ANTI-IDIOTYPE INDUCED REGULATION OF HELPER CELL FUNCTION FOR THE RESPONSE TO PHOSPHORYLCHOLINE IN ADULT BALB/c MICE

By K. BOTTOMLY,* B. J. MATHIESON, AND D. E. MOSIER

From the Laboratory of Immunology and Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Antibodies produced by BALB/c mice to phosphorylcholine (PC)[1] have been shown to be of restricted avidity and to express a dominant idiotype characteristic of the BALB/c PC binding myeloma, TEPC 15 (T15) (1–5). Even though the response to PC is idiotypically homogeneous, analysis of the anti-PC response at the precursor level reveals a significant proportion of T15-negative precursors (6, 7). The dominant idiotypic pattern of the anti-PC response can be altered by the administration of anti-T15 antibodies to adult or neonatal BALB/c mice, resulting in suppression of PC-specific clones bearing the T15 idiotype (8, 9). This has permitted an analysis of the induction or suppression of B-cell clones expressing a single (or a set of closely-related) V_H gene product (T15).

The role of T cells in regulating the expression of idiotype by PC-specific B cells is unclear. Because it has been reported previously in other systems involving predominant idiotypes that the induction of idiotype-bearing B-cell clones is controlled by idiotypically related helper and/or suppressor T cells (10–12), the present experiments were performed to determine the role of T cells in secondary anti-PC antibody responses in BALB/c mice. To do this, B cells from PC-primed mice were adoptively transferred with T cells from keyhole limpet hemocyanin (KLH)-primed donors and the plaque-forming cell (PFC) response to PC-KLH was measured. We found that T cells from anti-T15 treated mice were unable to cooperate effectively with PC-primed B cells. Furthermore, such T cells could suppress the helper activity of T cells from control KLH-primed mice in the response to PC-KLH and had an Ly phenotype characteristic of suppressor T cells in other systems.

Materials and Methods

Mice. BALB/cCum mice were obtained from Cumberland View Farms, Clinton, Tenn. Mice congenic with C57BL/6 (B6) for Lyt antigens, B6/Ly 1.1 and B6/Ly 2.1, 3.1, initially were obtained from Dr. E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York, and were bred in our colonies at NIH or were obtained from a colony maintained by Dr. Michael Potter at Litton Bionetics, Kensington, Md. DBA/2N mice were obtained from Small Animal Section, DRS, NIH.

Antigens. PC-KLH was prepared as described previously (2). *p*-diazonium phenylphosphorylcholine was synthesized by Dr. John Inman according to the method of Chesebro and

* Supported by National Institutes of Health Postdoctoral Fellowship AI05336-01.

[1] *Abbreviations used in this paper*: BGG, bovine gamma globulin; CFA, complete Freund's adjuvant; CGG, chicken gamma globulin; KLH, keyhole limpet hemocyanin; NRS, normal rabbit serum; PC, phosphorylcholine; PFC, plaque-forming cells; PnX, pneumococcal extract; r, regression coefficient; T15, TEPC 15; TNP, trinitrophenyl.

Metzger (13). This compound was reacted to either KLH, bovine gamma globulin (BGG), or chicken gamma globulin (CGG) in pH 9.2 borate-saline buffer. The degree of substitution was 24 mol PC per 100,000 daltons KLH (PC-KLH), 9 mol PC per mole of BGG, and 23 mol PC per mol of CGG. Trinitrophenyl $(TNP)_{12}$-KLH was a gift from Dr. Theo Kirkland. The plasma cell tumors TEPC 15, McPC 603, and MOPC 167 were obtained from Litton Bionetics, and were maintained by serial passage of tumor cells in BALB/c mice. The ascitic fluid containing myeloma proteins was collected and the PC-binding myeloma proteins were purified by passage over a PC-Sepharose immunoadsorbant. Pneumococcal extract (PnX) was a kind gift from Dr. Benjamin Prescott, National Institutes of Health, Bethesda, Md.

Anti-TEPC 15 Idiotype Antisera. Anti-TEPC 15 was raised in rabbits by repeated injections of purified TEPC 15 myeloma protein in complete Freund's adjuvant (CFA). The rabbit anti-T15 antiserum was absorbed with MOPC 384 (IgA, κ), McPC 603 (IgA, κ), and mixed gamma (γ_1, γ_{2a}, γ_{2b}, κ) - Sepharose immunosorbent columns to eliminate nonidiotypic antibodies. The antibodies were subsequently adsorbed to a TEPC 15 (IgA, κ)-Sepharose column and eluted by 0.1 M acetate buffer. The purified antibodies were determined to be idiotypically specific since they precipitated TEPC 15 myeloma proteins but not the closely related PC-binding IgA myeloma proteins McPC 603 and MOPC 167.

