABLE V
IL-2-Rich AOFS Supernatant Supports Anti-D10–dependent T Cell Proliferation in the Absence of Accessory Cells

Addition[‡]	Response		
	D10.G4.1 Proliferation:*		HT-2 Proliferation[§]
	—	Anti-D10	
—	38	6,001	384
1% IL-1	38	201,767	180
25% IL-2	2,234	50,648	43,922
12% IL-2	957	29,876	30,115
6% IL-2	447	21,200	13,211
3% IL-2	94	14,525	12,159

* 0.2-ml cultures containing 2×10^4 cloned T cells and anti-D10 1:800 or no antiserum. (—); mean cpm ^{3}H-TdR incorporation of triplicate cultures pulsed for the final 16 h of a 72-h culture period.
‡ Cultures contain no addition (—) or various amounts of an IL-1-rich P388D1 supernatant (IL-1) or an IL-2-rich AOFS supernatant (IL-2).
§ 0.1-ml cultures containing 1×10^4 HT-2 cells; mean cpm ^{3}H-TdR incorporation of triplicate cultures pulsed for the final 4 h of a 24-h culture period.

a very sensitive assay for the presence of contaminating feeder cells.

If anti-clone sera induce receptivity to IL-2 as well as IL-1, the addition of IL-2 should also induce proliferation in the absence of accessory cells. Because whole spleen cell supernatants can potentially contain IL-1 as well as IL-2, we have used a Con A-induced T cell hybridoma (AOFS 21.10.9) supernatant as a source of IL-2 (P. Marrack, personal communication). We do not have an assay for IL-1 in the presence of contaminating IL-2 but, given its T cell lineage, it is unlikely that AOFS produces IL-1. An AOFS supernatant will induce the proliferation of the IL-2-dependent cell line HT-2 (Table V). Note that an IL-1-rich P388D1 supernatant has no effect on HT-2 cells demonstrating the absence of IL-2 in this preparation (Table V). The experiment in Table V demonstrates that an AOFS supernatant can also support anti-D10-induced D10.G4.1 proliferation in the absence of accessory cells. The similarity between the AOFS supernatant dose response of anti-D10-induced proliferation and HT-2 proliferation supports the interpretation that both are mediated by IL-2. However, this response is consistently weaker than the response to anti-D10 in the presence of IL-1 or accessory cells. We have yet to determine whether this is a quantitative or qualitative difference. In summary, our data suggest that anti-clone sera induce receptivity to IL-1 and IL-2. The addition of IL-1 or IL-2 can then induce proliferation, the former presumably the result of the endogenous production of IL-2.

Anti-D10 Substitutes for Antigen in the Induction of T-Dependent Polyclonal B Cell Proliferation and Ig Secretion.

Helper T cell clones interact in vitro with B cells to induce B cell proliferation and antibody secretion (12). This interaction involves the recognition by the T cell of antigen and Ia glycoproteins on the B cell surface. At high antigen doses in vitro, these interactions bypass the B cell antigen receptor and induce poly-

TABLE VI
Anti-D10 Substitutes for Antigen in the Induction of B Cell Proliferation

D10*	B Cells‡	Ag§	Addition‖	Response¶		
				D10.G4 Exp I	D10.G4.1 Exp II	D10.G4.1 Exp III
−	H-2^k	+	—	22,718	1,517	4,364
+	H-2^k	−	—	16,262	2,007	6,697
+	H-2^k	+	—	156,178	40,136	80,392
+	H-2^k	−	NMS	8,477	ND	ND
+	H-2^k	−	Anti-D10	92,281	40,625	74,060
−	H-2^k	−	Anti-D10	ND	717	2,271
+	H-2^b	−	—	ND	70,184	63,717

* 2 × 10^4 mitomycin C-treated cloned T cells/0.2 ml culture.
‡ AKR/J or BALB.K (H-2^k), or C57Bl/6 (H-2^b) B cells prepared as described in methods section, 2 × 10^5 cells/0.2 ml culture.
§ 200 μg/ml conalbumin (+) or no antigen (−).
‖ NMS or anti-D10 1:200–1:800 (concentration of NMS and anti-D10 are identical in an individual experiment).
¶ Mean cpm ^{3}H-TdR incorporation of triplicate cultures, pulsed for the final 4 h of a 48-h culture period.

clonal stimulation. Mitomycin C-treated D10 will induce syngeneic B cell proliferation in the presence of conalbumin. D10.G4.1, which is alloreactive to H-2^b (Table I), will also induce H-2^{b}B cells to proliferate in the absence of antigen (Table VI). In the presence of anti-D10, however, B cells are triggered in a T-dependent manner in the absence of antigen (Table VI). In contrast to the T cell-dependent B cell proliferative response to antigen, this response shows no H-2 restriction between D10 and the B cell (data not shown).

