

DISTINCT FUNCTIONAL PHENOTYPES OF CLONED Ia-RESTRICTED HELPER T CELLS

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Antihapten antibody responses to hapten-protein conjugates involve the collaborative interaction of hapten-specific B cells and carrier-specific helper T (Th)¹ cells (1). Our previous analysis of the B cell response to the hapten phosphorylcholine (PC) in vitro, using cloned, carrier-specific, Ia-restricted Th cells has shown the following: (a) The interaction of the cloned Th cell and a PC-specific B cell requires T cell recognition of B cell surface Ia glycoproteins; (b) the hapten PC must be physically linked to the protein carrier for which the Th are specific; (c) effective interaction between a cloned Th and a B cell is influenced by the quantity of B cell surface Ia glycoproteins; and, (d) many of the PC-specific B cells activated do not bear the prototype idiotype determinant characteristic of the BALB/c PC-binding myeloma protein TEPC-15 (T15) (2).

Studies evaluating the responses to PC-conjugated proteins have shown that intact in vivo responses as well as in vitro responses induced by uncloned Ly-1 T cells are dominated by T15-bearing B cells (3–5). This seems surprising given that there is idiotype heterogeneity at the PC-specific precursor B cell level (6), this being convincingly documented recently by the finding (7) that only 48% of PC-specific precursors activated by lipopolysaccharide are T15-bearing. Our own data have suggested that one can activate non-T15-bearing B cells in T-dependent responses using cloned Ia-restricted Th cells (2, 8, 9), and that an increase in the T15-bearing anti-PC response may require additional T cell signals (10, 11). Together, these findings suggest that the activation requirements of T15-bearing and non-T15-bearing B cells may differ.

We have now generated and characterized a series of cloned, antigen-specific, Ia-restricted T cell lines with different functional phenotypes. In this report, the characteristic four types of cloned Ia-restricted Th cells differ in their ability to activate T15-bearing and non-T15-bearing B cells. Further analysis of these functionally distinct cloned Th cells on individual PC-specific B cells in limiting-dilution experiments showed that these Th differed in their ability to activate

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¹ *Abbreviations used in this paper:* BA, *Brucella abortus*; Id, idiotype; mAb, monoclonal antibody; OVA, ovalbumin; PC, phosphorylcholine; PFC, plaque-forming cell; TCGF, T cell growth factor; Th, T helper.

resting T15-bearing B precursor cells, and in their ability to drive clonal expansion.

Materials and Methods

Mice. BALB/cByJ mice were obtained from The Jackson Laboratory, Bar Harbor, ME, and were used between 8 and 12 wk of age. BALB.K and BALB.B mice were bred at Yale University.

Antigens. The preparation of antigens used in these experiments has been described previously (10, 12).

Antisera and Monoclonal Antibodies (mAb). The preparation, purification, and testing of rabbit anti-T15 idiotype (Id) antibodies have also been described previously (10, 12). In addition to polyclonal rabbit anti-T15, an anti-T15 mAb was used. The anti-T15 mAb was shown, by enzyme-linked immunosorbent assay and hemagglutination assays, to bind to T15 and, to a lesser degree, MOPC-511 myeloma proteins, but not to MOPC-167 proteins. Hybridoma producing anti-Thy-1.2 was kindly provided by Dr. J. Sprent, Wistar Institute, Philadelphia, PA. Monoclonal anti-Lyt hybridomas were kindly provided by Dr. P. Gottlieb, University of Texas, Austin (13). mAb were partially purified from ascites by precipitation with saturated ammonium sulfate, and were dialyzed against phosphate-buffered saline. GK1.5, specific for the L3T4a antigen (14) was grown in vitro, and culture supernatants were used as a source of antibody.

Preparation of Cells. Cloned T cell lines were generated according to the method of Sredni et al. (15). The preparation, cloning, and analysis of the various cloned T cells has been described extensively elsewhere (9, 16, 17). Basically, all the cloned T cells tested are phenotypically L3T4a⁺, 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 antigen, mitomycin C-treated feeder cells, and T cell growth factor (TCGF), TCGF is not needed during the Th assay. All cells were washed extensively before use. B cells from unprimed mice were treated simultaneously with GK1.5, anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.2 mAb plus complement.

Bulk Cell Cultures. For 96-well Costar (Cambridge, MA) 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, Th cells, and antigen are cultured in 1-ml volumes. Cultures were incubated at 37°C for 5 d, and assayed for plaque-forming cell (PFC) responses.

