DIRECT INTERACTIONS BETWEEN B AND T LYMPHOCYTES BEARING COMPLEMENTARY RECEPTORS

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After remaining elusive for many years, the receptor on MHC-restricted T cells responsible for antigen/MHC recognition has now been identified by means of antireceptor mAb (1–6). Most of these antibodies were detected by their ability to inhibit the functioning of the particular monoclonal T cell population against which they were raised. However, early studies by Infante et al. (7) with polyvalent antibodies capable of stimulating cloned alloreactive T cells have now been confirmed in our own studies using both antisera and mAb (3, 8–10). In our own studies, monovalent Fab fragments of antireceptor antibody were inhibitory (8), while several other investigators (11–13) have shown that inhibitory mAb coupled to Sepharose beads will stimulate the specific immunizing cloned T cell line.

The reason for the differences in the behavior of these cloned T cell lines is not known. In the present experiments, we have examined several possible mechanisms that might account for the behavior of our system, and can safely conclude that it represents an unusual property of the cloned T cell line used to raise the antireceptor antibody. The striking tendency of this cloned T cell line, D10.G4.1, to become activated upon encountering antireceptor antibodies may also account for its remarkable ability to induce antibodies that stimulate it to secrete lymphokines and, in the presence of IL-1, to proliferate. We can show that the B cell response to the receptor of D10.G4.1 is independent of other T cells both in vivo and in vitro. This has allowed us to prepare a battery of antireceptor mAb against D10.G4.1 that will be useful in further analyzing the interaction of antibody, antigen, and MHC-encoded molecules with the T cell receptor.

Materials and Methods

Animals. BALB/cByJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME; BALB.B and BALB.K mice were bred at Yale by Dr. Donal B. Murphy; nude mice were purchased from Life Sciences, North St. Petersberg, FL; Sprague-Dawley rats

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were purchased from Camm Research Lab Animals, Wayne, NJ.

Antibodies. The anti–D10 receptor mAb 3D3 has been extensively characterized (3, 8–10); its Fab fragment was prepared as described (8). mAb KJ-16 was a kind gift of Drs. Kappler and Marrack, and was used as a culture supernatant without further purification (6). Monoclonal anti–brain-associated T cell antigen (anti-BAT)[1] was prepared as an ascites in nude mice as previously described (14). Anti-D8 antibody was prepared as described previously (3).

Cloned T Cell Lines. Cloned T cell lines were prepared and maintained as previously described (3, 15). Cloned line D10.G4.1 (D10) is specific for hen egg conalbumin (CA) plus I-A^k and is alloreactive to I-A^b (3, 8). Cloned T cell line D8 from the same mouse is specific for hen OVA and I-A^k (3). A panel of BALB/cByJ, BALB.B, and BALB.K cloned T cell lines specific for OVA and self Ia was previously described (16).

T Cell Proliferation Assays. Typically, 2×10^4 cloned T cells were added to 200-μl cultures in flat-bottom microtiter trays in Click's EHAA medium with 10% FCS. Cell growth was stimulated by mAb or by antigen plus 3×10^5 syngeneic, mitomycin C–inactivated spleen cells, as previously described (3, 15). In some experiments, antibodies were added to T cells before the addition of antigen and feeder cells. After 48 h of culture, 1 μCi [^{3}H]TdR was added to each well, and the cells harvested 16 h later. Incorporated TdR was determined in triplicate cultures.

Interleukins and Interleukin assays. IL-1 was prepared by stimulating P388.D1 cells with 1 μg/ml LPS for 25 h (3). Activity was determined by costimulation of the growth of D10 cells (9). IL-2 was either the crude supernatant of rat spleen cells stimulated for 24 h with Con A or recombinant-derived human IL-2 donated by Dr. Richard Robb, E. I. du Pont de Nemours, Glenolden, PA. IL-2 was assayed by culturing with 10^4 HT-2 indicator cells for 36 h, followed by a 16-h pulse with [^{3}H]TdR.