It was possible that anti-D10 induced B cell proliferation by the interaction of antibody-coated D10 with B cell Fc receptors. Again, our model is the RaMBr system in which the T-dependent induction of B cell proliferation has been reported to be mediated via the Fc (23). Moreover, Lamers et al. found that, at very high doses, the monoclonal anti-Fc receptor 2.4G2 would also induce B cell proliferation (24). However, the data summarized in Table VII demonstrate that 2.4G2, at nonstimulating doses, has no effect on the anti-D10 or antigen response, while totally eliminating the RaMBr response. The induction of B cell proliferation by anti-clone sera is clone specific. Anti-D8, but not anti-D10, will induce D8-dependent B cell proliferation in the absence of antigen (Table VIII). Similarly, anti-D8 has no effect on D10-driven B cell proliferation (Table VIII).

D10.G4.1 will also drive B cells to secrete antibody in an antigen-specific manner. The requirement for antigen is eliminated, however, with the addition of anti-D10 (Fig. 4). Thus, anti-D10 and anti-D8 have properties similar to antigen, but require neither syngeneic Ia nor Fc receptor to activate cloned T cells or, in concert with cloned T cells, to activate B cells.

Production and Characterization of a Monoclonal Anti-D10 Antibody.

The above results demonstrate that one can produce clone-specific antisera that replace the need for antigen, Ia, and accessory cells in T cell activation and in T cell-dependent B cell proliferation. However, they do not demonstrate that

TABLE VII
Anti-D10 Does Not Require Interaction with Fc Receptors to Induce T-Dependent B Cell Proliferation

D10.G4.1*	B Cells‡	Addition§	B cell proliferation in the presence of: Anti-FcR‖ –	+
–	+	—	1,874¶	1,535
+	+	—	3,187	2,930
+	+	Conalbumin	79,474	62,745
+	+	Anti-D10	94,251	74,905
+	+	RaMBr	49,531	1,810

* 2×10^4 mitomycin-treated cloned T cells/0.2 ml culture.
‡ 2×10^5 AKR/J B cells/0.2 ml culture.
§ Conalbumin 200 μg/ml; anti-D10 1:800; RaMBr 1:150.
‖ Presence (+) or absence (–) of monoclonal antibody 2.4G2, 1 μg/ml.
¶ Mean cpm ^{3}H-TdR incorporation of triplicate cultures, pulsed for the final 4 h of a 48-h culture period.

TABLE VIII
Induction of T-Dependent B Cell Proliferation by Anti-Clone Sera is Clone Specific

T Clone*	B Cells‡	Addition§	Response‖
D10.G4.1	+	—	4,005
D10.G4.1	+	Conalbumin	65,403
D10.G4.1	+	Anti-D10	59,164
D10.G4.1	+	Anti-D8	3,944
D8	+	—	5,822
D8	+	OVA	67,606
D8	+	Anti-D8	56,530
D8	+	Anti-D10	5,124
—	+	Conalbumin	3,440
—	+	OVA	3,500
—	+	Anti-D10	1,954
—	+	Anti-D8	1,084

* 2×10^4 mitomycin C–treated cloned T cells/0.2 ml culture.
‡ 2×10^5 AKR/J B cells/0.2 ml culture.
§ Conalbumin and OVA, 200 μg/ml; anti-D10 1:800; anti-D8 1:200.
‖ Mean cpm ^{3}H-TdR incorporation of triplicate cultures pulsed for the final 4 h of a 48-h culture period.

antibody of a single specificity can produce such effects. To approach this problem, BALB.K mice were primed with D10, boosted, and their spleen cells fused to P3X63-Ag8.653 cells to produce monoclonal antibodies. One such monoclonal anti-D10 antibody, and its subclones, is shown in Table IX. This monoclonal antibody induces D10.G4.1 to proliferate, while having no effect on D8. Proliferation in the absence of accessory cells requires IL-1. The titer of culture supernatant of this hybridoma in a functional assay is remarkable; supernatant at $1{:}10^5$ induces D10.G4.1 proliferation (Table IX). Thus, a monoclonal antibody can replace antigen and Ia-bearing antigen-presenting cells for

