REGULATORY IDIOTYPES

T Helper Cells Recognize A Shared V_H Idiotope on Phosphorylcholine-specific Antibodies*

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The role of T helper cells in a conventional hapten-carrier response is commonly perceived as providing an essential signal to the hapten-specific B cell. The function of T helper cells specific for an idiotype on B cells is less clearly understood, and the necessity of such an idiotype recognizing T helper cell does not appear to be absolute (1, 2). The existence of idiotype recognizing T helper cells, however, has been demonstrated in experimental systems where a hapten is conjugated to the idiotype and the hapten-idiotype complex is used as an antigen (3). In a previous study (4) we have generated idiotype-recognizing T helper cells by new and indirect routes, i.e., by priming with anti-idiotypic antibody. These experiments were done in the phosphorylcholine (PC)[1] response system of BALB/c, which is dominated by the TEPC 15 myeloma protein (T15) idiotype (5).

Recently (6) we have shown that priming of BALB/c mice with PC-*Limulus polyphemus* hemocyanin (Hy) induces helper T cells that recognize trinitrophenylated T15 and MOPC 167 myeloma protein (M167), both PC-binding myelomas that are idiotypically and structurally different (7). The appearance of idiotype-specific T helper cells after exposure to antigen (PC-Hy) must be considered biologically significant. The role of these T helper cells could be to promote the dominant expression of an idiotype (1) and to regulate the expression of B and T cell idiotypes via network interactions.

In the present study we analyzed the relative frequencies and the fine specificity of individual idiotype-recognizing T helper cells generated in BALB/c mice by PC immunization. Our data indicate that a common antigenic determinant found on T15 and a T15-negative myeloma protein is the target for T cell recognition. This shared determinant consists of structures that constitute the binding site for PC. The conserved and shared nature of this determinant makes it likely that it serves as a regulatory idiotype.

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[1] *Abbreviations used in this paper:* BBS, borate-buffered saline; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DME, Dulbecco's modified Eagle's medium; DNP, dinitrophenyl; ELISA, enzyme-linked immunosorbent assay; Hy, *Limulus polyphemus* hemocyanin; IFA, incomplete Freund's adjuvant; M167, MOPC 167 myeloma protein; M315, MOPC 315 myeloma protein; PBS, phosphate-buffered saline; PC, phosphorylcholine; T15, TEPC 15 myeloma protein; TNP, 2,4,6-trinitrophenyl.

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Materials and Methods

Mice. BALB/c females, 6–8 wk old, were obtained from Cumberland View Farms, Clinton, TN. Athymic, *nu/nu* BALB/c were obtained from Harlan Sprague-Dawley, Madison, WI.

Myeloma Proteins. The plasma cell tumors, T15, M167, and MOPC 315 (M315), were obtained from Dr. M. Potter, National Cancer Institute, National Institutes of Health, and maintained by serial passage as ascites in BALB/c mice. The myeloma proteins T15, M167, and M315 were purified from the ascitic fluid by antigen affinity-column chromatography.

Hybridoma Proteins and Antisera. The hybridoma protein, F6, was the gift of Dr. M. Wittner, La Rabida-University of Chicago Institute. F6 has specificity for a T15 idiotypic determinant (8). Rabbit anti-mouse $F(ab')_2$ was prepared in our facility by multiple footpad injections of BALB/c $F(ab')_2$ in complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA). The antiserum was adsorbed on PC and 2,4,6-trinitrophenyl (TNP)-conjugated Sepharose 4B (Pharmacia Fine Chemicals, Pharmacia Inc., Piscataway, NJ). Rabbit anti-mouse κ chain and IgA were purchased from Litton Bionetics, Inc. (Kensington, MD).

Antigens. Hy was purchased from the Millipore Corp., Bedford, MA. Trinitrophenylated myeloma proteins were prepared by the reaction of 2,4,6-trinitrobenzene sulfonic acid (Sigma Chemical Co., St. Louis, MO) with purified T15 or M167, which had had their hapten-binding sites blocked with 0.1 M PC chloride (Sigma Chemical Co.). The hapten was removed after trinitrophenylation by dialysis against 0.1 M borate-buffered saline (BBS). Serological analyses of the trinitrophenylated myeloma proteins are shown in Table I. TNP-conjugated bovine serum albumin (TNP-BSA) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) was prepared by the reaction of BSA with TNBS. PC_5-BSA was prepared by the reduction of *p*-aminophenylphosphorylcholine (Biosearch, San Rafael, CA) to diazophenylphosphorylcholine and subsequent reaction with BSA.