Immunization and Production of mAb. Nude or BALB.K mice or Lewis rats were immunized intraperitoneally with 10^7 viable D10 cells. At various times later, they were boosted intravenously with live D10 cells, and 3 d later their spleen cells were fused with the nonsecreting myeloma P3X63/Ag-8.653 as previously described (3, 17). Cultures were fed after the first week with RPMI 1640 plus 5% FCS, and supernatants were tested for their ability to stimulate D10 cells plus IL-1 or to inhibit their response to CA plus I-A^k. Positive wells were expanded, cloned by limiting dilution, and rescreened for stimulation of D10 plus IL-1.

In Vitro Production of Anti-D10 Antibody. B cells from mice primed in vivo with 10^7 D10 cells 2–4 wk previously were prepared by treating spleen cells with anti-BAT antibody followed by rabbit complement, as previously described (18). These cells were cultured alone or with 10^4 mitomycin C–treated D10 or D8 cells for 6 d. Culture supernatants were tested at a dilution of 1:6 for their ability to stimulate D10 cells in IL-1; positive wells were rescreened for blocking by 3D3 Fab fragment.

Results

D10 Cells Are Activated by KJ-16, an Anti–T Cell Receptor mAb that Inhibits the Responses of Other Cloned T Cell Lines. The cloned T cell line D10 responds to soluble 3D3 antireceptor mAb at 1 ng/ml; aggregation of this antibody on Sepharose beads does not alter its behavior (J. Kaye, unpublished observations). However, it was possible that aggregates in the preparation of 3D3 were responsible for the ability of this antibody to stimulate D10. To examine this question, we obtained an mAb, KJ-16, specific for shared portions of the T cell receptor, from Drs. Kappler and Marrack. This antibody reacts with T cells of clone D10, but not T cells of clone D8, as determined by FACS analysis (not shown). Soluble KJ-16 stimulates D10 cells to secrete IL-2 (not shown) and, in the presence of IL-1, to proliferate, as shown in Table I. Although not as active in this regard as

[1] *Abbreviations used in this paper:* BAT, brain-associated T cell antigen; CA, hen egg conalbumin.

TABLE I

mAb KJ-16 Stimulates Cloned T Cell Line D10 But Not D8 to Proliferate

Cloned T cell line*	IL-1‡	T cell proliferative response to:				
		0	3D3§	KJ-16 1:10‖	KJ-16 1:100	Anti-D8¶
D 10	−	218	12,665	4,662	5,965	—
	+	250	173,340	39,153	38,450	—
D 8	−	104	—	132	130	3,610
	+	82	—	146	114	52,293

* 2×10^4 cloned T cells per well in 200-μl cultures. Triplicate cultures pulsed after 48 h with 1 μCi [^{3}H]TdR and harvested 16 h later. Mean cpm is reported.

‡ 4% of supernatant from LPS-stimulated P388.D1 cells.

§ mAb 3D3 final concentration 5 ng/ml.

‖ KJ-16 culture supernatants added at 1:10 or 1:100 final dilution.

¶ Anti-D8 serum prepared as previously described (3) and added at 1:150 final dilution.

soluble 3D3 antibody, this behavior of KJ-16 is in marked contrast to its ability to inhibit the proliferation of several other cloned T cell lines to stimulation by antigen plus self Ia, as reported previously (6, 16) and shown in Table II. Thus, we have examined the responses of several cloned T cell lines to KJ-16 and find that, of cloned lines reactive with this antibody, only D10 responds to the soluble antibody by IL-2 secretion and proliferation.