Anti-Ly Antisera. Anti-Ly sera were the generous gift of Doctors F-W. Shen and E. A. Boyse (Memorial Sloan Kettering Cancer Center), or were prepared by us. These sera were similar to those described elsewhere (14); anti-Ly 1.2, C3H/An versus CE normal thymocytes; anti-Ly 2.2 (C3H/An × B6/Ly 2.1)F_1 versus ERLD; a B6 tumor, or (C3H/HeN × B6/Ly 2.1)F_1 versus B6 normal thymocytes.

As a precautionary measure, all sera routinely were absorbed with cells from thymus, spleen, and lymph nodes of the appropriate B6 congenic mice before use in the elimination experiments. The anti-Ly 1.2 and anti-Ly 2.2 sera were absorbed also with lymphoid cells from DBA/2 (Ly 1.1^+, Ly 2.1^+) which express high levels of murine leukemia virus-associated cell surface antigens.[2] For each anti-Ly reagent, two sequential absorptions were done with ⅛ diluted antiserum at 4°C for 1 h each. The total lymphoid cell pellet for absorption was equal to the undiluted serum volume.

Specificity control for Ly 2.2 was achieved by an additional absorption of a sample of anti-Ly 2.2 with B6 and B6/Ly 1.1 lymphoid cells (i.e. Ly 2.2^+ cells) as well as DBA/2 cells. This reagent is referred to as absorbed anti-Ly 2.2.

Immunizations. BALB/cCum mice, 8 wk of age, to be used as T-cell donors were pretreated with either 0.1 ml normal rabbit serum (NRS) or 50 μg of rabbit anti-T15 antibodies, intraperitoneally. 1 wk later, they were immunized with either 200 μg of KLH or CGG in CFA. BALB/c mice to be used as B-cell donors were immunized with either 100 μg of PC-BGG or TNP-KLH in CFA. All donors were used 6 wk after priming.

Separation of Splenic T cells. T cells were obtained by passage of primed spleen cells over nylon wool columns by the method of Julius et al. (15). The yield of passed cells was usually 20% of the original spleen population. Staining of these cells with fluoresceinated rabbit anti-mouse immunoglobulin revealed less than 2% contaminating Ig^+ cells.

Antiserum and Complement Treatment of Cells. B cells were obtained after treatment of primed spleen cells with 1:4 dilution of AKR anti-C3H antiserum (anti-Thy 1.2) for 30 min at 4°C. The cells were subsequently exposed to a 1:4 dilution of guinea pig complement preabsorbed with a BALB/c liver cell suspension, for 30 min at 37°C.

Nylon wool purified spleen cells were treated with anti-Ly antisera at 1/20 or 1/40 dilution (as appropriate for each serum). The cells were mixed with antiserum for 30 min at room temperature, washed twice by centrifugation and resuspended in medium. An equal volume of 1/10 diluted, selected nontoxic rabbit serum as a source of complement was added to the washed pellet and the mixture incubated at 37°C for 30 min. The T cells remaining after treatment were transferred without correcting for cell death along with B cells into irradiated recipients.

Adoptive Transfer. BALB/cCum mice irradiated with 400 rads were used as recipients. Graded numbers of primed cells plus 50 μg of antigen were injected i.v. into the irradiated recipients.

---

[2] H. C. Morse, III. et al. Differences in expression of XenCSA on lymphocytes of inbred mouse strains. Manuscript submitted for publication.

TABLE I

Adoptive Secondary Anti-PC Response to PC-KLH: The Effect of Helper Cell Number

B-Cell donor ($\times 10^{-6}$) PC-primed	T-Cell donor ($\times 10^{-6}$) KLH-primed	Geometric mean (×/+ relative SE)* PC-PFC/spleen‡
5	—	886 (1.21)
—	4	492 (1.32)
5	0.5	2,698 (1.09)
5	1	4,003 (1.33)
5	2	5,929 (1.07)
5	4	10,829 (1.26)

* The geometric mean (standard error) for each group represents the responses of 6–21 individual BALB/c mice in five individual experiments.

‡ 95–100% of the anti-PC PFC were shown to be of the T15 idiotype by inhibition of plaque formation by using rabbit anti-T15 antibodies.

Their spleens were assayed for PFC 8 days after cell transfer. All groups contained three to five mice. All experiments presented were repeated at least three times with similar results.