Isolation of Immunoglobulin Heavy and Light Chains. Isolated T15 and M167 heavy and light chains were prepared from affinity-purified, reduced, and alkylated T15 and M167 myeloma proteins. 10 mg lyophilized protein was resuspended in 5–10 ml 4 M guanidine·HCl (Schwarz/Mann, Spring Valley, NY), 1 M propionic acid, pH 3 and incubated at 37°C for 60 min. The chains were separated by passage over a G-100 Sephadex column (Pharmacia Inc.). Heavy and light chains were analyzed for purity by sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis by the method of Steck et al. (9).

Priming of Mice. BALB/c mice, 6–8 wk old, were given 100 μg Hy in CFA i.p.; 4 wk later, they were given 100 μg PC PC-Hy in IFA i.p., and were used as T cell donors 4–8 wk later.

Donor T Helper Cell Preparation. Donor spleen cells were treated with 0.16 M NH_4Cl buffer

TABLE I

Analysis of TNP-conjugated Myeloma Proteins

	Detected with antisera against:*			
	IgA	Fab	k	T15 Id
Binding to PC-BSA by:‡	+	+	+	+
TNP-T15	+	+	+	−
TNP-167				
Binding to M315 by:	+	+	+	+
TNP-T15	+	+	+	−
TNP-167				

* PC_5-BSA and purified M315 were added to 96-well polyvinyl microtiter trays at 200 ng protein/0.1 ml BBS/well. The trays were incubated overnight at 4°C and sealed with 1% BSA-PBS. TNP-coupled myeloma proteins were added in 0.1 ml 1% BSA-PBS, and the trays were incubated overnight at 4°C.

‡ Rabbit antisera (1:250 dilution) against IgA, Fab, and k of mouse Ig were applied for 2 h at 37°. The assays were developed with alkaline phosphatase conjugated goat anti-rabbit in an ELISA. A monoclonal anti-idiotypic antibody for T15 was iodinated and added similarly. Positive reactivity was determined by radioimmunoassay.

to lyse erythrocytes. T cells were prepared by passage of donor spleen cells over nylon wool columns (10). Ly-2^+ T cells were removed by treatment of the nonadherent cells with the hybridoma antibody 3.239.2 (anti-Ly-2.2), a gift of Dr. F. Fitch, University of Chicago, and low toxicity rabbit complement (Accurate Chemical & Scientific Corp., Westbury, NY). The purity of the Ly-2.2-depleted cell population was tested by immunofluorescent staining with fluorescein-conjugated goat anti-mouse Ig, a gift of Dr. D. Levitt, La Rabida-University of Chicago Institute, and staining with 3.239.2 and fluorescein-conjugated goat anti-rat Ig, a gift of Dr. M. Loken, La Rabida-University of Chicago Institute. 6% of the cells were Ig^+ and <1% of the cells were Ly-2.2^+.

Splenic Fragment Cultures. The protocol for limiting dilution culturing of T cells in the presence of excess B cells has been defined in detail previously (4, 6). Briefly, purified T helper cells were transferred to recipient 6–8-wk-old athymic, *nu/nu* BALB/c mice. 24–48 h later, the recipient spleens were diced into 1-mm cubes, and the fragments were cultured individually in sterile 96-well culture plates (Costar, Data Packaging, Cambridge, MA) in Dulbecco's modified Eagle's medium (DME) (Grand Island Biological Co., Grand Island, NY) enriched with 10% agammaglobulin horse serum (Grand Island Biological Co.). The spleen fragments were immunized with TNP-conjugated myeloma proteins at 10^{-8} M TNP (1.4×10^{-9} M protein). Inhibitors of T cell recognition, PC chloride (Sigma Chemical Co.), and PC_5-BSA were added to the fragment culture at the time of immunization at 10^{-7} M PC; similarly, T15, M167, and their separated heavy and light chains were used at 1.4×10^{-8} M protein. 3 d later culture supernatants were observed, and at 3-d intervals the supernatants were collected and assayed for the presence of anti-TNP antibody by enzyme-linked immunosorbent assay (ELISA). Supernatants that had anti-TNP antibody over two collections were considered positive.