D10 Cells Require Neither APC nor Cell-Cell Interaction in Responding to Antireceptor Antibody. While soluble antireceptor antibody clearly activates D10 cells, it is possible that residual APC present in the culture are involved in this response. Alternatively, D10 cells may present antireceptor antibodies to one another, one cell serving to aggregate the antibody for another. To examine these questions, D10 cells were purified from culture by Ficoll-Hypaque centrifugation 3 wk after last receiving feeder cells, and then added to cultures containing 3D3 and IL-1. Both IL-2 secretion and D10 proliferation were determined and plotted against the cell number per well (Fig. 1). On this log-log plot, the response is linearly related to the cell number, and the titration curve has a slope of approximately one. Thus, only a single cell type, D10, appears to be limiting. In addition, when feeder cells are used as a source of IL-1 (Table III), their FcR for IgG1/IgG2b recognized by mAb 2.4G2 were required for the response to rabbit anti-mouse brain sera, but not for responses to the IgG1 antibody 3D3. Thus, FcR on T or B cells do not play a detectable role in this response. To confirm the finding that cell interactions are not required for D10 responses to 3D3, we plated D10 cells at 0.3 cells/well in flat-bottom microtiter plates, identified cultures containing single cells in an inverted microscope, and added 3D3 plus IL-1, or medium alone to such wells. Wells were inspected daily, and the number of cells per well was recorded. Soluble 3D3, at 10 ng/ml, plus IL-1 (5%) stimulated the great majority of D10 cells to divide for at least 3 d without further additions (Fig. 2), with a division rate of >1 per 24 h. This strongly suggests that 3D3 can stimulate D10 cells directly in the absence of APC, and that the great majority of cells in bulk cultures of D10 cells can respond in this way.

D10 Cells Give Rise to a High Frequency of Stimulating mAb. In our initial study of D10 cells, we observed a very high titer of stimulating, clone-specific antibodies in the sera of BALB.K mice immunized with D10 cells (3). We were able to

TABLE II

mAb KJ-16 Inhibits Response of Cloned T Cell Lines to Antigen Presented by Syngeneic Feeder Cells

Cloned T cell line*	Stimulator cells‡	Antigen§	T cell proliferative response in presence of:‖	
			0	KJ-16¶
8B5	C57BL/6	−	2,343	2,491
		+	42,748	16,911
8B6	C57BL/6	−	701	661
		+	45,727	26,433
8B6	C57BL/6	−	10,869	1,820
		+	144,480	37,502
8D3	BALB/c	−	6,957	4,945
		+	144,480	55,987
8D11	BALB/c	−	2,967	3,164
		+	51,293	19,275
AK-5	BALB.K	−	928	458
		+	22,427	3,007
AK-8	BALB.K	−	363	311
		+	31,074	1,198
AK-1**	BALB.K	−	6,856	8,106
		+	42,370	33,055
JBr-2**	BALB.K	−	470	553
		+	15,742	23,446

* Clones 8B5 and 8B6 are BALB.B cells specific for OVA/I-A^b, while 8D3 and 8D11 are BALB/c cells specific for OVA/I-A^d (16); clones AK-1, AK-5, and AK-8 are AKR/J clones, and clone JBr-2 is a B10.BR clone, all specific for CA/I-A^k (our unpublished results). 2×10^4 T cells added per well. Each cloned line was tested at least twice; representative data are shown.

‡ 5×10^5 mitomycin C–treated spleen cells were added per well.

§ OVA or CA was added to 100 μg/well.

‖ 200-μl cultures were established in the presence or absence of KJ-16, which was added to cloned T cells for 60 min at 37°C before adding stimulator cells and antigen. Triplicate cultures pulsed after 48 h with 1 μCi [^{3}H]TdR and harvested 16 h later. Mean cpm are shown; SD (< 20% of mean) are omitted.

¶ Medium or KJ-16 culture supernatant was added to a final concentration of 1:4.