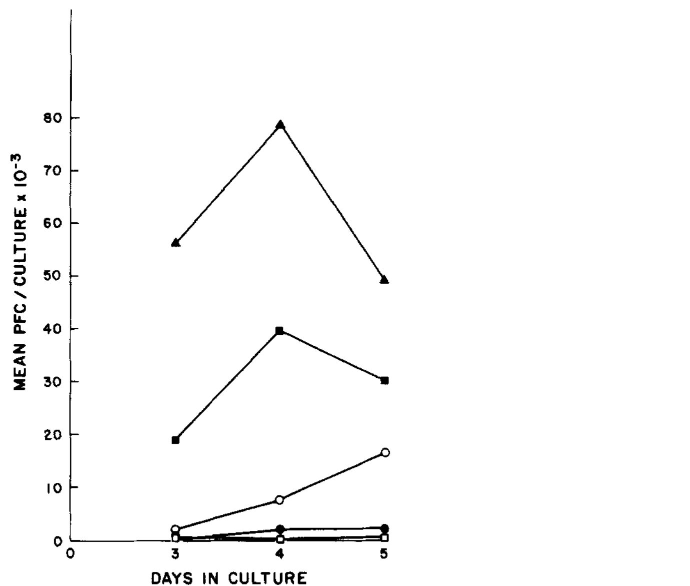

FIGURE 4. Anti-D10 induces T-dependent polyclonal B cell antibody secretion. 3 × 10^4 D10.G4.1 were cultured with 4 × 10^5 BALB.K B cells in 0.2-ml cultures containing; no addition (○), 200 μg/ml conalbumin (■), or anti-D10 1:500 (▲). Control cultures contained B cells and conalbumin (●) or B cells and anti-D10 (□) in the absence of cloned T cells. After a 3-, 4-, or 5-d culture period, triplicate cultures were pooled and assayed in a plaque-forming cell assay using protein A coupled sheep erythrocytes.

T cell activation in this system. We are using this monoclonal antibody to isolate the molecule, presumed to be the antigen-Ia receptor, from D10.G4.1 cells.

Discussion

In an attempt to characterize the proteins involved in helper T cell antigen recognition we have raised antisera and a monoclonal antibody in BALB.K mice against two AKR-derived cloned helper T cell lines, D10 and D8. BALB.K anti-D10 serum, BALB.K anti-D8 serum, and monoclonal antibody 3D3 are potent inducers of T cell proliferation. However, these antibodies react only with the immunizing cell line, exhibiting no cross-reactivity between D10 and D8 cells. In addition, anti-D10 serum does not functionally react with normal or mitogen-stimulated AKR T cells. These results and the fact that D10 and D8 are functionally identical but differ in antigen specificity and alloreactivity, suggested to us that these antisera may react with a variable portion of the antigen/Ia receptor(s). Why such an immunization protocol should produce only antibodies directed against "idiotypic" and not framework determinants, is unknown. Three possibilities are apparent; *a*) T cell receptor constant regions may be less polymorphic than immunoglobulins, with no polymorphism between AKR and BALB.K strains; (*b*) T cell receptor allotypes may be poorly immunogenic or (*c*) anti-T cell receptor allotypes are not detected in the assays used. We have also been able to produce a similar anti-D10 activity in two out of four (AKR × B6)F1 mice and are currently testing AKR anti-D10 sera for activity. White et al. (2)

TABLE IX

Clone-specific BALB.K Anti-D10 Monoclonal Antibody 3D3 Induces T Cell Proliferation; Proliferation is Accessory Cell–independent in the Presence of an IL-1-rich Supernatant

Exp	T Clone*	IL-1-Rich supernatant‡	Antibody§	Response‖
I.	D10.G4.1	−	—	162
	D10.G4.1	+	—	295
	D10.G4.1	−	3D3 1:20	7,194
	D10.G4.1	+	3D3 1:20	100,100
	D10.G4.1	−	Anti-D10	1,421
	D10.G4.1	+	Anti-D10	80,681
	D8	+	—	232
	D8	+	Anti-D8	58,284
	D8	+	3D3 1:20	170
II.	D10.G4.1	−	—	134
	D10.G4.1	+	—	176
	D10.G4.1	−	3D3 1:100	3,080
	D10.G4.1	+	3D3 1:100	105,313
	D10.G4.1	+	3D3 1:12,500	116,280
	D10.G4.1	+	3D3.1 1:20	114,080
	D10.G4.1	+	3D3.2 1:20	149,600
III.	D10.G4.1	−	—	567
	D10.G4.1	+	—	1,599
	D10.G4.1	−	3D3 1:10^5	1,118
	D10.G4.1	+	3D3 1:10^5	57,518
	D10.G4.1	+	3D3 1:10^6	457

* 2×10^4 cloned T cells cultured in 0.2 ml.
‡ Presence (+) or absence (−) of 2% IL-1-rich P388D1 supernatant.
§ Anti-D10 1:8,000; anti-D8 1:200; 3D3 is a monoclonal BALB.K anti-D10 hybridoma culture supernatant; 3D3.1 and 3D3.2 are agar clones of 3D3.
‖ Mean cpm ^{3}H-TdR incorporation of triplicate cultures pulsed for the final 16 h of a 72-h culture period.

also found few limitations in the strain combinations capable of producing specific blocking antibodies directed against helper T cell hybrids. Thus, genetic mapping of the determinants recognized by these sera has, so far, been impossible.