ELISA. An ELISA detecting anti-hapten was modified for use in 96-well polyvinyl microtiter plates (Cooke Engineering Co., Alexandria, VA). Briefly, 0.1 ml hapten-carrier conjugate (200 ng/ml in phosphate-buffered saline [PBS]) was added to each well. After an incubation at 4°C overnight, the wells were washed with PBS and sealed with an excess of 1% BSA-PBS for 1 h at room temperature. A 0.025-ml supernatant sample was added and diluted with 0.075 ml 1% BSA-PBS in each well. Serial dilutions of purified BALB/c anti-TNP antibody served as the standards when assaying culture supernatants. After an overnight incubation at 4°C, the wells were washed with PBS and filled with 0.1 ml rabbit anti-mouse Ig (N. L. Cappel Laboratories, Cochranville, PA) diluted 1:1000 in 1% BSA-PBS. The trays were incubated for 2 h at 37°C in a humidified chamber. The wells were washed with PBS, filled with 0.1 ml alkaline phosphatase- (Sigma Chemical Co.) conjugated goat anti-rabbit Ig (Litton Bionetics, Inc., Kensington, MD), and incubated overnight at 4°C. The trays were washed with PBS and developed with 0.1 ml Sigma 104 phosphatase substrate (30 mg/50 ml diethanolamine buffer) (Sigma Chemical Co.) added to each well. Absorbance at 405 nm was measured on a Titertek Multiscan spectrophotometer (Flow Laboratories, Inc., Rockville, MD).

Results

Precursor Analysis of Idiotype-recognizing T Helper Cells. In a recent report (6), we have shown that priming of BALB/c mice with PC-Hy induces T helper cells that recognize idiotypically different anti-PC myelomas as carriers for a TNP-specific B cell response. The idiotypes used as trinitrophenylated antigens were T15 and M167, which are serologically idiotype-different (11). These findings pose the question of whether T15 and M167 are recognized by two different T helper cell populations or one T helper cell clone recognizes a shared idiotope present on T15 and M167.

To answer this question, we compared the frequencies of T helper cells that recognize T15 and M167 myelomas proteins as hapten carriers. Both proteins are IgAk antibodies that bind PC, but they have different serologically defined idiotypic specificities (11). Performing these experiments required that the splenic foci system for the analysis of individual T cells be defined with regard to efficiency, which is dependent on the splenic homing of the donor cells and the effective stimulation of

the cultured lymphocytes. To determine the homing efficiency of T helper cell populations, we had to remove suppressing T cells. To accomplish this, the donor cells were treated with monoclonal anti-Ly-2 antibody and complement. These Ly-2-depleted T cells were radiolabeled with ^{51}Cr and injected into athymic, *nu/nu* BALB/c recipients. 24 and 48 h later, the recipient spleens were removed and counted for ^{51}Cr. The results of this experiment are shown on Table II. About 30% of the ^{51}Cr-labeled Ly-2$^-$ T cells were recovered. The recovery compares favorably with that reported by Zatz and Lance (12), who injected ^{51}Cr-labeled B and T lymphocytes into unirradiated normal recipients and assayed the recipient spleens 6 and 24 h later.

To determine the frequencies of Ly-2$^-$ T cells from PC-Hy-primed BALB/c, which recognizes T15 and M167, graded numbers of donor Ly-2$^-$ T cells were transferred to athymic, *nu/nu* BALB/c recipients. The splenic fragment cultures were immunized with either TNP-T15 or TNP-M167 and assayed for anti-TNP antibodies. As shown in Table III, the response to the trinitrophenylated antibodies increases with the number of injected T cells. The negative logarithm of nonresponding cultures ($-$Ln F_0) was plotted vs. the number of transferred cells, according to Lefkovits and Waldmann (13) (Fig. 1). Using a splenic homing efficiency of 30%, the frequencies of T15 and M167-recognizing Ly-2$^-$ T cells are estimated to be 1/490,000 Ly-2$^-$ T cells and 1/410,000 Ly-2$^-$ T cells, respectively. We wish to emphasize the similarity in frequencies between cells that recognize T15 and those that recognize M167. The frequencies are indistinguishable from one another within the experimental variation. Thus, this finding is compatible with the notion that the same population of T cells recognizes the T15 and the M167 carriers. To further define these helper T cells, the fine specificity in the carrier idiotype recognition was investigated.

