SUBPOPULATIONS OF B CELLS DISTINGUISHED BY CELL SURFACE EXPRESSION OF Ia ANTIGENS

Correlation of Ia and Idiotype During Activation by Cloned Ia-restricted T Cells*

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The major histocompatibility complex (MHC)[1] has been shown to play a significant role in regulating cell-to-cell interactions. Antigens encoded by the I region of the MHC (Ia antigens) are essential in cellular interactions between T cells and antigen-presenting cells (APC) (1–4) and between T and B cells (5–8). It is clear that while Ia glycoproteins are expressed on all B cells, the density of Ia antigens on B cells is heterogeneous (9–10). Not only have subsets of B cells been defined by means of B cell surface Ia expression (10–13), but it has been suggested that the quantitative expression of surface Ia may be critical in B cell activation (14). Recent studies (15) have shown that T cell interactions with APC are influenced by Ia density, with relatively small changes in Ia expression influencing the antigen-specific T cell proliferative response. While this has been clearly demonstrated for T cell interactions with antigen-bearing APC, few studies have focussed on the effect of B cell Ia density on T helper (Th) cell-B cell interactions, and none have examined the expression of a dominant cross-reactive idiotype (Id) as a function of B cell surface Ia antigen expression.

Th cells have been shown in many studies to recognize antigen in the context of B cell Ia (6, 16). For the present experiments, a series of monoclonal ovalbumin (OVA)-specific T helper cells were generated whose interaction with B cells has been shown to depend on recognition of B cell Ia glycoproteins (16). The cloned Th cells, which were used to induce a T-dependent antiphosphorylcholine (anti-PC) antibody response in vitro, appeared to be identical to the "classic" Th cell (17), requiring hapten-carrier (PC-OVA) linkage for optimal B cell activation. This in vitro system allowed an analysis of the role of B cell Ia density in the response to PC-OVA. The T-dependent anti-PC plaque-forming cell (PFC) response generated in the presence of cloned Th cells differed from the PC-specific responses generated in vivo (18–20) or in vitro (20) using carrier-primed whole splenic Ly-1$^+$ T cell populations in that the anti-PC PFC responses induced

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[1] *Abbreviations used in this paper*: APC, antigen-presenting cell; BA, *Brucella abortus*; Id, idiotype; MHC, major histocompatibility complex; OVA, ovalbumin; PC, phosphorylcholine; PFC, plaque-forming cell; T15, TEPC-15; TCGF, T cell growth factor; Th, helper T cell; TNP, trinitrophenyl.

by the Th cell clones were idiotypically heterogeneous.

These studies suggest either that the monoclonal Th cell activated all available PC-specific precursor B cells, only a minority (30%) of which bear the TEPC-15 (T15) Id, or alternatively that Ia-recognizing Th cells require a threshold level of Ia on the B cell surface and these activate only that fraction of the PC-specific B cells of highest Ia density to secrete antibody. Both of these possibilities seemed reasonable in that the studies of Cosenza (20) using limiting dilution analysis demonstrated that the majority (75–98%) of PC-specific precursors are T15 Id bearing, whereas the somewhat more idiotypic heterogeneity of PC-specific precursor B cells was reported by Gearhart et al. (21). Since greater idiotypic heterogeneity was noted in our studies than in either of the previous reports on PC-specific precursors, studies were carried out to evaluate whether or not the level of Ia expression on a B cell might influence its ability to interact with a cloned Ia-recognizing Th cell.

To test for the importance of Ia density in B cell activation by Ia-recognizing T cell clones, B cells were treated with a monoclonal anti-Ia antibody and complement that kills only B cells with a high density of Ia antigens. The activation requirements of the remaining B cells were tested. The results demonstrated (*a*) that the total anti-PC PFC response was reduced after anti-Ia antibody and complement pretreatment even if equivalent numbers of B cells were tested and (*b*) that primarily non–T15-bearing B cells were affected by this pretreatment. These results indicated that the non–T15-bearing B cells are enriched in the B cell pool expressing high levels of Ia glycoproteins and that these B cells are most easily activated by the Ia-recognizing cloned Th cells.

Materials and Methods

Mice. BALB/cByJ and (BALB/c × C57Bl/6)F1 mice were obtained from The Jackson Laboratory, Bar Harbor, ME and were used between 8 and 12 wk of age. BALB.B mice were bred at Yale University.

Antigens. The preparation of antigens used in these experiments has been described previously (22, 23).