Hemolytic PFC Assay. Spleen cells were assayed for direct anti-PC or anti-TNP PFC by the modified Jerne hemolytic plaque technique (16), by using 1:20 dilution of guinea pig complement (Flow Laboratories, Inc., Rockville, Md.). The indicator cells were sheep erythrocytes coupled with a PnX (17) or TNP (18). Indirect plaques were determined by inhibition of IgM antibody with a 1:1,000 final dilution of goat anti-mouse IgM serum, and enhancement of IgG PFC with a 1:100 final dilution of rabbit anti-mouse γ_1 and γ_2 kindly provided by Dr. Richard Asofsky, National Institutes of Health.

Results

Effect of T-Cell Dose on the Adoptive Secondary Anti-PC Antibody Response. To study the regulatory effects of T cells on the anti-PC antibody response of primed B cells, conditions were established for an adoptive secondary anti-PC response to PC-KLH. The results in Table I demonstrate low background responses by either PC-BGG primed B cells or KLH-primed T cells transferred separately into irradiated recipients and boosted with PC-KLH. When these two cell types are mixed, responses substantially greater than the sum of the responses given by either population alone are obtained. Furthermore, these responses are proportional ($r = 0.99$) to the number of KLH-primed T cells transferred. The anti-PC PFC were shown to be specific for PC and to be predominantly (95%) of the T15 idiotype by inhibition of plaque formation. Thus, in this system, the secondary response to PC is dependent on the presence of T cells and is virtually entirely of the T15 idiotype.

Effect of Anti-T15 Idiotype on the Helper Activity of KLH-Primed T Cells. To determine the effect of anti-idiotype antibody on T cells involved in regulation of the anti-PC antibody response, T-cell donors were treated with either anti-T15 antibody or with NRS before priming with KLH. These T cells were then tested for their ability to collaborate with PC-specific B cells. T cells from NRS-pretreated donors induced a substantial anti-PC response of which 95% was $T15^+$ (Table II). In contrast to this response, KLH-primed T cells from anti-T15-pretreated donors were unable to effectively collaborate with PC-primed B cells. An apparent decrease in helper activity was seen at all T-cell numbers tested. In addition, expression of the T15 idiotype in the suppressed response was somewhat less than in the control response.

TABLE II

Inhibition of the Anti-PC Response after Anti-Idiotype Treatment of the T-Cell Donor

B-Cell donor ($\times 10^{-6}$) PC-primed	T-Cell donor ($\times 10^{-6}$)		Geometric mean (×/+ relative SE)* PC-PFC/Spleen	%T15
	NRS, KLH-primed	Anti-T15, KLH-primed		
5	—	—	589 (1.39)	
—	4	—	519 (1.30)	
—	—	4	321 (1.26)	
5	1	—	2,036 (1.08)	96
5	2	—	4,675 (1.24)	95
5	4	—	9,003 (1.34)	94
5	—	1	201 (1.71)	90
5	—	2	616 (1.39)	84
5	—	4	812 (1.41)	85
5	2	2	1.598 (1.20)	90
5	4	4	3,236 (1.63)	91

* The geometric mean (standard error) for each group represents the responses of 12–32 individual BALB/c mice in six replicate experiments.

To determine whether this anti-T15-pretreated T-cell population lacked helper activity or had gained suppressor activity, a mixture of anti-T15 and NRS-pretreated T cells was transferred with PC-primed B cells. It would be expected that a mixture of 2×10^6 NRS-pretreated and 2×10^6 anti-T15-pretreated T cells would produce a response greater than 5,000 PFC per spleen if the two responses were simply additive. Yet, it can be seen that such a mixture gave only 1,600 PFC per spleen. Therefore, the failure of T cells from anti-T15-pretreated donors to collaborate optimally seems to be due at least in part to the generation of suppressor T cells which are capable of regulating the PC-specific response.

Specificity of Suppressor T Cells Generated by Anti-T15 Pretreatment. Having established that T cells from anti-T15-treated, KLH-primed donors could effectively suppress the T15 idiotype positive anti-PC antibody response to PC-KLH, we next asked whether the suppressive T cell was specific for the carrier, KLH, or for the T15 idiotype. To assess carrier-specificity we tested the ability of anti-T15 treated, KLH-primed T cells to help the response to the hapten TNP coupled to KLH.

The experiment shown in Table III again demonstrates that T cells from anti-T15-treated, KLH-primed donors have far less ability to help PC-primed B cells respond to PC-KLH than do NRS-treated, KLH-primed T cells. By contrast, both populations of KLH-primed T cells help equally the anti-TNP response of TNP-primed B cells to TNP-KLH. It should be noted that the response to PC-KLH is composed entirely of direct (IgM) PFC, while the response to TNP-KLH is primarily indirect (IgG), PFC, yet neither the direct nor the indirect response to TNP was affected by T cells from anti-T15-pretreated donors. The specificity of the PC-primed B cells is shown when they are challenged with an inappropriate antigen such as TNP-KLH. Either no response is generated, or a very small response is seen which may be due to a primary anti-TNP response. The results illustrated in Table III suggest that the suppressor T cells generated by anti-T15 treatment do not act exclusively upon carrier (KLH)-specific helper T cells.