** Clones AK-1 and JBr-2 are KJ-16 negative.

isolate a stimulating mAb from just 60 parent wells showing hybridoma growth; a second positive well was lost before cloning. We have now repeated this experiment with two more BALB.K mice, and have found 19 positive parent wells from a total of 560 showing growth. We have been able to clone 17 of these parent wells, and all yielded mAb that stimulate clone D10. Five of the nine cloned cell lines tested are completely inhibited by 3D3 Fab and are thus directed at an epitope on the receptor close to or identical to that recognized by 3D3, as shown in Table IV, and the response to the other four is partially but significantly inhibited by 3D3 Fab. These results stand in stark contrast to the difficulties we and other laboratories have had in raising mAb to most cloned T cell lines (2, 4, 5). For instance, Kappler et al. (12) report screening 15,000 parent wells to obtain two antireceptor mAb. We find only stimulating mAb; we

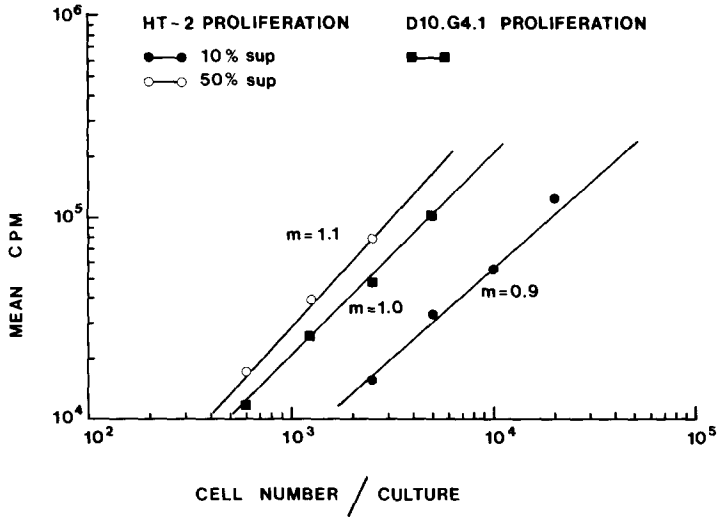

FIGURE 1. Dose-response analysis of the activation of D10 cells by 3D3 and IL-1. 0.2-ml cultures containing various numbers of D10 cells, 1:1,000 human IL-1, and 50 ng/ml 3D3 were established, and [^{3}H]TdR incorporation after 3 d (■) was determined by pulsing the culture for the final 16 h. In addition, culture supernatants at 50% (○) and 10% (●) were analyzed for their ability to induce proliferation in HT-2 cells (10^4 cells/well) in 24-h cultures pulsed for the final 4 h with 1 μCi [^{3}H]TdR. Slopes (m) were determined by linear regression analysis for best fit.

TABLE III

3D3 Does Not Require Interaction with FcR to Induce D10 Proliferation

T cells*	Accessory cells‡	Stimulus§	Anti-FcR‖	Response¶	
				Exp. 1	Exp. 2
D10	BALB.K	—	−	63	3,135
D10	BALB.K	3D3	−	14,970	56,155
D10	BALB.K	3D3	+	13,353	45,985
D10	BALB.K	RAMBr	−	13,306	59,686
D10	BALB.K	RAMBr	+	63	15,619
D10	—	3D3	−	504	1,811

* 2×10^4 cloned T cells per 0.2-ml culture.
‡ 2×10^5 mitomycin C–treated spleen cells per 200-μl culture.
§ 1:1,000 3D3 culture supernatant or rabbit anti–mouse brain serum (RAMBr) at 1:100.
‖ 1 μg/ml mAb 2.4G2.
¶ Mean cpm [^{3}H]TdR incorporation of triplicate cultures pulsed for the final 16 h of a 65 h culture period.

have never observed an mAb to D10 cells that inhibited their activation by CA plus I-A^k, although we have screened nearly 1,000 parent wells for such an activity.