Hemolytic (PFC) Assay. The cultures were assayed for direct anti-PC PFC by the modified Jerne hemolytic plaque technique (18), as described previously (12).

Inhibition of Plaque Formation. The proportion of anti-PC PFC shown to be of the T15 Id was determined by inhibition of plaque formation using both rabbit anti-T15 and anti-T15 mAb in the agarose suspension. Only the PFC shown to be inhibited by free PC were considered to be PC-specific. In all experiments, anti-T15 inhibition of the responses to PC-conjugated *Brucella abortus* (PC-BA) was determined. 90–100% of the PFC induced by PC-BA are inhibited by the addition of anti-T15 antibodies. Such inhibition confirmed, in each experiment, that the concentration of anti-T15 antibodies added to the agarose was sufficient to inhibit all T15-specific PFC.

Limiting-dilution Analysis. Cultures were set up in flat-bottomed Costar microtiter plates and contained a total of 5×10^5 or 10^6 cells/well. Varying numbers of B cells, 10^3 cloned Th cells and 0.1 µg/ml PC-OVA (PC-conjugated ovalbumin) were cultured with the number of filler cells required to achieve 5×10^5 (10^6) cells/well. In all cases, the filler cells were spleen cells from unprimed, syngeneic donors, which were pretreated with GK1.5, anti-Ly-1.2, anti-Lyt-2.2, and anti-Thy-1.2 mAb plus complement, and given 1,200-rad irradiation. 60 cultures were set up for each cell concentration. The procedure was similar to those previously described (19–21). All cultures were analyzed by PC-PFC assay. A clone was defined as consisting of 4 PFC/well. By plotting the negative logarithm of the fraction of nonresponding cultures ($-\ln F_0$) on the ordinate, and the number of B cells/well on the abscissa, the linearity of a semilog plot is consistent with only a single cell type being diluted out. This plot is also a convenient way to estimate the frequency of B

precursor cells by interpolating at 37% nonresponding cultures (average of 1 precursor cell/well by Poisson distribution). The average clone size c , is defined by: $c = PFC/N$; where $N = w \times u$, w being the number of wells assayed, $u = -\ln F_0$ and PFC being the total number of PFC found in w wells.

Results

Cloned Th cell lines have been prepared from a variety of mouse strains by immunization with OVA, in vitro stimulation of lymph node T cells with antigen, and cloning in agar 3 d after the initial cultures were established. Lines were recloned by limiting dilution, and are maintained by the addition of antigen, syngeneic mitomycin C-treated spleen cells, and TCGF at weekly intervals.

All the cloned Th cell lines are phenotypically Lyt-1⁺,2⁻, L3T4a⁺, and have been shown to be antigen specific and Ia-restricted (9, 16, 17). As previously reported (2, 11, 22), the first 40 cloned Th lines treated fell into two functional categories. Cloned Th cells of the first category induced anti-PC PFC responses that were idiotypically heterogeneous (2, 11). Further analysis of these clones suggested that, at low antigen concentration, the T cells interacted most efficiently with high-Ia-density B cells; these being primarily non-T15-bearing (2). Certain cloned lines tested, however, did not give rise to anti-PC PFC under any conditions, and one cloned T cell line of this type was shown to suppress the activation of a subset of PC-specific B cells, apparently by acting directly upon the B cell in an antigen-specific, Ia-restricted fashion (22). These Lyt-1⁺ cloned T cells have suppressive rather than helper activity. Subsequently, upon generation of a larger panel of cloned Th cells, we have identified two more functional types of cloned Th lines; all the cloned Th cells derived so far fall into four different patterns, as determined by B cell activation. These Th cell types are described in detail below.

Behavior of Four Types of Cloned, Ia-restricted T Cell Lines: Responses in Bulk Cultures. Cloned, OVA-specific T cells were added to highly purified syngeneic B cells at various T cell and PC-OVA antigen doses. All of the cloned Th cell lines tested fell into four functional types based on the following criteria: (a) their ability to induce a PC-specific PFC response, (b) their requirement for the presence of antigen during induction of B cell responses, and (c) their activation of primarily T15-bearing or non-T15-bearing B cells.