Antisera and Monoclonal Antibodies. The preparation, purification, and testing of rabbit anti-T15 Id antibodies have been described previously (22, 23). In addition to rabbit anti-T15 antibodies, a monoclonal anti-T15 antibody was used. The monoclonal anti-T15 was shown by enzyme-linked immunoabsorbent assay (ELISA) and hemagglutination assays to bind to T15 and to a lesser degree MOPC-511 myeloma proteins, but not to MOPC-167 proteins. Hybridoma anti-Thy-1.2 was kindly provided by Dr. J. Sprent, Wistar Institute, Philadelphia, PA. Monoclonal anti-Lyt hybridomas were kindly provided by Dr. Paul Gottlieb (24). Monoclonal antibodies specific for $A_e^{b,k,r,s}$:E_α complexes (Y17) (25), I-A^d (26), I-A^b (27), and H-2.5 (16) are described in detail elsewhere.

Preparation of Cells. Cloned T cell lines were generated according to the method of Sredni et al. (28). The preparation, cloning, and analysis of the various cloned T cells has been described extensively elsewhere (16, 29–31). Basically, all the cloned T cells tested are phenotypically Thy-1^+, Lyt-1^+, 2^- and have been grown for >1 yr, maintaining a stable phenotype and specificity pattern even after extensive recloning. Although the maintenance of these lines requires mitomycin C-treated feeder cells and T cell growth factor (TCGF), TCGF is not needed during the helper cell assay. Before use the T cells are washed extensively. T cells from unprimed mice are treated simultaneously with anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.2 monoclonal antibodies plus complement. Splenic adherent cells are prepared as described by Cowing et al. (32). Briefly, T cell-depleted spleen cells are allowed to adhere on plastic tissue culture dishes at 37°C for 2 h.

Nonadherent cells are removed by gentle washing and are replated on a second plastic dish. After 2–3 cycles, the nonadherent cells are depleted of APC function. The adherent population is incubated for 6 h, removed, and used as a source of APC.

Cell Cultures. For 96-well Costar plates, 3.0×10^5 B cells are cultured along with varying numbers of cloned Th cells and antigen. For 24-well Costar plates, 3×10^6 B cells, 1×10^5 Th cells and antigen are cultured in 1 ml volume. Cultures were incubated at 37°C for 5 d and assayed for PFC responses.

Hemolytic (PFC) Assay. The cultures were assayed for direct anti-PC PFC or antitrinitrophenyl (TNP) PFC by the modified Jerne hemolytic plaque technique (33) as described previously (22).

Inhibition of Plaque Formation. The proportion of anti-PC PFC shown to be of the T15 idiotype was determined by inhibition of plaque formation using both rabbit anti-T15 and monoclonal anti-T15 antibodies in the agarose suspension. Only the PFC shown to be inhibited by free PC were considered to be PC-specific.

Analysis of PFC Phenotype. Cultured cells were treated with 100 μl of monoclonal anti-H-2.5 antibody for 30 min at 4°C. The treated cells were washed and incubated for 45 min at 37°C with absorbed rabbit complement. Control aliquots of each culture were treated with complement alone, and after treatment, test and control aliquots were resuspended to a standard volume before the PFC assay.

FACS Analysis. A fluorescence-activated cell sorter (FACS IV; BD FACS Systems, Sunnyvale, CA) was used to examine antigens on spleen cell surfaces. The fluorescent reagents used for these experiments were prepared as described previously (25).

Results

Monoclonal, Ovalbumin-specific T Cells Provide Help for a Primary Anti-PC Response In Vitro. For most T-dependent responses, antigen-specific, Ia-recognizing Th cells have been shown to be required for B cell activation to secretion (5, 6, 16). The precision of this interaction can be analyzed most effectively by evaluating the interaction of cloned Th cells and unprimed B cells in vitro. In these studies, monoclonal Lyt-1^+,2^- T cells which have been shown to recognize the antigen OVA in the context of syngeneic Ia (16, 29) were assayed for their ability to induce anti-PC PFC responses. As seen in Fig. 1, cloned T cells were capable of generating substantial anti-PC PFC responses in the presence of PC-OVA. By contrast to the response to PC-*Brucella abortus* (BA), a T-independent antigen, the response to PC-OVA required T cells, and increased in magnitude with increasing monoclonal T cell number. The in vitro response was generated over a broad range of antigen concentrations (Fig. 2), and as was seen in vivo, required the hapten, PC, to be physically linked to the carrier, OVA (30, 31, and data not shown). These data demonstrate that monoclonal Ly-1 T cells that proliferate specifically to OVA in the context of syngeneic Ia glycoprotein antigens can activate B cells to secrete anti-PC antibody.