To determine whether the suppressor T cell was specific for the T15 idiotype, we tested the ability of anti-T15-treated, KLH primed T cells to suppress a response to

TABLE III

Specificity of Suppression after Anti-Idiotype Treatment

B-Cell donor ($\times 10^{-6}$)	T-Cell donor ($\times 10^{-6}$)			Antigen	Geometric mean PFC (×/+ relative SE)		
PC-primed	TNP-primed	NRS, KLH-primed	Anti-T15, KLH-primed		Direct anti-PC PFC/spleen	Direct anti-TNP PFC/spleen	Indirect anti-TNP PFC/spleen
5	—	—	—	PC-KLH	617 (1.85)		
—	5	—	—	TNP-KLH		783 (1.47)	183 (1.62)
—	—	4	—	PC-KLH	743 (1.58)		
—	—	4	—	TNP-KLH		1,716 (1.06)	66 (1.73)
5	—	4	—	PC-KLH	43,414 (1.07)		
5	—	4	—	TNP-KLH		3,214 (1.19)	599 (1.05)
5	—	—	4	PC-KLH	10,490 (1.30)		
—	5	4	—	TNP-KLH		6,336 (1.30)	11,105 (1.35)
—	5	—	4	TNP-KLH		4,871 (1.37)	15,303 (1.01)

Results of one of three replicate experiments.

PC on the carrier CGG. The results in Table IV (lines 5–14) demonstrate that CGG-primed T cells, like KLH-primed T cells, collaborate effectively with PC-specific B cells when challenged with PC-CGG. As seen with anti-T15-treated, KLH primed donors, T cells from anti-T15-treated, CGG-primed donors are unable to collaborate effectively with PC-specific B cells and are capable of suppressing a normal response to PC-CGG. Both carriers show a certain degree of cross-priming, in that T cells from KLH-primed donors can induce a substantial PC-CGG response (line 6) and CGG-primed T cells can induce a PC-KLH response (line 11). The responses which we ascribe to cross priming are not decreased when T cells from anti-T15 pretreated donors are used (lines 8 and 13).

When T cells from anti-T15-treated, CGG-primed donors that can suppress the response to PC-CGG are mixed with KLH-primed T cells, they are unable to suppress a response to PC-KLH generated by the interaction of KLH-primed T cells and PC-specific B cells (line 19). Likewise, suppressor T cells generated by anti-T15 treatment and KLH priming do not affect the collaboration between CGG-primed T cells and PC-specific B cells in response to PC-CGG (line 21). Furthermore, the responses induced by KLH-primed T cells in the presence of anti-T15-treated, CGG-primed T cells, and the responses induced by CGG-primed T cells in the presence of anti-T15 treated, KLH-primed T cells are considerably greater than expected. The mixtures of either the two carrier-primed cell populations or the two anti-T15 treated, carrier primed populations (lines 15–18) give PFC responses somewhat greater than expected.

Having established that mixtures of primed T-cell populations resulted in augmented PFC responses, we tested that ability of unprimed T cells to induce the same responses. As seen in Table V, it is clear that unprimed T cells do not affect the response to PC-KLH. Unprimed T cells alone are incapable of inducing a response to PC-KLH. Similarly, addition of unprimed T cells to T cells from KLH-primed, anti-T15 treated, KLH-primed, or CGG-primed donors had no effect on the responses to either PC-KLH or PC-CGG. These results clearly indicate that priming of the T-cell donors is necessary both for the cross-priming evident in Table IV and the augmen-