Binz and Wigzell (24) have reported T cell proliferation induced by a heteroantiserum raised against putative T cell allo-receptors and Infante et al. (1) have described activation of alloreactive clones with "antiidiotypic" sera. The ability of anti-D10 and anti-D8 to stimulate T cells has allowed us to compare the activation of T helper cells by antigen and Ia with that induced by putative anti-antigen/Ia receptor antibodies. The anti-D10 or anti-D8 induction of T cell proliferation is dependent on accessory cells. Splenic adherent cells are enriched for these accessory cells, as well as for antigen-presenting cells. The requirement for accessory cells, however, is eliminated in the presence of IL-1. Taken together these results suggest that the accessory cell for an anti-D10 or anti-D8 response may be an IL-1-producing antigen-presenting cell. We have not yet determined whether the accessory cells involved also express Ia molecules. The accessory cell dependence of an anti-D10 response is not Fc receptor-mediated, as dem-

onstrated by blocking studies with monoclonal antibody 2.4G2. Anti-D10 may therefore activate D10 to induce, via a soluble mediator or directly, IL-1 production from the accessory cell population. The lack of genetic restriction between the T cell and accessory cell in antibody-mediated responses would suggest that if direct cell contact is required, it is not mediated through a specific Ia recognition event. However, we have not ruled out the possibility that recognition of nonpolymorphic portions of Ia molecules is involved in this process. Mitogen activation of T cells also appears to involve induction of IL-1 from an accessory cell population in the absence of specific Ia recognition (26). Our data also suggest that anti-D10 will induce receptivity to IL-2, independently of IL-1. It is unlikely, however, that when anti-D10 induces proliferation in the presence of accessory cells that this is the result of accessory T cell-released IL-2, since adherent cells are enriched for accessory cells. In addition, anti-Thy-1 and complement-treated spleen cells are not depleted of accessory cells for an anti-D10-response although deficient in IL-2 production induced by Con A (data not shown). By analogy, the induction of receptivity to IL-2 by antigen and Ia may also be the result of direct activation, independent of IL-1. Meuer et al. (3) have described a monoclonal antibody specific for an individual cloned human cytolytic T cell line which, although it inhibits antigen-induced proliferation, also induces increased receptivity to IL-2.

Infante et al. (1) have described similar clone-specific antisera raised against alloreactive T cell clones. These antisera induce IL-2 production and T cell proliferation (although variably) in the absence of accessory cells, and therefore apparently independent of IL-1. We are currently testing the requirements for the induction of IL-2 secretion by the antibodies described here. The antisera we have prepared also induce low level proliferation, in some experiments, in the absence of added accessory cells. However, minor feeder cell contamination of the cloned T cell population can create the appearance of an accessory cell-independent response. In experiments in which soluble antigen and IL-1 did not induce T cell proliferation, the anti-D10 or anti-D8 response in the absence of IL-1 was negligible (Table IV). These findings also clarify an issue raised by Durum and Gershon (15). These authors proposed that Ia recognition was required in antigen-driven T cell proliferation only for the induction of IL-1 secretion. Our more discriminating system, using cloned antigen-Ia specific T cells and IL-1, demonstrates that IL-1 and antigen are not sufficient for T cell activation. Thus, Ia is required for antigen recognition by helper T cells.

We have also demonstrated that a monoclonal antibody can induce D10.G4.1 proliferation. This finding may have profound implications concerning the mechanism of activation of helper T cells by antigen in association with Ia molecules. Anti-immunoglobulin sera activate B cells, but only at concentrations well above the saturation level of binding (27). In addition, cross-linking of B cell surface immunoglobulin seems to be involved in this triggering process. Unless there are multiple determinants recognized by 3D3 on a single target molecule, monoclonal antibody 3D3 could not extensively cross-link the T cell surface. Also, based on the titer of 3D3 culture supernatants, very low concentrations of antibody are sufficient to activate the T cell. If indeed this antibody binds the antigen/Ia receptor, the interaction of these receptors and antigen-Ia

complexes may therefore be more analagous to the interaction of a hormone with its receptor than to the interaction of a multi-determinant antigen with B cell surface immunoglobulin. Anti-hormone receptor antibodies can, in some systems, mimic the hormone in the activation of a target cell and, in fact, cause pathology by this mechanism in autoimmune disease (28).