The antigen-dose-response curves for two examples of each type of cloned T cell line are shown in Fig. 1. Type 1 clones (Fig. 1A) are similar to those we have already reported (2, 11); at all antigen doses, the response is made up of PC-PFC, 20–50% of which are T15-bearing. Type 2 clones (B), in contrast, induce a PFC response of greater magnitude, and 80–95% of the PC response is T15-bearing. There is also a difference in the antigen-dose-response curves of these two types of cloned T cell lines: type 1 cloned lines give a peak response at about 1 $\mu\text{g/ml}$ PC-OVA, and plateau or decline at higher doses; type 2 cloned T cell lines give progressively higher responses at increasing antigen dose. Type 3 clones (Fig. 1C), which include clone Cla, do not help at any cell or antigen dose. Some of these type 3 clones, like Cla, actually suppress the B cell response to PC-conjugated protein induced by other cloned T cell lines, as reported previously (22). Finally, like several other investigators, we have obtained occasional cloned

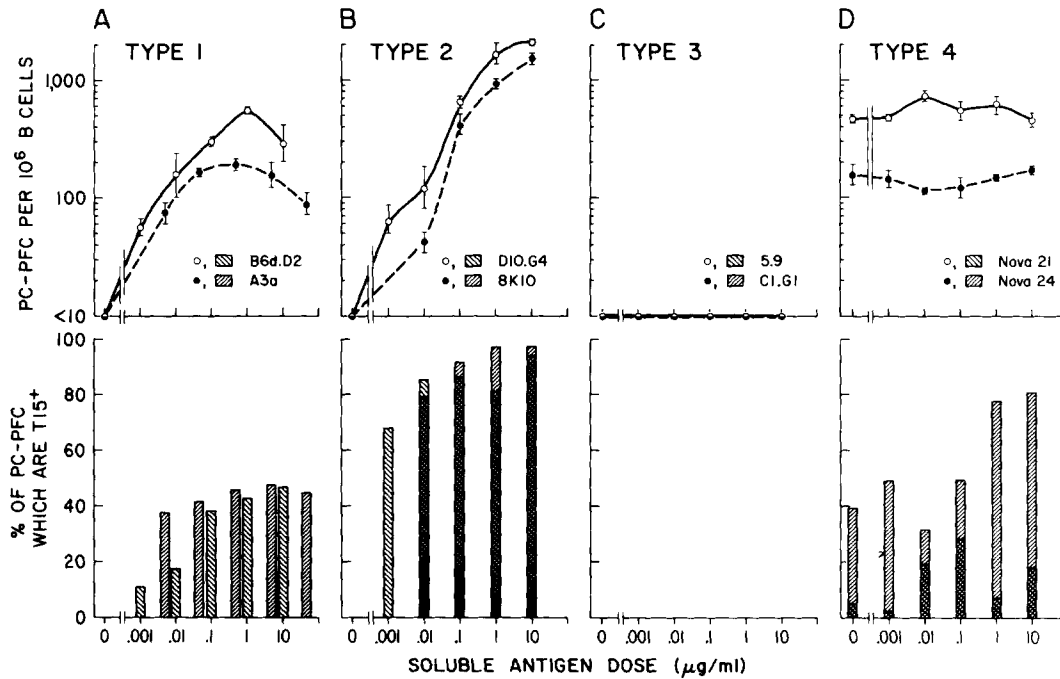


FIGURE 1. Antigen dose-response curve for types 1-4 Th cell induction of a PC-specific PFC response. The antigen concentration is expressed in $\mu\text{g/ml}$. The hapten-carrier antigens used for each clone are as follows: PC-OVA for B6d.D2, A3a, 8K10, NOVA 21, NOVA 24; PC-conalbumin for D10.G4. Each panel (top and bottom) represents one functional phenotype. The upper graph is the number of PC-specific PFC induced in the presence of 5×10^4 T clones and varying concentrations of antigen. The lower graph shows what percent of the total PC-PFC at each antigen dose used is T15-bearing. Only those PFC inhibitable with free PC are considered to be PC-specific.

T cell lines that are not protein antigen-specific but are specific for autologous Ia antigens (D). Such self-Ia-specific cloned T cell lines induce either high or low levels of T15 idiotype-bearing PC-PFC responses independent of antigen dose, although a slight antigen effect may be observed with such apparently autoreactive cloned T cell lines. We term such cloned lines type 4.

Fig. 2 shows the T cell dose-response curves for the four types of cloned T cell lines as well. Again, it can be observed that the highest responses are generated by high doses of type 2 cloned T cell lines (Fig. 2 B), while the type 1 cloned lines (A) give a somewhat lower response. More strikingly, at all cell doses, type 1 cloned lines give responses that are 20-40% T15 Id⁺ anti-PC PFC, while type 2 cloned lines, at all cell doses tested, give responses that are >80% T15 Id⁺. Type 3 cloned T cell lines give no response at all cell doses, while type 4 cloned lines show a clear T cell dose-dependent B cell response, in which the contribution of the T15 idiotype is affected more by the actual cloned line used than the dose of cells tested.