TABLE IV

Specificity of Suppression after Anti-Idiotype Treatment

B-Cell donor ($\times 10^{-6}$)	T-Cell donor ($\times 10^{-6}$)				Antigen	Geometric mean (×/+ relative SE) PC-PFC/spleen	Expected response
PC-primed	KLH-primed	Anti-T15, KLH-primed	CGG-primed	Anti-T15, CGG-primed			
1. 5	—	—	—	—	PC-KLH	145 (2.13)	
2. 5	—	—	—	—	PC-CGG	142 (2.04)	
3. —	4	—	—	—	PC-KLH	245 (1.88)	
4. —	—	—	4	—	PC-CGG	195 (1.05)	
5. 5	2	—			PC-KLH	8,903 (1.07)	
6. 5	2	—			PC-CGG	3,206 (1.31)	
7. 5	—	2			PC-KLH	1,671 (1.53)	
8. 5	—	2			PC-CGG	4,016 (1 22)	
9. 5	2	2			PC-KLH	4,193 (1.04)	10,574
10. 5			2	—	PC-CGG	10,622 (1.07)	
11. 5			2	—	PC-KLH	997 (1.29)	
12. 5			—	2	PC-CGG	3,854 (1.01)	
13. 5			—	2	PC-KLH	1,182 (2.03)	
14. 5			2	2	PC-CGG	4,309 (1.32)	14,476
15. 5	2	—	2	—	PC-KLH	16,724 (1.23)	9,900
16. 5	2	—	2	—	PC-CGG	17,229 (1.33)	13,828
17. 5	—	2	—	2	PC-KLH	13,747 (1.12)	2,853
18. 5	—	2	—	2	PC-CGG	11,245 (1.39)	7,870
19. 5	2	—	—	2	PC-KLH	28,897 (1.13)	10,085
20. 5	2	—	—	2	PC-CGG	7,696 (1.31)	7,060
21. 5	—	2	2	—	PC-CGG	37,439 (1.31)	14,638
22. 5	—	2	2	—	PC-KLH	15,894 (1.16)	2,268

tation seen with mixtures of primed T cells. As was also seen in Table IV, the responses due to cross-priming are not suppressed by suppressor T cells generated by anti-T15 treatment and KLH priming. These results suggest that the suppressor T cells generated by anti-T15 treatment and KLH priming suppress only a response to PC on the appropriate carrier, in this instance, KLH.

These experiments demonstrate that the suppressor T cells generated by anti-T15 pretreatment fail to act upon KLH or CGG-specific helper T cells. Likewise, the cross carrier-priming evident in Table IV and V is also unaffected by the suppressor T cells. Elicitation of the suppressive activity present with T cells from anti-T15 treated mice seems to require duplication of the environment in which they were generated originally; viz., both the idiotype and the original carrier must be present. The suppression therefore is carrier-related, but not carrier-specific.

Ly Phenotype of Suppressor T Cells Induced by Anti-T15 Treatment. The final series of experiments dealt with two questions: (*a*) the surface Ly phenotype of the suppressor and helper T cells in this system, and (*b*) whether or not the anti-T15-pretreated,

TABLE V

Unprimed T Cells do not Influence the Response to PC-KLH

B-Cell donor ($\times 10^{-6}$) PC-primed	T-Cell donor ($\times 10^{-6}$)				Geometric mean (×/+relative SE) Response PC-PFC/spleen after boosting with	
	Unprimed	KLH-primed	Anti-T15, KLH-primed	CGG-primed	PC-KLH	PC-CGG
5	—	—	—	—	568 (1.28)	ND*
—	4	—	—	—	272 (1.18)	ND
—	—	2	—	—	602 (1.06)	ND
—	—	—	2	—	416 (1.30)	ND
—	—	—	—	2	ND	503 (1.27)
5	4	—	—	—	424 (1.41)	262 (1.20)
5	—	2	—	—	18,785 (1.14)	3,617 (1.33)
5	—	—	2	—	5,816 (1.06)	3,002 (1.41)
5	—	—	—	2	2,986 (1.21)	21,107 (1.20)
5	4	2	—	—	20,002 (1.36)	4,212 (1.22)
5	4	—	2	—	5,491 (1.16)	3,982 (1.37)
5	4	—	—	2	2,869 (1.42)	23,010 (1.43)

* ND, Not determined.

KLH-primed T cells contained helper T cells specific for PC-KLH that could be revealed by removing the suppressor cells.

Purified T cells from anti-T15-pretreated, KLH-primed donors were tested by cytotoxicity for their expression of Lyt 1 and Lyt 2 surface antigens. Spleen cells from either NRS- or anti-T15-pretreated, KLH-primed donors were first passed over nylon wool. The purified T-cell populations were then exposed to anti-Ly 2.2, absorbed anti-Ly 2.2, or anti-Ly 1.2 antiserum and rabbit complement. The cells remaining were subsequently tested for their ability to collaborate with PC-specific B cells in an adoptive secondary response to PC-KLH. The ability of NRS-pretreated T cells to collaborate with PC-specific B cells was unaffected by anti-Ly 2.2 treatment (Table VI). By contrast, anti-Ly 2.2 treatment of anti-T15-pretreated T cells restored their ability to collaborate with PC-specific B cells and eliminated their ability to suppress collaboration between NRS-pretreated, KLH-primed T cells and PC-specific B cells.