Evidence that D10 Receptors Interact Directly with Complementary Molecules on B Cells in Inducing the Anti-D10 Antibody Response. The unique characteristics of the D10 clone described above, namely its ability to be stimulated by soluble antireceptor mAb and its striking ability to generate antisera and mAb that will activate it via its receptor for CA plus I-A^k (3), taken together with the ability of

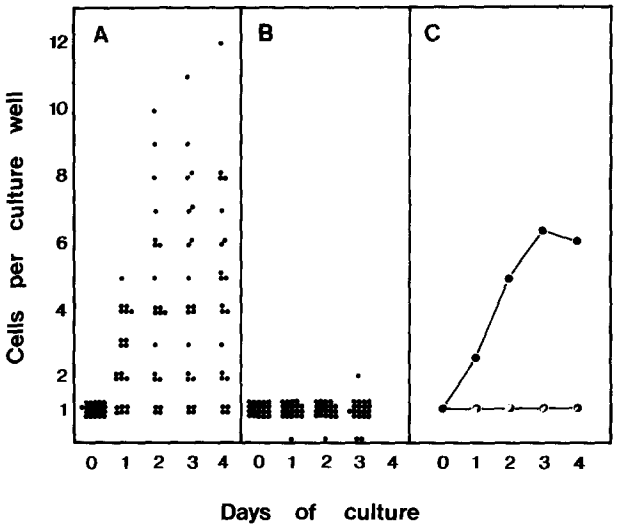

FIGURE 2. Direct determination of the ability of single D10 cells to respond to 3D3 and IL-1. Flat-bottom microtiter plates were seeded with 0.3 D10 cells per 0.2-ml culture; some were also given 10 ng/ml 3D3 and 5% murine IL-1 (*A*). Individual wells were inspected and seen to contain one D10 cell at time 0. Cultures were stimulated with 3D3 plus IL-1 (*A*), or left unstimulated (*B*). Mean number of cells per well in *A* (●) or *B* (○) is plotted in *C*.

3D3-stimulated D10 cells to activate purified resting B cells to proliferate and to secrete Ig (18), suggested that D10 cells might stimulate B cells bearing complementary Ig receptors by a direct receptor-receptor interaction. To test this hypothesis, B cell responses to D10 cells were examined in the absence of other T cells. This was done in two ways. First, nude mice were immunized with D10 cells in the same way as BALB.K mice, and their spleen cells were harvested and fused with myeloma cells. Of 297 wells with growth, 15 primary wells were found to stimulate D10 cells. After a growth crisis, four of these yielded subclones that stimulated D10 cells. We have not yet determined whether these mAb are specific for D10 receptors, although this seems likely. Thus, nude mice will make a stimulating antibody response to the receptor on D10 cells under conditions analogous to those in euthymic mice, and the yield of positive wells is ~1/20, although in this case the recloning efficiency was lower for unknown reasons.

To more directly assess the ability of D10 cells to directly activate B cells, 10^4 mitomycin C–treated D10 cells were cultured with 4×10^5 purified B cells from D10-primed BALB.K mice. Supernatants of such cultures were tested for their ability to stimulate D10 cells to proliferate in the presence of IL-1. Whereas 2% (1/48) of B cell cultures without D10 cells gave significant levels of stimulation, 17 of 192 wells (8.3%) with D10 cells gave significant levels of proliferation. Of these, 15 of 17 stimulated strongly on repeat testing, and were inhibited by 3D3-Fab, while the one positive control supernatant was negative on repeat testing (Table V).

A second experiment compared D10 cells with D8 cells, and found that the 3D3-inhibitable anti-D10 antibody response occurred using D10 cells, but not D8 cells. Thus, purified B cells can be stimulated by clone D10 cells to make

TABLE IV
Anti-D10 mAb that Stimulate D10 Growth Are Directed at the Same or Closely Associated Sites as 3D3

mAb*	D10 proliferative response in the presence of:‡	
	0	3D3-Fab§
Exp. 1		
0	2,800	2,800
3D3	136,000	4,100
193.6	112,700	6,100
193.8	42,600	2,600
193.9	94,900	2,700
193.5	35,000	17,000
193.10	107,500	47,000
193.13	77,700	13,000
Exp. 2		
0	5,000	16,000
3D3	380,000	22,000
193.12	177,000	17,000
193.14	17,000	11,000
193.16	115,000	119,000
Exp. 3		
0	10,700	22,800
3D3	324,000	23,500
193.16	189,000	60,700

* 25 μl 3D3 at 100 ng/ml or unknown supernatant of BALB.K anti-D10 mAb.