Limiting-dilution Analysis of Type 1 and Type 2 Cloned Th Cells. To further characterize differences in the activation of T15 Id⁺ PC-specific B cells by different types of cloned Th cell lines in bulk cultures, the activation of individual

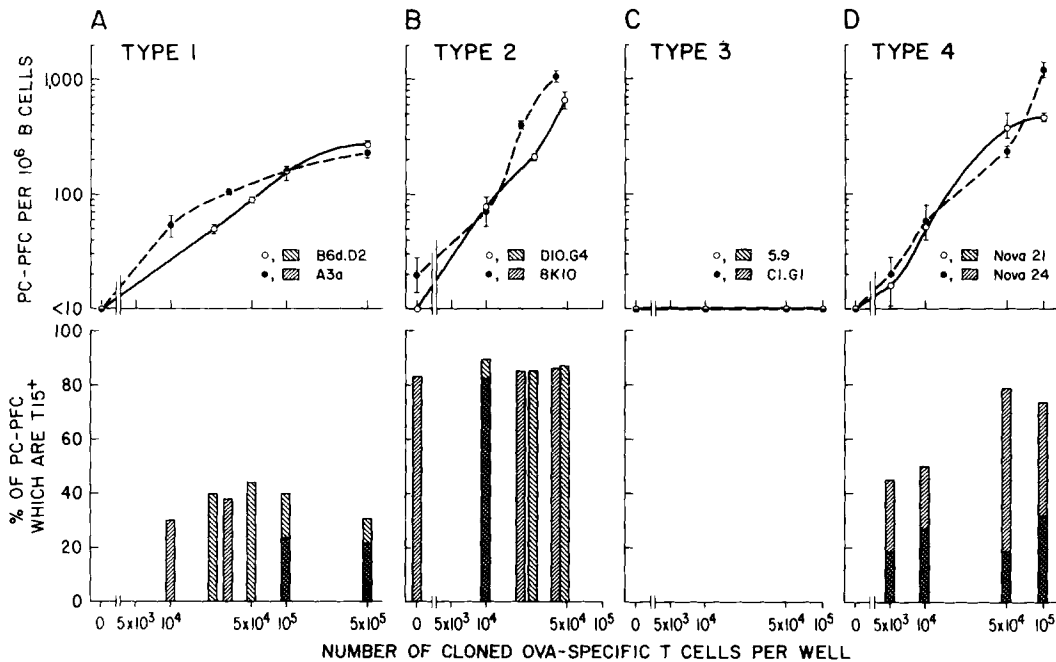


FIGURE 2. T cell dose-response curve for types 1-4 Th cell induction of a PC-specific PFC response. Each panel (top and bottom) represents one functional phenotype. The upper graph shows the number of PC-specific PFC induced by 0.1 $\mu\text{g}/\text{ml}$ of PC-OVA or PC-conalbumin and varying concentrations of cloned T cells. The lower graph shows what percent of the total PFC response at each T cell dose used is T15-bearing.

PC-specific B cells was examined using limiting-dilution analysis with two examples of type 1 and two examples of type 2 cloned Th cell lines. We wished to determine whether or not type 2 cloned Th cell lines activated more T15-bearing, PC-specific B precursor cells, or caused greater clonal expansion of those T15-bearing cells that were already activated. In fact, as shown below, both effects contributed to the T15 Id dominance seen when type 2 cloned Th cell lines are used in bulk culture responses.

Fig. 3 shows limiting-dilution analysis of B cells using two different type 1 cloned Th cell lines (NOVA 25 and 8D13). Fig. 4 shows the same analysis for two different type 2 cloned Th cell lines (4.19 and D10.G4). Note that the X-axis differs in the two figures. The logarithm of the fraction of negative wells, when plotted against the number of B cells/well, decreased linearly, insuring that the B cells were limiting and that the frequency of precursors could be estimated according to a Poisson distribution. Moreover, T cell-depleted irradiated filler cells and T cell clones were added at a density that would optimally support the cell cultures, and yet would ensure the activation of B cells only in the presence of a single T cell type. The frequency of PC-specific B cells induced by each Th type is seen in Table I. Type 1 cloned T cells activate either roughly equal numbers of T15-bearing and non-T15-bearing PC-specific B cells, or a greater number of T15⁻ precursors. In contrast, type 2 cloned T cells activate 4.5 times as many T15 Id⁺ as T15 Id⁻ B cell precursors. Both types of cloned T