Cloned Th Cells Induce an Anti-PC Response That Is Idiotypically Heterogeneous. Since the T-dependent anti-PC response in vivo and in vitro has been shown to be characterized by the secretion of antibody predominantly bearing the T15 Id (18–20), it seemed important to determine whether or not the PC-specific B cells activated in the presence of monoclonal Th cells were mainly T15 bearing. A summary of previously published data (30, 31) is presented in Table I. Using extensively recloned T cells, the response to PC-OVA was considerably more diverse idiotypically than the T-independent response to PC-BA and more diverse than has been seen in vivo (22) or in vitro using whole T cell populations

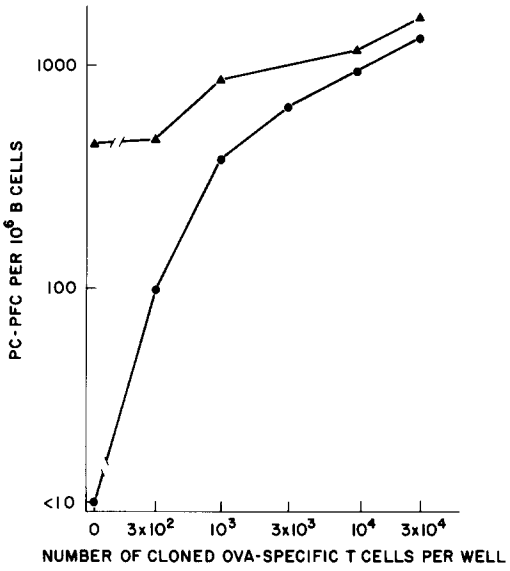

FIGURE 1. Helper activity of cloned Th cells in vitro. Varying numbers of monoclonal T cells were added to 3.0×10^5 B cells along with either 0.1 μg/ml PC-OVA (●) or 10^{-2} dilution PC-BA (▲) in 96-well Costar plates. The geometric mean (x/÷ relative standard error) of PC-specific PFC was determined after 5 d. Only those PFC inhibitable with free PC are considered to be PC specific.

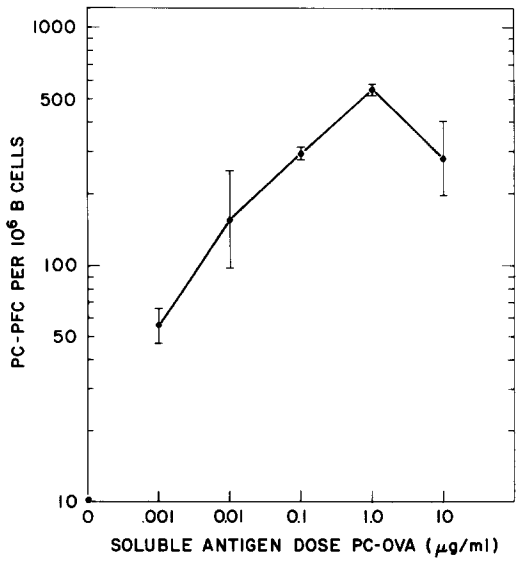

FIGURE 2. Soluble antigen dose required for a PC-specific in vitro response. 3×10^6 B cells and 1×10^5 monoclonal Th cells (in this experiment B6d.D2/B4) were cultured along with varying concentrations of PC-OVA in 24-well Costar plates. See legend for Fig. 1.

TABLE I

*T15 Idiotype Expression of the PC-specific Response Induced In Vitro By Monoclonal Helper T Cells**

T cell clone	Haplotype of the Th cell clone	Range of the frequency of T15 idiotype expressed on PC-PFC in response to:‡	
		PC-OVA	PC-BA
		%	
B6d.D2/B4	d	7–39	81–97
4.19	d	20–55	89–95
D10/G4	k	21–48	85–92
D4b	b	15–45	89–99
B6B5/B2	b	12–51	83–95
A3a.10.4	d	16–40	85–95

* 3×10^6 B cells of strains syngeneic with the Th cell clone used were cultured with either 0.5, 1.0, or 2.0×10^5 cloned Th cells along with 0.1 μg/ml PC-OVA or 10^{-2} dilution PC-BA.

‡ PC-PFC were assayed on day 5 and the proportion shown to be $T15^+$ PFC was determined by plaque inhibition with anti-T15 antibody, subtracting the remaining PFC from the total PC-PFC response. The frequency of T15-bearing PC-PFC equals the number of $T15^+$-PFC divided by the total PC-PFC (× 100). The range compares the frequency data of five individual experiments. Only those PFC inhibitable with free PC are considered to be PC specific.

to induce PC-specific PFC responses (20). This idiotypic heterogeneity has been observed with all T cell clones tested (40).