RaMBr sera are also mitogenic for D10 and D8. These sera recognize a nonpolymorphic determinant on the Thy-1 molecule (29). Although BALB.K and AKR strains are allelic at the Thy-1 locus, anti-D10 and anti-D8 mitogenic activities are not the result of anti-Thy-1 antibodies for the following reasons: (*a*) D10 and D8 are both Thy-1.1^{+}1.2^{-} but anti-D10 and anti-D8 are clone specific; (*b*) AKR Con A blasts or the Thy-1.1^{+} thymoma BW5147 do not absorb out anti-D10 mitogenic activity while removing anti-Thy-1 mitogenic activity from RaMBr and anti-Thy-1 cytotoxic activity from anti-D10; (*c*) (AKR × B6)F1 anti-D10 is also mitogenic for D10 but not D8; and (*d*) under conditions where RaMBr immunoprecipitates Thy-1 from surface-iodinated D10.G4.1, BW5147 absorbed anti-D10, while still biologically active, does not (data not shown). It is possible, though, that Thy-1 acts as a carrier protein, increasing the immunogenicity of the AKR T cells.

The finding that anti-clone sera substitute for antigen and Ia in the helper T cell-dependent activation of B cells is novel. Blocking studies with 2.4G2 suggest that B cell activation in this system is not mediated through an Fc receptor. Other possible mechanisms of B cell activation, particularly the release of a soluble factor, are currently under investigation. We plan to extend these studies to include antigen-specific responses, and an examination of the activation state of B cells capable of responding to T helper cells activated by these antisera. The fact that anti-D10 and anti-D8 exhibit clone specificity in the induction of T-dependent B cell activation suggests that these antisera are reacting with the same determinant to induce both T cell and T-dependent B cell activation. Preliminary studies with the monoclonal anti-D10 antibody have confirmed this (data not shown).

In preliminary biochemical studies using biosynthetically labeled cloned T cells we have found that these antisera precipitate a number of proteins. One protein is precipitated by anti-D10 from D10, but not D8 cells. Two-dimensional SDS-PAGE analysis (first dimension nonreducing, second dimension reducing conditions) reveals that this protein has an apparent molecular weight of 80,000 and is comprised of 40,000-dalton disulfide bonded subunits. Similar findings have been reported by two other laboratories using clone-specific monoclonal antibodies (3, 30). Two-dimensional gel analysis (first dimension nonequilibrium pH gradient electrophoresis, second dimension SDS-PAGE) of anti-D10 immunoprecipitates reveals that this protein is a heterodimer consisting of an acidic and a basic subunit. Immunoprecipitation studies using the clone-specific monoclonal anti-D10 antibody 3D3 have yielded similar results. A protein with similar characteristics has previously been isolated from a T cell tumor and was also demonstrated to be a constituent of normal T cells (31).

These studies demonstrate that cloned T cell lines can be activated by clone-specific antibodies, including a monoclonal antibody, in the complete absence of other cell types, provided exogenous IL-1 (or IL-2) is added. Thus, this system

is ideally suited for the biochemical analysis of the complex process of T cell activation, as only a single, cloned cell type is present. These antibodies should also serve as a means for isolating and characterizing the receptor involved in antigen-Ia recognition.

Summary

Two antisera and a monoclonal antibody raised in BALB.K mice against cloned, major histocompatibility complex (MHC)-restricted, antigen-specific helper T cell lines are described. These antibodies are specific for individual cloned T cell lines and are potent inducers of T cell proliferation. The induction of T cell proliferation by these antibodies requires the presence of an adherent accessory cell. There is no H-2 restriction between this accessory cell and the cloned T cell, nor is this antibody-induced proliferation blocked by a monoclonal anti-Fc receptor antibody. The requirement for an accessory cell, however, is eliminated in the presence of an IL-1- or IL-2-rich supernatant. Thus this system allows the analysis of helper T cell activation with only a single cell type present. Anti-T cell sera also induce T cell-dependent B cell proliferation and immunoglobulin secretion. The induction of T cell-dependent B cell activation by these sera does not require H-2-matched T cells and B cells. The specificity of these antibodies and their ability to stimulate cloned helper T cells in the absence of antigen and antigen-presenting cells strongly suggest that these antibodies are directed against antigen and/or Ia recognition sites on the T cell.

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