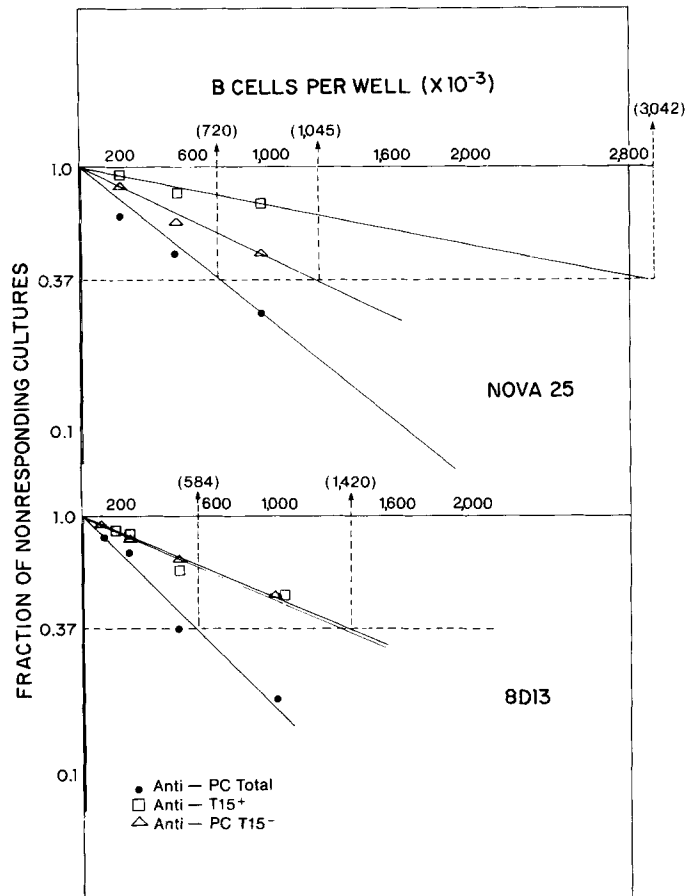


FIGURE 3. Frequency of PC-specific B precursor cells induced by type 1 cloned Ia-restricted T cells and antigen. Varying concentrations of B cells (5×10^4 – 10^6) were cultured with 10^5 cloned T cells and irradiated, T cell-depleted syngeneic filler cells to equal a total of 5×10^5 or 10^6 cells/well. Each point in the figure is based on assays of 60 cultures. The number of PC-PFC was determined on day 5 of culture. The best fit lines have been calculated by the method of least squares. The numbers in parenthesis represent the estimated frequency of B precursor cells by interpolating at 37% nonresponding cultures.

cell lines activate nearly identical numbers of non-T15-bearing B cells. Thus, a major difference between the two types of cloned Th cell lines is the ability of type 2 cloned Th cells to activate approximately five times as many T15⁺ PC-specific B cells as are activated by type 1 cloned Th cells.

In Table II, the average number of PC-specific PFC produced per activated B cell in these same experiments is presented. Not only do type 2 cloned Th cells induce more T15-bearing B cells than do type 1 cloned Th cells, but in addition, each activated T15-bearing B cell proliferates more extensively when helped by a type 2 Th cell than when helped by a type 1 Th cell.

Taken together, these differences should lead to ~15-fold greater T15 Id expression in bulk culture responses when the cloned Th cell line used is type 2 rather than type 1. This difference is approximately the difference required to