‡ 10^4 D10 T cells in 200 μl Click's EHAA plus 10% FCS and 5% P388.D1 supernatant as a source of IL-1. After 48 h, 1 μCi [^{3}H]TdR was added per culture, and means of triplicate ^{3}H incorporation determined 16 h later.

§ The Fab fragment of 3D3, 5 μg/ml (Exps. 1 and 2) or 25 μg/ml (Exp. 3), was added to culture. In control experiments, the D10 response to KJ-16, Con A, PHA, or recombinant IL-2 is not inhibited by >25% by 3D3-Fab.

antireceptor antibody in vitro in the absence of contaminating T cells. The precursor frequency of such B cells appears to be low. This is not a polyclonal antibody response, as polyclonal responses require either antigen or antireceptor antibody (18).

Finally, rats immunized with D10 cells will also produce antibodies that stimulate clone D10 cells specifically (Table VI). While T cells are present in these rats, it seems likely that this again reflects direct receptor-receptor interaction of T and B cells across a species difference.

Discussion

The cloned T cell line D10 can be stimulated by soluble antireceptor mAb to secrete IL-2 and, in the presence of IL-1, to increase its level of receptors for IL-2 and proliferate in response to its own IL-2 (3, 8–10). This stands in contrast to our own experience with cloned T cell lines, and to that of other investigators.

TABLE V

Stimulation of D10 Cells by Supernatants of BALB.K B Cells Cultured with D10 Cells: Inhibition by 3D3-Fab

Supernatant*	Proliferative response of D10 cells plus IL-1‡	
	Without 3D3-Fab	With 3D3-Fab§
0	476	300
3D3‖	111,370	270
1A10	29,142	451
1A12	9,433	3,884
1B7	15,058	2,139
1C6	14,196	1,665
1G12	37,260	928
2C11	18,785	310
2D8	46,212	614
2E11	35,153	465

* Supernatants of cultures of 4×10^5 BALB.K splenic B cells cultured with 10^4 mitomycin C–treated D10 cells for 6 d; only representative wells shown.

‡ 25 μl of supernatant was added to 125 μl with 10^4 D10 cells and 6% IL-1 for 48 h. 1 μCi [^{3}H]TdR was added for the final 16 h of culture, and responses were determined.

§ 5 μg/ml Fab fragments were added to cultures. In control experiments, 3D3-Fab inhibited 3D3-driven D10 responses by 95–100%, Con A responses by 0–8%, PHA responses by 0–16%, and recombinant IL-2 responses by 0–26%.

‖ 3D3 added at 100 ng/ml.

TABLE VI

Rat Anti-D10 Sera Induce D10 But Not D8 Proliferation in the Presence of IL-1

T Clone*	IL-1‡	Antiserum§	Response‖		
			Exp. 1	Exp. 2	Exp. 3¶
D10	−	—	154	54	33
	+	—	68	37	158
	+	RaD10.1 1:100	20,260	4,182	1,001
	+	RaD10.1 1:300	3,675	ND	ND
	+	RaD10.2 1:100	32,167	13,972	24,943
	+	RaD10.2 1:300	16,325	ND	ND
D8	−	—	75	17	64
	+	—	177	34	128
	+	RaD10.1 1:100	46	35	96
	+	RaD10.2 1:100	83	35	79
	+	Anti-D8 1:200	ND	21,656	ND

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

‡ 1% of an IL-1-rich P388.D1 supernatant.

§ Rat anti-D10.G4.1 (RaD10); 1 and 2 denote sera from individual animals; preimmune normal rat serum does not stimulate D10 cells (data not shown).

‖ Mean cpm [^{3}H]TdR incorporation of triplicate cultures pulsed for the final 16 h of a 65 h culture period.

¶ Exps. 1 and 2 are duplicate experiments using the identical sera; Exp. 3 uses a later bleed from the same animals.