It seemed likely that B cell Ia expression would be important in the T-dependent activation of B cells to secrete antibody since Matis et al. (15) had demonstrated that the amount of Ia expressed by APC directly influences the proliferative potential of a cloned antigen-specific T cell and that this effect can be overcome in some combinations by increasing the antigen concentration. To probe similar effects in T-B interactions, we used in some experiments a limiting but nearly optimal antigen concentration to study the importance of Ia expression and to more closely parallel physiological conditions.

Interaction of T Cell Clones and PC-specific B Cell Is MHC-restricted and Depends on Recognition of Syngeneic B Cell Ia Glycoproteins. The T cell clones have been shown previously to both proliferate specifically to OVA in the context of syngeneic Ia glycoproteins (16, 29) and to help only syngeneic PC-specific B cells (16). However, certain studies have suggested that the subset of B cells bearing the Lyb-5 alloantigen and missing in the CBA/N mouse can be activated by Ia-restricted Th cells that are allogeneic to the B cell source. This interaction appears to be most efficient at high antigen concentrations (34, 35) and to depend on Th cell recognition of Ia glycoproteins on the surface of APC. Furthermore, the CBA/N defective mouse fails to make T15-bearing anti-PC antibodies, which emphasizes the association of T15-PFC with the $Lyb\text{-}5^+$ subset of B cells (36, 37). Thus, it was important to determine if the activation of both T15-bearing and non–T15-bearing B cells to secrete antibody required recognition of B cell Ia antigens. To determine if the T cell clones recognized B cell Ia rather than APC Ia, monoclonal Th cells were allowed to interact with F1,

fully syngeneic, allogeneic, or a mixture of allogeneic and syngeneic B cells. After 5 d, the B cells activated to secrete antibodies in the presence of either an Ia^d-recognizing or an Ia^b-recognizing Th clone were analyzed by treating the PFC with Y-3 (monoclonal anti–H-2.5 antibody, reacting with 100% of $H\text{-}2^b$ B cells) plus complement. It can be seen in Table II that cloned Th cells activated only syngeneic B cells. B6 Th clones failed to activate BALB/c B cells and BALB/c Th clones failed to activate BALB.B B cells, yet both Th clones generated PC-PFC responses using CB6F1 B cells. Similarly, when the cloned T cells were cultured with mixtures of B cells from allogeneic and syngeneic donors, with APC of both haplotypes present in all wells, all of the anti-PC PFC generated were syngeneic to the cloned Th cells as assessed by Y-3 plus complement treatment of the PC-PFC. The requirement for MHC identity was found for both T15-bearing and non–T15-bearing B cells, and the restricted interactions occurred at both 0.1 and 20 μg/ml of PC-OVA.

The Th cell clones used for these studies have been shown to proliferate to

TABLE II

*Interaction Between OVA-specific Th Cell Clones and PC-specific B Cells Is MHC-restricted**

B cells tested	B cell haplotype	Dose of PC-OVA	Y3 + complement treatment	PC-PFC/10^6 B cells from B cells cultured in the presence of OVA-specific T cell clones:‡			
				B6 Th cell clones		BALB/c Th cell clones	
				Total PFC	$T15^+$-PFC	Total PFC	$T15^+$-PFC
		μg/ml					
CB6F1	d × b	0.1	−	80	42	118	62
			+	0	0	0	0
		20.0	−	86	50	208	112
			+	0	0	20	4
BALB.B	b	0.1	−	46	26	0	0
			+	0	0	0	0
		20.0	−	62	38	0	0
			+	2	0	0	0
BALB/c	d	0.1	−	0	0	280	142
			+	0	0	292	172
		20.0	−	0	0	374	124
			+	0	0	364	168
BALB.B + BALB/c	d + b	0.1	−	64	32	94	30
			+	0	0	128	40
		20.0	−	58	30	140	62
			+	0	0	154	58

* 10^5 Th cell clones (B6d.D2/B2, d haplotype; or B6B5/B2, b haplotype) were added to a total of 3 × 10^6 B cells prepared as described in Materials and Methods. B cells were taken from CB6F1, BALB/c, and BALB.B unprimed donors. B cells, Th cell clones, and 0.1 μg/ml or 20 μg/ml of PC-OVA were cultured in 24-well Costar plates.

‡ PC-specific PFC were assayed on day 5 of the response. PFC bearing $H\text{-}2^b$ determinants (CB6F1 or BALB.B) were determined by treating the PFC either with complement alone or with monoclonal anti-H-2.5 antibody (Y3) plus complement. The PC-PFC responses shown here are the result of three wells pooled and are representative of five experiments with similar results.