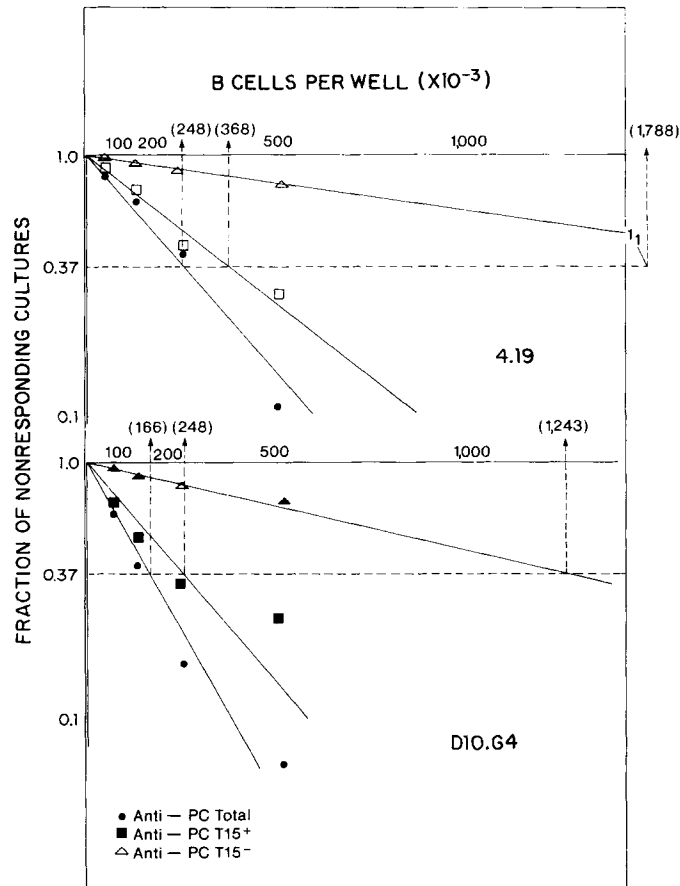


FIGURE 4. Frequency of PC-specific B precursor cells induced by type 2 cloned Ia-restricted T cells and antigen. See Fig. 2 legend.

TABLE I
Frequency of Responding PC-specific Precursors Induced by Type and Type 2 Monoclonal T Cells

T cell type	Precursors per 10^7 B cells*		
	T15 ⁺	T15 ⁻	T15 ⁺ :T15 ⁻ †
Type 1: Nova 25	3.3	9.5	0.35
8D13	7.0	7.0	1.0
Type 2: 4.19	27	6	4.5
D10.G4	41	9	4.5

* Cultures were set up as described in Materials and Methods. The estimated number of precursors activated in the presence of each clone was derived from Figs. 3 and 4, and is expressed per 10^7 B cells above.

† The ratio of T15⁺:T15⁻ precursors is determined by dividing the number of T15⁺ precursors per 10^7 B cells by the number of T15⁻ precursors per 10^7 B cells.

TABLE II
Clonal Expansion of PC-specific Precursors Induced by Type 1 and Type 2 Monoclonal T Cells

T cell type		Average clone size*		
		T15 ⁺	T15 ⁻	T15 ⁺ :T15 ⁻
Type 1:	Nova 25	18	20	0.9
	8D13	7	11	0.64
Type 2:	4.19	52	12	4.3
	D10.G4	233	78	3.0

* Average clone size is determined as described in Materials and Methods. For all T cell types tested, $n = 60$.

change such a response from 30% T15 Id⁺ to 80–90% T15 Id⁺, if the number of T15 Id⁻ anti-PC PFC remains constant, as we have consistently observed (2, 8, 10, 17).

Discussion

When examining a large number of cloned T cell lines obtained from mice immunized with a protein antigen and having in common specificity for self Ia molecules, four functionally distinct types of cloned T cell lines were observed, two of which we have previously reported (2, 11, 22). The unique characteristics of these four types of cloned T cell lines are summarized in Table III. Three important findings have emerged from the present analysis. First, cloned T cell lines fall into at least four distinct types based on their activation of PC-specific B cells that bear the T15 Id. Second, these functional phenotypes are stable properties of a cloned T cell line. Third, cloned T cell lines can selectively activate distinct subsets of PC-specific B cells; in these studies, B cell subsets are delineated simply by the expression or lack of expression of the T15 Id. Type 2 clones, which induce predominantly T15-bearing B cells, so do by activating five times as many T15-bearing, PC-specific B precursor cells as the type 1 clones, which induce primarily non-T15-bearing B cells, and by increasing the proliferative capacity of the activated T15-bearing precursors. These two effects, taken together, can fully account for the differences between these two types of clones previously observed in bulk culture.

In these studies, type 1 clones are the predominant cell type produced. This is consistent with previous *in vivo* studies of Th populations isolated from B cell-deficient mice or from B mice repopulated with selected alloreactive T cells. The ability of Id-specific, non-major histocompatibility complex-restricted T cells from normal mice to restore T15 Id dominance in responses helped by such T cell populations or, more recently, type 1 cloned Th lines, has led to the proposal that Id-specific Th cells participate in T15-dominated anti-PC responses. Whether T cell populations isolated from normal mice are predominantly type 1 or type 2, and whether type 4 clones play a role in such responses, remains to be determined. It seems likely that the procedure used to produce cloned T cell lines will strongly bias the results obtained, and thus frequency of clone type is not likely to be an accurate guide to the *in vivo* situation. For instance, the agar