Fine Specificity of T Helper Cells for T15 and M167. The data on the idiotype specificity of T helper cells induced by PC-Hy priming (6) suggested that a shared idiotypic determinant on T15 and M167 is being recognized. To further support this hypothesis, a series of inhibition experiments was done where the interaction of T helper cells with the idiotype carrier was blocked by adding putative inhibitors during the in vitro immunization. Inhibitors used were PC, unconjugated T15 and M167 idiotypes, and isolated heavy and light chains of T15 and M167.

TABLE II

Splenic Homing of Primed BALB/c T Cells Injected into Athymic nu/nu BALB/c Mice

^{51}Cr-donor T cells*	Number of T cells injected	cpm injected‡	cpm recovered in spleen§	
			24 h after cell transfer	48 h after cell transfer
Ly-2$^-$ PC-Hy Primed	9.6×10^5	1,229	391	369

* Donors were primed with PC-Hy and Ly-2.2$^-$ T cells prepared as described in Materials and Methods. 5×10^6 cells were suspended in 0.2 ml DME with 10 fetal calf serum and 0.1 ml ^{51}-chromium sulfate (1 mCi/ml) and incubated for 60 min at 37°C.

‡ The cells were washed four times and resuspended to 1 ml. 0.2 ml of the cell suspension was counted and another 0.2 ml was injected into an athymic, *nu/nu* BALB/c recipient.

§ At 24 and 48 h after cell transfer, the recipients were killed and their spleen cells assayed for radioactivity in a gamma counter.

TABLE III

Dose Response of Ly-2⁻ T Cells from PC-HY Primed BALB/c that Help a Response to TNP-T15 or TNP-M167

BALB/c Ly-2^- T cell donor*	Number of donor cells injected‡	In vitro antigen§	Anti-TNP-positive wells‖
	× 10^5		
PC-Hy	0	TNP-T15	1
	2		8
	5		25
	7.5		30
	10		44
PC-Hy	0	TNP-M167	2
	2		15
	5		26
	8		46
	10		52

* 8-wk-old BALB/c mice were given 100 μg Hy in CFA i.p.; 4 wk later, they were given 100 μg PC-Hy in IFA i.p., and were used as spleen cell donors 4 wk later.

‡ T cells were prepared by nylon wool passage of spleen cells, and the nonadherent cells were collected. Ly-2^- T cells were prepared by treatment of nylon wool-purified T cells with hybridoma product 3.239.2 (anti-Ly-2.2) and rabbit complement. 6% of the donor T cells were Ig^+ and <1% of the donor Ly-2.2-depleted T cells were Ly-2^+ as judged by immunofluorescence.

§ Donor cells were injected intravenously in graded doses into athymic, *nu/nu* BALB/c recipients. 24–48 h later, the recipient spleens were diced and the fragments were cultured individually.

‖ The splenic fragments were immunized in vitro with TNP_7-T15 at 10^{-8} M TNP. 96 fragment supernates/group were assayed from anti-TNP antibodies by ELISA.

Because the PC-binding site is highly conserved in different anti-PC antibodies (14), we reasoned that this site might bear the shared idiotypic determinant. To test this possibility, PC and PC-BSA were used to block the T cell recognition of the idiotypic determinant on the PC-binding antibody molecules. As seen in Table IV, both free PC and PC-BSA blocked the T cell-mediated help for B cells responding to TNP-T15 and TNP-167, as no anti-TNP positive fragments were detected in the presence of these inhibitors. These results show that the PC binding site on T15 and M167 are targets for recognition by T helper cells.

If a shared determinant on T15 and M167 is the major target for interaction of T helpers, then unconjugated T15 and M167 should be equally effective inhibitors of T15 recognition in vitro. Dilutions of purified T15 and M167 were added to fragment cultures at the time of immunization with TNP-T15. Using 1, 5, and 10 times T15 and M167 in molar excess over the antigen concentration, the expected inhibitions are 50%, 80%, and 90%, respectively. The results shown in Table V indicate that the interaction of PC-primed T cells with TNP-T15 and TNP-167 can be equally and effectively blocked by unconjugated myeloma idiotypes. The failure to achieve close to 90% inhibition can be explained by the partial denaturation of the PC binding site during storage or purification of the myeloma proteins (H. Köhler, unpublished data).