OVA in the context of self-I-A (16, 29). To demonstrate that the helper cell activity of the clones depended on I-A antigen recognition, monoclonal anti-I-A^d was added to cultures containing BALB/c-derived Th cells and B cells. The response to PC-OVA was completely inhibited by the addition of monoclonal anti-I-A^d (Table III). By contrast the response of BALB.K B cells was unaffected by the presence of anti-I-A^d antibody. These data strongly suggest that Th cell recognition of B cell Ia glycoproteins is critical for all PC-specific B cells to be activated to secretion in vitro.

Monoclonal Ia-recognizing Th Cells Selectively Activated Non–T15-bearing B Cells Expressing a High Density of Ia Glycoproteins. Since in this system the cloned Th cells require B cell Ia recognition for successful T-B collaboration, and since B cells are heterogeneous in the density of Ia expressed, it seemed possible that B cell activation depended on a particular density or threshold of Ia glycoproteins on the B cell surface. Thus, it was important to determine whether or not the cloned Th cells acted selectively on B cells that expressed higher amounts of cell surface Ia glycoprotein antigens. These results also suggested the possibility that a subset of T15-bearing B cells, which appear to be more difficult to activate in vitro, were relatively deficient in Ia expression.

To separate B cells by surface Ia density, advantage was taken of the observation that the monoclonal anti-A_e:E_α antibody Y17 reacted with a subpopulation of B cells in CB6F1 mice (25). This was shown to be the subpopulation of B cells with the greatest density of cell surface A_e:E_α complexes (25). Thus, at plateau titers, Y17 plus complement will kill ~50% of CB6F1 B cells, reflecting heterogeneity in B cell surface Ia density.

In the experiment seen in Table IV, CB6F1 B cells were treated with Y17 plus complement or with complement alone. The resulting B cells were cultured at the same viable cell density along with cloned I-A^d-recognizing Th cells. It can be seen that Y17 plus complement drastically reduces the number of non–T15-bearing PC-specific PFC whereas the T15-bearing PFC response appears unaffected. However, since the cell numbers are restored to control levels before culturing, some T15-bearing B cells must also be eliminated by Y17 plus

TABLE III

*Inhibition of Th Cell-dependent B Cell Responses By Anti-I-A Antibody**

Source of cells		Antibody	Geometric mean (×/÷ relative SE) PC-specific PFC/10^6 B cells
B cells	Th cell clones		
BALB/c	B6d.D2	None	144 (130)
		Anti-I-A^d (1/50)	0
		Anti-I-A^d (1/500)	24 (1.12)
		Anti-I-$A_e^kE_\alpha$ (1/10)	151 (1.08)
BALB.K	D10/G4	None	231 (1.10)
		Anti-I-A^d (1/50)	209 (1.15)

* Set Table I for culture conditions. Anti-I-A^d (MKD6) and anti-I-A_eE_α (Y17) were added to the culture on day 0. The concentration of Y17 antibody used has been shown to totally inhibit the T cell proliferative response of pidgeon cytochrome C-specific B10.A T cell colonies to pidgeon cytochrome and (BI0.A(5R) × B10.D2)F1 spleen cells (15).

TABLE IV

*Pretreatment of B Cells with Y17 and Complement Reduces T15-bearing PFC Response**

Source of cells		Antigen	Y17 + complement‡ treatment	Geometric mean (×/÷ relative SE) PFC/10^6 B cells§			
B cells	Th cell clones			Total PC-PFC	$T15^+$-PFC	$T15^-$-PFC	TNP-PFC
Experiment 1							
CB6F1	B6d.D2/B4	PC-OVA	−	166 (1.21)	47 (2.06)	*110* (1.04)	
			+	62 (1.29)	48 (1.41)	*11* (1.35)	
CB6F1	B6d.D2/B4	TNP-OVA	−				260 (1.03)
			+				160 (1.08)
CB6F1	None	PC-BA	−	501 (1.06)	333 (1.02)	165 (1.23)	
			+	291 (1.01)	203 (1.03)	88 (1.11)	
BALB/c	B6d.D2/B4	PC-OVA	−	432 (1.11)			
			+	459 (1.21)			
Experiment 2							
CB6F1	B6d.D2/B4	PC-OVA	−	135 (1.10)	60 (1.25)	*74* (1.07)	
			+	72 (1.00)	57 (1.01)	*15* (1.04)	
CB6F1	B6d.D2/B4	TNP-OVA	−				186 (1.09)
			+				156 (1.08)

* See Table I for culture conditions. The cultures contained a total of 3×10^6 complement-treated or (Y17 + complement)-treated B cells, 0.5×10^5 B6d.D2/B4, along with 0.1 μg/ml PC-OVA, 0.1 μg/ml TNP-OVA, 10^{-2} dilution PC-BA.