TABLE III
*Types of Ia-restricted T Cell Clones Delineated by Their Ability to Activate
 Subsets of PC-specific B Cells*

Properties	Type 1	Type 2	Type 3	Type 4
<u>Phenotype</u>				
Ly-1 ⁺ , 2 ⁻ , L3T4a ⁺	+	+	+	+
<u>Specificities</u>				
Ia-restricted	+	+	+	+
Antigen-specific	+	+	+	-
<u>Functions</u>				
Antigen plus Ia-induced T cell proliferation	+	+	+	-
Induction of B cell proliferation	+	+	+	+
Induction of Ig secretion	+	+	-	+
Suppression of Ig secretion	-	-	+	-
B cell cytoysis	+/-	-	+	?
B cell cytotoxicity	+/-	-	+	?
<u>Requirements for function</u>				
Hapten-carrier linkage	+	+	+	-
Threshold level of B cell surface Ia antigen	High	Low	High	Variable
<u>B cells influenced</u>				
T15 ⁺ :T15 ⁻	1:1 (1:2)	10:1	1:1 (1:2)	Variable

Detailed information on the properties of Ia-restricted clones can be found in the following references: Specificities, (2, 16, 22, and 30); Functions, (2, 11, 16, 22, 30, 34, and 35); Requirements for function, (2, 8, and 16).

cloning procedure employed in these studies gives rise to a frequency of alloreactive T cell lines among antigen plus Ia-specific clones that is similar to that found among unselected T cells (16), a finding consistent with the results obtained in alloblast-repopulated nude mice (23). In contrast, cloned Th derived from cell lines have a low to negligible frequency of alloreactive cells (24). Also, the procedure used here involves frequent addition of exogenous IL-2, whereas the commonly used procedure of Shigeta and Fathman (25) does not; it seems likely that this procedure selects strongly for high-rate lymphokine-secreting T cells, which probably are predominantly type 2 Th cells. Thus, analysis of cloned populations can give information about available functionality and specificity repertoires, but cannot give accurate information about their relative proportions among normal cells.

The limiting-dilution analysis shown in Fig. 3 and 4, and summarized in Tables I and II did not seek to establish an absolute frequency of PC-specific B cells, but rather to examine differences in the activation of T15-bearing and non-T15-bearing B cells by the different types of cloned T cell lines. Interestingly, the apparent precursor frequency changes depending on the cloned Th cell line used in the analysis. As this difference is exclusively in B cells that are PC specific and bear the germ line-encoded T15 Id, these differences cannot be in the Ig

expressed by the B cell, but rather must relate to some difference in activation requirements between subsets of PC-specific B cells. This suggests that the two types of cloned Th must differ in their ability to provide this activation requirement for T15-bearing B cells.

One variable on which our previous studies had focused was the amount of Ia glycoprotein expressed per B cell. We showed that T15-bearing, PC-specific B cells had, on the average, lower levels of Ia glycoprotein per cell than did PC-specific B cells that were non-T15-bearing (2). These studies were performed with type 1 cloned Th cell lines, and the low level of Id expressed in bulk cultures was thought to reflect such Th cells' preferential activation of high-Ia antigen density, T15⁻ B cells. Likewise, cloned type 3 T cells preferentially suppressed the high Ia-density, T15⁻, PC-specific B cells (22). Thus, for both type 1 and type 3 T cells, the efficiency of T-B interaction depends on a threshold level of B cell surface Ia; the mechanics of the interaction being similar. The signal delivered, once the interaction has occurred, is help in the case of type 1, and suppression in the case of type 3. It might be suggested that the difference between type 1 and 2 cloned T cell lines is that type 2 Th cells can activate B cells having a lower density of Ia antigens than can the type 1 Th cells. The mechanism by which Ia-restricted T cells activate antigen-binding B cells has been extensively investigated, and is still highly controversial. We have previously shown that, in the response to the hapten PC, T-B interaction is cognate; that is, only B cells bearing the Ia antigen for which the cloned Th cell line is specific contribute to the PC-specific antibody response, even when two MHC-disparate B cells are mixed (2). A similar result using different cloned T cell lines and a different approach to cell mixing led Moisiere and Feeney (26) to the same conclusion. Recent studies by Tite et al. (27), Ashwell et al. (28), De Franco et al. (29), and Tite and Janeway (30) may lead to a synthesis of conflicting views in this field. These studies suggest that the role of B cell Ia glycoprotein is to present antigen complexed to Ia molecules to Th cells specific for such complexes. The Th cell then releases a group of molecules that locally influence other cells, most particularly the B cell to which it is bound by means of its receptor for antigen and Ia. Thus, under limiting conditions of stimulation, the antigen binding, Ia-matched B cell is the only cell likely to be detectable as having been activated. Thus, the cognate interaction is the recognition, by the Th cell, of antigen plus Ia on the B cell surface, all subsequent steps being mediated by nonspecific, short-range factors.