The present studies show that D10 cells differ from other cloned lines prepared by the same technique in our laboratory, since mAb KJ-16, specific for a determinant found on receptors of ~25% of T cells in most strains, stimulated D10 cells in soluble form but inhibited several other cloned T cell lines with which it reacted (all six tested herein). However, the ability of soluble antibody to activate rather than inhibit cloned T cell lines is not confined to D10; Infante et al. (7) reported antisera that stimulate cloned alloreactive T cells, and we have observed similar behavior with a second cloned T cell line, D8, as reported earlier (3) and confirmed here (Tables I and VI). We observe no difference in the response of D10 cells to soluble or Sepharose bead–conjugated 3D3 (not shown). Both preparations stimulate to the same titer, and both require IL-1 to observe D10 growth.

A second, and perhaps related, unusual characteristic of D10 cells is their ability to induce stimulating antireceptor antibodies in vivo. As judged by our own experience over several years with numerous cloned T cell lines, and the difficulty other investigators have experienced in attempting to raise antireceptor antibodies to cloned T cell lines, this is a remarkable property of D10 cells. This is confirmed in the present experiments by the finding of a frequency of 1 hybridoma in 30 that stimulates clone D10 cells in four separate fusion experiments. That a similar frequency is observed using nude mice suggests that T cells other than D10 itself are not required for this response. This is strongly supported by the findings presented in Table V, in which B cells are stimulated in vitro by D10 cells to secrete anti-D10 antibody.

The basis for the unusual ability of D10 cells to be stimulated by antireceptor antibody is clearly the property of single cells, and does not reflect either the antibody or occult cell interactions. The cellular basis for this unusual characteristic is not known. We are presently preparing variants of D10 that respond to Sepharose-bound 3D3 but not to soluble 3D3. Comparison of the variant to the parent line may give insight into this question.

The ability of D10 cells to activate B cells to secrete stimulating antireceptor antibodies, many (and perhaps all) of which are clearly directed at the antigen-recognizing site on the receptor, since they are inhibited by 3D3-Fab (8), is believed to reflect a direct interaction between the receptor on D10 cells and the complementary B cell Ig. We propose that this interaction leads to aggregation of the D10 receptor, which in turn activates D10 cells to function as Th cells. This argument is strongly supported by our previous finding that soluble 3D3 can activate D10 cells to induce B cell proliferation and Ig secretion (18), and that this is as potent a stimulus of B cell responses as is antigen plus self Ia on the B cell surface. 3D3-Mediated stimulation of B cells is not dependent on FcR on B cells, as shown previously also for anti-D10 antisera (3 and J. Kaye, unpublished results). Thus, we believe that the production of antibody stimulatory for D10 cells serves as a measure of direct stimulatory interactions of T cell and B cell receptors.

However, this receptor-receptor interaction should, in theory, apply to all cloned T cell lines. Why is D10 unusual in its ability to stimulate the B cells so contacted to secrete antibody and fuse to become hybridomas? The answer may lie in three unusual characteristics of D10 cells. The first is their ability to be