If the activity of the anti-Ly 2.2 was removed by absorption (Materials and Methods), the T cells from anti-T15-pretreated donors were unable to collaborate effectively with PC-specific B cells. The suppression is even more pronounced in this group treated with the absorbed anti-Ly 2.2 antiserum.

Within the limitations of cytotoxic elimination experiments, we can conclude that the Ly phenotype of the suppressor cells present in anti-T15-pretreated, KLH-primed donors is Ly 1^-, Ly 2^+. It also is possible that the suppressor cells have only relative differential expression of Lyt antigens, such that these cells have decreased Lyt 1 expression and increased Lyt 2 expression. Based on these data, it can be inferred that treatment of the anti-T15-pretreated T-cell population with anti-Ly 1.2 should remove all helper activity without altering suppressor function. The final set of experiments (Table VI) indicate that anti-Ly 1.2 treatment of anti-T15-pretreated T-cell populations eliminates collaboration with PC-specific B cells, yet the cells remaining are capable of suppressing the anti-PC response normally induced by NRS-pretreated T cells.

TABLE VI

Ly Phenotype of Suppressor T Cell by Treatment with Anti-Ly Antisera

B-Cell donor ($\times 10^{-6}$) PC-primed	T-Cell donor ($\times 10^{-6}$)*				Geometric mean ($\times$/+ relative SE) PC-PFC/spleen
	NRS, KLH-primed	T cells treated with	Anti-T15, KLH-primed	T Cells treated with	
5	—	—	—	—	1,027 (1.05)
—	4	—	—	—	2,598 (1.60)
5	4	—	—	—	32,811 (1.36)
5	—	—	4	—	7,207 (1.27)
5	4	—	4	—	17,059 (1.12)
5	4	Anti-Ly 2.2	—	—	27,098 (1.20)
5	—	—	4	Anti-Ly 2.2	38,720 (1.16)
5	4	Anti-Ly 2.2	4	Anti-Ly 2.2	41,913 (1.10)
5	—	—	4	Anti-Ly 2.2 absorbed‡	3,076 (1.58)
5	4	Anti-Ly 2.2 absorbed‡	4	Anti-Ly 2.2 absorbed‡	11,750 (1.79)
5	—	—	4	Anti-Ly 1.2	808 (1.85)
5	4	—	4	Anti-Ly 1.2	18,444 (1.09)

* The T cells remaining after anti-Ly antisera plus complement treatment of the 4 million starting T-cell population were transferred.

‡ Anti-Ly 2.2 was specifically absorbed with B6 and B6/Ly 1.1 lymphoid cells.

It is clear from these experiments that the surface phenotype of helper T cells necessary for a secondary anti-PC response is Ly 1^+, Ly 2^-. Furthermore, pretreatment of the T-cell donor with anti-T15 does not eliminate this population of helper T cells since removal of suppressor cells restores normal help for the anti-PC response.

Discussion

In these studies, we have tested the concept that the T15 idiotype bearing PC-specific B cells responding in a secondary adoptive transfer experiment can be regulated by T cells which recognize or bear the T15 idiotype. We have shown that helper T-cell activity from KLH-primed donors is necessary for a secondary anti-PC response to PC-KLH. Pretreatment of the T-cell donors with purified anti-T15 antibodies before KLH priming substantially reduces the ability of their T helper cells to collaborate with PC-primed B cells in the generation of a normal $T15^+$ anti-PC response. This regulation of the secondary response to PC by pretreatment of T-cell donors with anti-T15 is clearly due to the generation of Ly 2^+ suppressor T cells and not due to the lack of helper T-cell activity since removal of suppressor activity with anti-Ly 2.2 antiserum allows the expression of normal levels of helper function. The expression of the Ly 2^+ phenotype on a subpopulation of suppressor T cells is consistent with the findings of other workers (19–23).

It might be expected that suppression of the dominant T15 idiotype through either direct interaction with the B cell or interaction with an idiotype-specific helper T cell would result in the expression of an anti-PC response with alternate idiotypes. Analysis of the anti-PC response at the precursor levels shows that BALB/c mice do have $T15^-$ precursors and are genetically capable of expressing alternate idiotypes (6, 7). The expression of $T15^-$ idiotypes seen after neonatal suppression with anti-T15 requires 4–6 mo (24), so it seems clear that the suppression of the dominant T15 idiotype is

not immediately compensated for by expression of alternate idiotypes. Furthermore, the PC-priming of the B cells generates a predominantly $T15^+$ precursor population. These factors may explain the diminished response to the hapten PC seen in the presence of anti-T15-generated suppressor T cells, since an 8 day assay does not allow enough time for the expression of an anti-PC response with alternate idiotypes.