If this synthesis of the data is correct, then two possibilities for the difference between type 1 and type 2 clones of Th suggest themselves. First, type 2 Th may have higher-affinity receptors, requiring lower levels of antigen plus Ia on the B cell surface to become activated. Alternatively, type 2 Th may release molecules that induce higher levels of Ia antigen expression on B cells, thus allowing B cells that have a low level of Ia glycoprotein to become activated by a cloned Th cell that has a receptor identical to that of a type 1 cloned line. An obvious problem with this argument is that, if the release of the Ia antigen-inducing factor requires recognition of antigen plus Ia at a certain concentration on the B cell in the first place, it is not clear how such a factor could contribute to the process. However, this may be a difficulty only if the release of factors by Th cells is an

all-or-none process, which antigen dose-response curves of T cell activation almost surely prove is not the case. Preliminary data show that type 2 cloned T cell lines do release a potent Ia antigen-inducing factor, presumably similar to that recently reported by Roehm and coworkers (31); whether this is the major difference between type 1 and type 2 cloned Th cells awaits testing of a panel of such cloned lines.

One difference between type 1 and type 2 cloned lines on one hand, and type 3 cloned T cell lines on the other, is that type 3 secrete large amounts of lymphotoxin, and can kill both B lymphoma cells and LPS-activated normal B cells. Thus, this distinction is clearly due to differences in the pattern of mediator molecules secreted by the different types of cloned T cell lines. One might expect that type 1 cloned T cell lines combined the activities of type 2 and type 3 cloned lines, and this may be so. However, we believe that the answer will be more complex, since type 3 cloned lines suppress exclusively T15 Id⁻ B cells, when mixed with type 1 Th cells. Thus, any type 3-like behavior in type 1 cloned lines should lead to dominant T15 Id expression, which is not what is observed.

Type 4 cloned T cell lines actually fall into two subtypes as well, some giving high T15 anti-PC responses, others giving low T15 anti-PC responses in the absence of added antigen. Whether these correspond to autoreactive versions of type 2 and type 1 clones, respectively, remains to be determined.

Recently, Asano and Hodes (32) reported cloned T cell lines with a suppressive activity similar to that reported for type 3 clones such as Cla (22). Thus, type 3 clones, as a distinct functional phenotype, can be identified in a variety of experimental systems. Also, Mosier and Feeney (26), and Hathcock et al. (33) have described Ia-restricted cloned Th that induce T15 Id-dominated anti-PC responses; we would classify such cloned Th as type 2.

Having classified cloned Th cell lines into four distinct types, three of which are antigen specific, we are presently determining the pattern of lymphokine released by several cloned lines of each type. It is our expectation that each type will release a distinctive set of lymphokines, the sum of whose effects is to generate the type of PC-specific antibody response we have observed.

Summary

Analysis of activation of phosphorylcholine (PC)-specific B cells by a large number of different cloned, self Ia-specific helper T cell (Th) clones has permitted the classification of such T cells into four distinct functional types. Types 1 and 2 induce B cells to secrete anti-PC antibody in an antigen-specific, Ia-restricted fashion. Type 3 cells induce antigen-specific, Ia-restricted B cell proliferation, but do not lead to specific antibody formation, and have been shown previously (22) to have suppressor functions. Type 4 cells are autoreactive, and induce antigen-independent B cell activation and antibody secretion. The distinction between type 1 and type 2 Th clones was analyzed in detail. In bulk cultures, type 1 cloned lines generate an idiotypically heterogeneous anti-PC antibody response, whereas type 2 cloned lines induce a larger response that is dominated by the T15 idotype. In limiting-dilution analyses, type 2 cells induce fourfold more T15⁺, PC-specific precursor B cells than do type 1 cells, and in addition, induce larger burst sizes for T15⁺, PC-specific B cells. Type 4 clones

can also be subdivided into cells that are type 1-like, and cells that are type 2-like. These differences in functional phenotype are seen over a broad range of antigen and cell doses. Detailed analysis of the behavior of these distinct functional types of Th should allow a better understanding of the functional properties of mixed populations of antigen-primed, Ia-restricted Th cells.

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