Finally, it was of interest to determine if the idiotypic target determinants for T cell help can be detected on the isolated Ig chains. Thus, isolated light and heavy chains

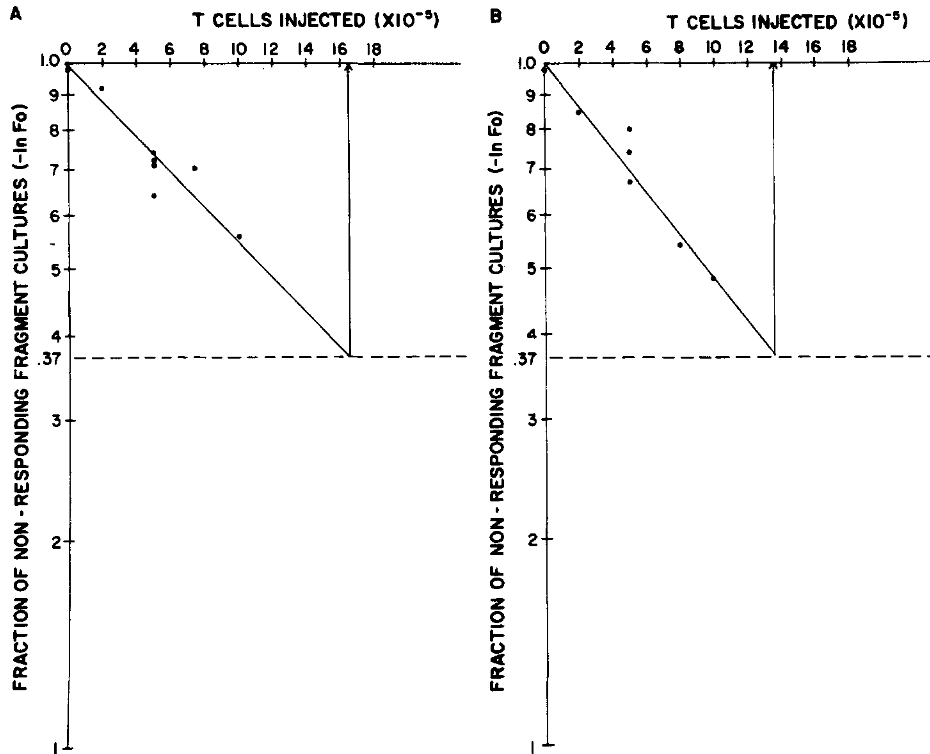

FIG. 1. Number of transferred T helper cells plotted against the fragment of nonresponding fragments. Fragment cultures immunized with (A) TNP-T15 or (B) TNP-M167.

TABLE IV

Inhibition of In Vitro Recognition of TNP-T15 Antigen by PC Inhibitors

T cell donor	Donor treatment*	In vitro antigen‡	In vitro inhibitor§	Number of positive fragments‖
BALB/c	PC-Hy	TNP_7-T15	—	29
BALB/c	PC-Hy	TNP_7-T15	PC	0
BALB/c	PC-Hy	TNP_7-T15	PC-BSA	0

* Donor mice were primed and Ly-2.2-depleted T cells prepared as in Table II. 5 × 10^5 T cells were injected intravenously into athymic *nu/nu* BALB/c recipients.

‡ TNP_7-T15 was used as an in vitro antigen at 10^{-8} M TNP.

§ The hapten, PC, was added to cultures as inhibitor at 10^{-7} M PC. The hapten-carrier conjugate, PC-BSA, was used as an in vitro inhibitor at 10^{-7} M PC. Inhibitors and antigen were removed after 3 d of culture.

‖ Fragments cultured were assayed as in Table III.

of T15 and of M167 were added as inhibitors to fragment cultures responding to TNP-T15. As seen in Table VI, only the free heavy chains of T15 and M167 inhibited the response to TNP-T15. Because the inhibition by heavy chains is not complete, the light chains, though by themselves not inhibiting, might be needed for complete expression of the target determinant. Alternatively, the isolated chains may be partially denatured and cannot fully express their natural conformation.