The experiments presented in Table IV raise several interesting questions about the specificity of regulatory T cell networks and require detailed consideration. First, the observation that suppressor T cells with an Ly 1^-, 2^+ phenotype can be induced by anti-idiotype treatment suggests that one T cell in the network expresses idiotypic determinants on its cell surface receptors. The exact mechanism of the generation of the suppressor T cell is unclear. Second, the carrier-related specificity of the anti-idiotype induced suppression requires several assumptions to formulate even a potential explanation. We envision the priming of mice with either KLH or CGG in CFA as leading to at least three events: (*a*) induction of T helper cells of conventional carrier specificity, (*b*) induction of idiotype-specific helper T cells, and (*c*) induction of polyclonal T-cell activation by CFA as well as the protein carriers. After the perturbation of preexisting PC regulatory networks by anti-T15 injection, the anti-T15-treated, carrier-primed T-cell donors potentially could contain carrier-specific T helper cells, idiotype-specific T helper cells, carrier-specific T suppressor cells, idiotype- or anti-idiotype-specific T suppressor cells, and polyclonally-activated T cells of unknown specificity. How might these T cells interact to give the results obtained in the experiments illustrated in Table IV? The crucial mixing experiments are represented in lines 15–22. In lines 15 and 16, it is seen that mixtures of control KLH- or CGG-primed T cells produce an anti-PC response somewhat greater than expected whether the antigen is PC-KLH or PC-CGG. This modest synergy might be explained by one or both of two mechanisms; preexisting polyclonally-stimulated idiotype-specific T helper cells present in both donors in low numbers might synergize in the T-cell mixture to enhance the anti-PC response, or carrier-specific activated T helper cells may recruit either specific or nonspecific (polyclonally-activated?) T helper cells from the inappropriate carrier-primed T-cell donor, which would explain the cross priming seen in lines 6 and 11. The results for lines 17 and 18 show that mixtures of T cells from anti-idiotype-treated donors provide a substantial source of T helper function which is much greater than that anticipated by the reactivity of either population alone. It should be recalled that neither of these populations have a detectable reduction in their KLH- or CGG- specific helper function, since TNP-KLH or TNP-CGG collaborative responses are normal (Table III and our unpublished observations), but their ability to help for a PC-specific response is impaired substantially (lines 7 and 12) when homologous hapten-carrier conjugates are used for boosting. The cross-priming evident in lines 6 and 11 and failure of cross-primed T cells to be influenced by anti-T15 induced suppressors (lines 8 and 13) may also explain the degree of enhancement seen in lines 15 through 18. The apparent interaction between T cells from different donors is even more pronounced when one donor has not been treated with anti-idiotype antibodies (groups 19 and 21), reemphasizing that some helper component is suppressed in groups 17 and 18 despite the unanticipated high level of helper function. The dilemma is posed by the source of idiotype-specific help (or absence of idiotype-specific suppression) in lines 15 through 22, since KLH- or CGG-specific helpers by themselves cannot generate adequate

helper function in anti-T15-treated donors. One possible way to escape this dilemma is to postulate that the Ly 1^-, 2^+ suppressor T cells are induced by the combination of anti-T15 treatment and carrier-priming and have a conjoint specificity, i.e. their activation for expression of suppressor function requires exposure to both idiotype and carrier, perhaps simultaneously, even though they were induced by separate exposure. An alternative explanation is that both anti-idiotype treatment and carrier-priming induce populations of specific suppressor T cells which separately are relatively ineffective, but which can synergize for suppression when both are activated. The enhancement of T helper function in lines 17 and 18 then would be explained by a failure of suppressors from anti-T15-treated, KLH-primed donors to suppress the interaction between idiotype-specific help and CGG carrier-specific help and vice versa. This potential explanation also would account for the very high responses in lines 19 and 21, since anti-idiotype induced idiotype-specific helper cells would escape suppression because they would be activated by PC on the inappropriate carrier for suppressor stimulation.

A general explanation is that all the classes of T helper and suppressor cells mentioned above exist in a dynamic equilibrium, and that activating one component of the interacting system destroys the equilibrium throughout the system. The signals which allow communication between components of the system may be relatively non-specific, while activation of many of the individual members of regulatory network is envisioned as antigen-specific (e.g. hapten, carrier, or both).