stimulated by soluble as well as Sepharose-bound antireceptor antibody. If a Th cell is inhibited by soluble antibody, then even if it is activated to help a B cell whose Ig is complementary to the T cell receptor and hence activates the T cell, the Th cell will be inhibited by the antibody once the B cell commences Ig secretion, and will thus stop providing helper signals. D10 cells, by contrast, will continue to be stimulated in this situation, and will continue to secrete helper lymphokines. Second, D10 secretes potent helper lymphokines upon receptor crosslinking by antibody, as determined by its ability to activate resting and *xid* B cells in the absence of direct cell-cell contact (18). Furthermore, D10 produces much more IL-2 than any other cloned T cell line we have observed. Thus, D10 is simply a more efficient Th cell than most other cloned T cell lines. In analyzing responses to phosphorylcholine, Kim et al. (19) confirmed this conclusion. Third, and perhaps most important, D10 cells have no cytotoxic capacity, whereas most of our cloned T cell lines can kill or inhibit the growth of B lymphoma lines bearing appropriate antigen plus Ia complexes (20–22). Recently, Lancki and Fitch (23) have shown that a cytolytic T cell line can kill a hybridoma making antireceptor antibody, while Kranz et al. (24) have shown that cells chemically coupled with antibody directed at the receptor of a cytolytic T cell are also killed by such cells. Thus, it may be especially difficult to raise mAb directed at receptors on such cells, and dead or inactivated T cells might be the best immunogen in these cases. In the case of Th cells, we find that most of the cloned lines we have produced make cytotoxic molecules, such as lymphotoxin (22, 25) or lytic lymphokine (26). While such cells can serve as Th cells, they do so far less well than does D10 (19). Such cytolytic, Ia-restricted T cells clearly kill activated B cells, and can be shown to suppress both specific (27, 28) and polyclonal (J. Tite, unpublished results) B cell responses. The combination of activation by soluble antireceptor antibody, high helper efficiency, and lack of cytolytic capacity may well account for the striking ability of D10 cells to induce antireceptor antibodies in mice and rats by direct interaction with B cells bearing complementary receptors.

This experimental system may provide an interesting model for studying the interaction of T and B lymphocyte receptors in the generation and expression of the T and B cell repertoires. The assay system is highly sensitive and specific, and the expansion of our panel of antireceptor mAb could yield antibodies to recurrent T cell idiotypes; recurrent Ig idiotypes have played a central role in examining the B cell receptor repertoire. Clearly, in the D10 system, both the B cell and the T cell are activated to proliferate and secrete. As argued above, this response is probably an exaggeration of the normal response occurring when T cells and B cells with complementary receptors interact. While Eichmann et al. (29), using idiotype-immunized T cells, have shown that such cells can drive antigen-independent activation of idiotype-bearing B cells to secrete antibody, a finding confirmed subsequently by Pierce et al. (30) and by Cerny and Caulfield (31), many investigators have not observed direct B cell activation by antiidiotypic T cells (32–39). Rather, such studies have revealed an influence on the activation of B cells by antiidiotypic T cells; full B cell activation requiring a conventional, antigen plus Ia–specific Th cell as well. It is interesting to speculate that antiidiotypic Th cells might resemble D10 in their interaction with B cells. Such

cells have been shown in several systems to depend upon B cells or their products for their differentiation (32, 39). Thus, Ig appears to play a role in shaping the antiidiotypic T cell repertoire. Antiidiotypic T cells, in turn, may further shape both the B and the T cell repertoire through further receptor-receptor interactions (40, 41). Having a simple and highly sensitive experimental system to explore these questions should make it easier to monitor these effects.

Summary

A murine cloned Th cell line specific for the antigen conalbumin in the context of self I-A molecules can be activated by low concentrations of soluble antireceptor mAb. By using an antireceptor mAb to shared antigenic determinants on T cell receptors, we have shown that the ability to be activated by soluble antireceptor mAb is an unusual, although not unique, feature of this cloned T cell line. This activation does not involve occult APC, FcR, or interaction between individual cloned T cells, as limiting-dilution analysis shows that individual cells of this clone will grow in the presence of the antireceptor antibody and IL-1 as stimulus. This cloned T cell line is highly immunogenic in vivo, giving rise to antireceptor antibodies that stimulate its growth in both mice and rats. This response is not dependent upon exogenous T cells. Rather, the clone directly interacts with complementary B cells, as shown by the production of mAb in nude mice, and by production of stimulating antireceptor antibodies by purified B cells cultured with cloned Th cells in vitro. Several features of this cloned Th cell line, most especially its ability to be activated, rather than inhibited, by antireceptor antibodies, may account for its striking ability to directly activate B cells bearing complementary receptors. The direct interaction of the cloned Th cell with B cells bearing complementary receptors may serve as a model for receptor-receptor interactions in the generation of both T and B cell repertoires.

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