‡ Experiment 1: 60% of CB6F1 and 19% BALB/c B cells were eliminated by Y17 + complement pretreatment; experiment 2: 52% of CB6F1 were eliminated by Y17 + complement pretreatment.

§ The number of PC-PFC shown to be T15 negative was determined by inhibiting T15-bearing PFC with anti-T15 antibodies. The number of T15-positive PFC was determined by subtracting the T15-negative PFC response from the total anti-PC response for each well. Two to five identical wells were set up for each group, and the experiment repeated five times with similar results.

complement pretreatment.

The reduction in the anti-PC PFC response cannot be accounted for by blocking by residual Y17 since Y17 antibody will not block the interaction of the I-A^d-recognizing Th cell clone with B cells (Table III). Similarly, pretreatment with Y17 alone before in vitro culture has no effect on the subsequent B cell response (data not shown). By contrast, although the response to PC-BA is reduced by Y17 plus complement pretreatment, there is no selective reduction of non–T15-bearing B cells, which in any case contribute little to this T-independent B cell response.

To demonstrate that the B cells which express high levels of Ia had been reduced by pretreatment with Y17 plus complement, the treated cells were evaluated for Ia expression by FACS analysis. The treated cells were stained with either biotin-conjugated anti-I-A^d, anti-I-A^b, or Y17 and fluoresceinated avidin. As seen in Fig. 3, the staining profile of Y17 plus complement-treated B cells using anti-I-A^d antibody differed from B cells treated with Y17 or complement only. B cells of high fluorescence intensity were diminished in amount after pretreatment with Y17 plus complement. This confirmed previous results where pretreatment with Y17 and complement has been shown to reduce high Ia density B cells as seen by staining with monoclonal anti-I-A^d antibody (26). While the data are not shown, staining the pretreated B cells with monoclonal anti-I-A^b antibody or Y17 (monoclonal anti-A_e^b:E_α) revealed a reduction in the number of B cells of high fluorescence intensity. Although pretreatment with Y17 and complement clearly blocks the subsequent staining of the B cells with Y17 antibody, monoclonal anti-I-A^b and anti-I-A^d antibodies see distinct determinants (29).

Thus, it would appear that non–T15-bearing B cells reside in the B cell pool

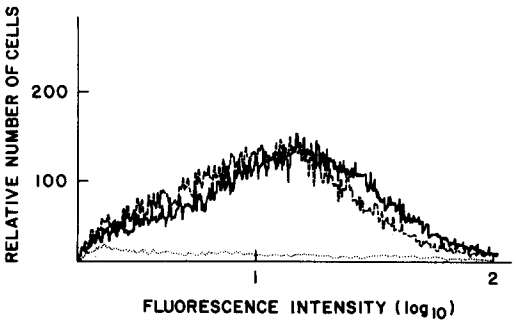

FIGURE 3. Immunofluorescence staining of CB6F1 B cells with monoclonal anti-I-A^d antibody. B cells were pretreated with complement (C) only (—) or Y17 + C (---) and then incubated with biotin-conjugated anti-I-A^d antibody followed by fluorescein-conjugated avidin. (Y17 + C)-pretreated cells were also stained with fluorescein-conjugated avidin without anti-I-A^d (···).

TABLE V

*Attempt to Reconstitute APC Function After Y17 Plus Complement Pretreatment**

Source of cells			Y17 + complement§ treatment	Geometric mean (×/÷ relative SE) of PFC/10^6 B cells			Percent T15
B cells	Th cells	APC‡		Total PC-PFC	$T15^+$-PFC	$T15^-$-PFC	
CB6F1	4.20	None	−	623 (1.02)	305 (1.02)	318 (1.03)	48
			+	399 (1.10)	284 (1.03)	105 (1.17)	71
CB6F1	4.20	10^3	+	469 (1.03)	339 (1.11)	127 (1.17)	72
		10^4	+	447 (1.11)	338 (1.20)	104 (1.17)	76
		10^5	+	443 (1.07)	340 (1.10)	102 (1.01)	77
CB6F1	None	10^5	+	0	0	0	—

* The culture conditions are described in Table I, using 1.0×10^5 Th cell clones.
‡ Preparation of APC is described in the Materials and Methods.
§ 30% of CB6F1 B cells were eliminated by Y17 + complement pretreatment.

expressing high levels of cell surface Ia and can be easily eliminated by Y17 plus complement. These data suggest that there may be a distinction in the amount of cell surface Ia between T15-bearing and non–T15-bearing B cells. Since pretreatment of B cells with Y17 plus complement reduced the total anti-PC response even after restoration of viable B cell numbers to the control level, it would appear that the presence of B cells expressing high levels of Ia is required for an optimal T-dependent PFC response in the presence of the cloned Th cells. Since the cloned Th cells have an absolute requirement for B cell Ia recognition, and appear to activate preferentially high density Ia B cells, a threshold of B cell Ia may be required for B cell activation to secretion.