Summary

An adoptive secondary antibody response to phosphorylcholine (PC) can be generated by the transfer of keyhole limpet hemocyanin (KLH)-primed T cells, PC-bovine gamma globulin-primed B cells, and PC-KLH into irradiated syngeneic BALB/c mice. If the KLH-primed T-cell donors were pretreated with anti-idiotype antibodies directed against the BALB/c PC-binding myeloma TEPC 15, their T cells were unable to collaborate effectively with PC-primed B cells; moreover, they could suppress the helper activity of T cells from normal mice for the PC-KLH response. The Ly phenotype of these T cells was found to be Ly 1^-, 2^+. The specificity of the suppressor T-cell population induced by anti-T15 treatment appears to be both for idiotype (hapten) and carrier, since the suppressor T cells fail to interfere with the antibody response to PC on a heterologous carrier, nor do they suppress the response to trinitrophenol-KLH.

The authors wish to thank Doctors E. A. Boyse and F.-W. Shen for their generous supply of anti-Ly sera. We are indebted to Dr. R. A. Asofsky for his advice and for his review of the manuscript.

Received for publication 25 May 1978.

References

1. Cosenza, H., and H. Köhler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science (Wash. D.C.).* **176:**1027.
2. Lee, W., H. Cosenza, and H. Köhler. 1974. Clonal restriction of immune response to phosphorylcholine. *Nature (Lond.).* **247:**55.
3. Claflin, J. L., R. Lieberman, and J. M. Davie. 1974. Clonal nature of the immune response

to phosphorylcholine. II. Idiotypic specificity and binding characteristics of anti-phosphorylcholine antibodies. *J. Immunol.* **112:**1747.

4. Sher, A., and M. Cohn. 1972. Inheritance of an idiotype associated with the immune response of inbred mice to phosphorylcholine. *Eur. J. Immunol.* **2:**319.
5. Lieberman, R., M. Potter, E. B. Mushinski, W. Humphrey, Jr., and S. Rudikoff. 1974. Genetics of a new IgV_H (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med.* **139:**983.
6. Gearhart, P., N. Sigal, and N. Klinman. 1975. Heterogeneity of the BALB/c anti-phosphorylcholine antibody response at the precursor cell level. *J. Exp. Med.* **141:**56.
7. Cosenza, H., M. H. Julius, and A. A. Augustin. 1977. Idiotypes as variable region markers: analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* **34:**3.
8. Strayer, D. S., H. Cosenza, W. Lee, D. A. Rowley, and H. Köhler. 1974. Neonatal tolerance induced by antibody against antigen-specific receptors. *Science (Wash. D.C.).* **186:**640.
9. Strayer, D. S., W. M. F. Lee, D. A. Rowley, and H. Köhler. 1975. Anti-receptor antibody. II. Induction of long-term unresponsiveness in neonatal mice. *J. Immunol.* **114:**728.
10. Nisonoff, A., and S. A. Bangasser. 1975. Immunological suppression of idiotypic specificities. *Transplant Rev.* **27:**100.
11. Eichmann, K. 1975. Idiotypic suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* **5:**511.
12. Ward, K., H. Cantor, and E. A. Boyse. 1977. Clonally-restricted interactions among T and B cell subclasses. *In* Immune System: Genetics and Regulation. E. Sercarz, L. A. Herzenberg, and C. F. Fox, editors. Academic Press, Inc., New York. 397.
13. Chesebro, B., and H. Metzger. 1972. Affinity labeling of a phosphorylcholine binding mouse myeloma protein. *Biochemistry.* **11:**766.
14. Shen, F. W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics.* **2:**591.
15. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
16. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Wash. D.C.).* **140:**405.
17. Baker, P. J., P. W. Stashak, and B. Prescott. 1969. Use of erythrocytes sensitized with purified pneumococcal polysaccharides for the assay of antibody and antibody producing cells. *Appl. Microbiol.* **17:**422.
18. Rittenberg, M. B., and C. Pratt. 1969. Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* **132:**575.
19. Feldman, M., P. C. L. Beverly, M. Dunkley, and S. Kontianen. 1975. Different Ly antigen phenotypes on helper and suppressor cells. *Nature (Lond.).* **258:**614.
20. Jandinski, J., H. Cantor, T. Tadakuma, D. L. Peavy, and C. W. Pierce. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper activities are inherent properties of distinct T cell subclasses. *J. Exp. Med.* **143:**1382.
21. Cantor, H., F. W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization antigen-specific suppressor and helper activities are mediated by distinct T cell subclasses. *J. Exp. Med.* **143:**1391.
22. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
23. Tse, H. Y., and R. W. Dutton. 1977. Separation of helper and suppressor T lymphocytes.

II. Ly phenotypes and lack of DNA synthesis requirement for the generation of concanavalin A helper and suppressor cells. *J. Exp. Med.* **146:**747.

24. Augustin, A., and H. Cosenza. 1976. Expression of new idiotypes following neonatal idiotypic suppression of a dominant clone. *Eur. J. Immunol.* **6:**497.