Reduction in Anti-PC PFC Response After Y17 Plus Complement Pretreatment Is Not Due to Insufficient APC. Since $A_e{}^bE_\alpha$ is expressed on APC as well as B cells, it might be suggested that pretreatment of the B cell source depleted not only B cells but APC necessary for a substantial anti-PC response. To test this possibility, a source of plastic-adherent APC was added to the in vitro cultures. As seen in Table V, there was no change in the anti-PC PFC response in the presence of graded numbers of APC. The same source of APC could reconstitute an anti-

PC response where APC had been thoroughly depleted (data not shown). These data confirm the finding that the failure to activate non–T15-bearing B cells after Y17 plus complement pretreatment was due to an elimination of a high Ia density, non–T15-bearing B cell subset.

Discussion

The observations obtained in this system strongly suggest that the amount of Ia antigen on a B cell may be an important factor in determining its ability to respond to T cell help. By using monoclonal Th cells that recognize antigen in the context of B cell Ia glycoproteins, it can be shown that not all PC-specific B cells are activated equally in vitro. These conclusions are based on the findings that (*a*) elimination of a high Ia density B cell subset reduced the PC-specific PFC response even when an equivalent number of cells was cultured and (*b*) the B cells expressing the highest level of Ia antigens were mainly non–T15-bearing B cells, suggesting that there is a relationship between Ia and idiotype expression. Through the use of monoclonal Ia-recognizing Th cells, it is possible to distinguish between Id-bearing and non–Id-bearing B cells on the basis of the relative ease with which they are activated by such cells plus antigen to secrete antibody.

In an attempt to understand why the T-dependent anti-PC response in the presence of cloned "classic" Th cells is more idiotypically heterogeneous than the response induced using whole populations of carrier-primed Ly-1$^+$ Th cells, it may be suggested that the inability of cloned Th to elicit a T15-dominated response is due to the relative abundance of T15-bearing PC-specific precursor B cells. If only 20–40% of B cell precursors are T15 bearing, the Th cell clones may activate any precursors available and capable of binding PC. This would suggest that the Th clones are equally capable of helping T15-bearing and non–T15-bearing B cells. The non–T15-bearing B cells would be found predominantly in the Ia high cell pool and the T15-bearing B cells equally in high and low Ia-expressing B cell pools. The data of Gearhart et al. (21), using the splenic focus assay, has shown that 2–50% of PC-specific precursors are non–T15-bearing, supporting the concept that there is some idiotypic heterogeneity at the PC-specific precursor B cell level. We feel this explanation of our results is unlikely, however, because pretreatment of B cells with anti-Ia antibody and complement, to eliminate only those B cells expressing high quantities of cell surface Ia, results in a reduction of the total anti-PC PFC response even when the number of B cells per culture is maintained. One might expect that if the cloned Th cells activated all PC-specific B cells equally that the total number of PC-PFC would be maintained after anti-Ia antibody and complement pretreatment.

Alternatively, it might be suggested that monoclonal Th cells distinguish between subsets of B cells and activate only those B cells with which their interaction is most efficient. Several studies have shown that the anti-PC PFC responses induced in vivo (18–20) and in vitro (20) by carrier-primed Ly-1 T cells are predominantly T15 bearing. Cosenza et al. (20) here demonstrated, using limiting dilution analysis of PC-specific precursors, that the vast majority (75–98%) of precursors of PC-specific B cells express the T15 Id (20). This would suggest that T15-bearing B cells are not optimally activated in the presence

of cloned Th cells, but rather that the cloned Th cells preferentially induce PFC responses by B cells with high levels of Ia that are non–T15-bearing. The studies reported here strongly support the latter alternative which emphasizes that only those B cells expressing a critical density of Ia glycoproteins are activated in the presence of Ia-recognizing Th cell clones—a threshold of Ia density on the B cell surface being required for effective Th-B cell interaction under these experimental conditions. Observation of this requirement is optimized by the use of an Ia-recognizing Th cell clone. These findings confirm the previous findings of Henry et al. (14) that B cells which survive anti-Ia antibody plus complement treatment required more Th cells than untreated B cells in response to Lac-keyhole limpet hemocyanin, as might be expected if the Ia density were low, and that Ia molecules were critical for T-B interaction.

Further support for the notion that T15-bearing cells appear to be inefficiently activated by Ia-recognizing Th cell clones comes from studies in which the addition of antigen-primed Lyt-1^+ T cells to the cultures greatly increased the number of PFC-secreting T15-bearing antibody (38). The Ly-1 Th cell that augments the activation of T15-bearing B cells has been shown to bind specifically to and be recovered from T15-coated plates and appears to be specific for those B cells expressing the T15 Id (Dunn, E., and K. Bottomly, manuscript in preparation). One might postulate that the role of T15-specific Th cells is to interact specifically with T15-bearing B cells by recognition of T15-bearing immunoglobulin (Ig) on the B cell surface. Much as anti-Ig reagents have been shown to trigger B cells to proliferate and to increase expression of cell surface Ia glycoproteins (39, 40), anti-Ig Th cells (in this case T15 specific) might raise the density of Ia on T15-bearing B cells. This would then allow T15-bearing cells to be activated by Ia-recognizing Th cell clones. This explanation and others are currently being investigated to explore the differences in activation requirements seen between the T15-bearing and non–T15-bearing B cells.

Data from other systems using monoclonal Th cell populations have suggested that subsets of B cells differ in their activation requirements. In these systems, two subpopulations of B cells, distinguished by their expression of the Lyb-5 determinant, differ in their activation by Th cells. Only the activation of the Lyb-5^- subpopulation by Th cells is MHC restricted; the activation of Lyb-5^+ B cells occurs without direct interaction between Th cells and B cells (34, 35). However, in the data reported here, all the responses are I-A restricted and required direct interaction between the Th and B cell. Yet, it would seem that the T15-bearing B cells which are missing in CBA/N mice belong to the pool of Lyb-5^+ cells (36, 37). Together these findings suggest that both Lyb-5^+ and Lyb-5^- B cells are activated in the anti-PC PFC response, requiring direct interaction with the Th cell clone. Although these data appear contradictory, it seems likely that while Lyb-5^+ cells have been shown to be activated in an MHC-unrestricted fashion, they are not limited to this pathway (35) and may be activated directly by Th cells that recognize syngeneic B cell Ia determinants.

One may ask why T15-bearing and non–T15-bearing B cells are distinguished by Ia density. It is possible that T15-bearing B cells, which appear to be most efficient at binding PC (41) and perhaps have been exposed previously to the environmentally prevalent forms of PC, are not as dependent on Th cell signals

for activation. Several studies have demonstrated that the requirements for B cell activation are influenced by prior antigenic exposure (42–44). Furthermore, since T15-recognizing Th cells that act on activated T15-bearing B cells in what is probably an Ia-restricted fashion have been described by the studies of Cerny and Caulfield (45), it may be necessary to protect the system from autoactivation in the absence of antigen by having T15-bearing B cells express relatively low amounts of Ia glycoprotein. The relationship between Ia density and activation state is being explored at this time.

Summary

We have investigated in vitro the induction of antibody responses to phosphorylcholine (PC) by cloned T helper (Th) cell lines. The cloned Th cells are antigen specific, in this case ovalbumin (OVA), self-Ia recognizing, and induce antibody secretion only if the hapten, PC, is physically linked to the carrier (OVA) molecule. The plaque-forming cell (PFC) response generated in the presence of cloned Th cells is idiotypically diverse with 5–40% of the secreting B cells bearing the TEPC-15 (T15) idiotype. The interaction of the cloned Th cells and unprimed B cells requires recognition of B cell surface Ia glycoproteins for all B cells activated to secrete anti-PC antibody, whether they be T15-bearing or not. More importantly, however, effective interaction between a cloned Th cell and a B cell is determined by the quantity of B cell surface Ia glycoproteins. Our results indicate that quantitative differences in B cell surface Ia antigens are directly related to B cell activation by the cloned Th cell. The high Ia density B cells are most easily activated by cloned Th cells, and these appear to be mainly non–T15-bearing. These data suggest that the failure of cloned Th cells to effectively activate T15-bearing B cells in vitro may be due to the lower relative Ia density of these B cells and therefore to their inability to interact effectively with cloned Ia-recognizing Th cells. These results imply that monoclonal T cells may distinguish between T15-bearing and non–T15-bearing B cells based on their Ia density.

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