TABLE V

Inhibition of In Vitro Recognition of TNP-T15 by T15 and M167 Myeloma Proteins

T cell donor	T cell donor treatment*	In vitro antigen‡	In vitro inhibitor§	Inhibitor concentration	Anti-TNP-positive wells‖
				$\times 10^{-9}$ M	
BALB/c	PC-Hy	TNP-T15	—	—	34
BALB/c	PC-Hy	TNP-T15	T15	1.4	31
BALB/c	PC-Hy	TNP-T15	T15	7.0	20
BALB/c	PC-Hy	TNP-T15	T15	14.0	15
BALB/c	PC-Hy	TNP-T15	M167	1.4	39
BALB/c	PC-Hy	TNP-T15	M167	7.0	12
BALB/c	PC-Hy	TNP-T15	M167	14.0	15

* Donor mice were primed with 100 μg Hy in CFA i.p. 4 wk later, they were given 100 μg PC-Hy in IFA i.p. and used as T cell donors 4–8 wk later. T cells were prepared by nylon wool passage of spleen cells, followed by treatment with hybridoma product 3.239.2 (anti-Ly-2.2) and complement. 5×10^6 cells were injected intravenously into athymic, *nu/nu* BALB/c recipients.

‡ TNP_7-T15 was used as the in vitro antigen at 10^{-8} M TNP (1.4×10^{-9} M protein).

§ Purified myeloma proteins, T15 and M167 were used as in vitro inhibitors at 1.4, 7.0, and 14.0×10^{-9} M protein (1, 5, and 10 times the protein concentration of TNP-T15, respectively).

‖ 96 splenic fragments were cultured and assayed for anti-TNP antibody as in Table III.

TABLE VI

Inhibition of In Vitro Recognition of TNP-T15 by T15 and M167 Heavy and Light Chains

T cell donor	Donor treatment*	In vitro antigen‡	In vitro inhibitor§	Anti-TNP-positive wells‖
BALB/c	PC-Hy	TNP-T15	—	40
BALB/c	PC-Hy	TNP-T15	T15 H	27
BALB/c	PC-Hy	TNP-T15	T15 L	35
BALB/c	PC-Hy	TNP-T15	M167 H	24
BALB/c	PC-Hy	TNP-T15	M167 L	41

* Donor mice were primed with 100 μg Hy in CFA i.p. 4 wk later, they were given 100 μg PC-Hy in IFA i.p., and were used as T cell donors 4–8 wk later. T cells were prepared by nylon wool passage of spleen cells followed by treatment with hybridoma product 3.239.2 (anti-Ly-2.2) and complement. 5×10^6 cells were injected intravenously into athymic, *nu/nu* BALB/c recipients.

‡ TNP_7-T15 was used as an in vitro antigen at 10^{-8} M TNP (1.4×10^{-9} M protein).

§ Isolated T15 and M167 heavy (H) and light (L) chains were used as in vitro inhibitors at 1.4×10^{-8} M protein.

‖ 96 splenic fragments were cultured and assayed for anti-TNP antibody as in Table III.

Discussion

The network hypothesis postulates that B and T cells interact via idiotypic specificities of cellular receptors. Idiotypes have been demonstrated on T cells in several systems (15–17), and thus a T cell idiotype repertoire may exist similar to that of the B cell idiotype repertoire. Cellular circuits of T-B cell interactions via idiotypic specificities have been described for suppressor loops, but little information is at hand on similar idiotypic T-B interactions that help or enhance B cell responses. Idiotype-specific T helper cells have been invoked as promoters of the dominant expression of a clone (1, 18). This functional assignment, however, is not supported by the evidence indicating that clonal dominance can be maintained in reconstituted idiotype-defi-

cient animals (2). In addition, idiotype-recognizing T helper cells can presumably activate primed B cells into antibody production in the absence of antigen (19, 20).

Pierce et al. (21) have studied the T helper cells under limiting cell-dose conditions and have obtained evidence for antibody-specific immunoregulation. However, in their anti-TNP response system, no idiotypic specificities were used for marking T helper cells. The system used in this study and described earlier (4, 6) takes advantage of the idiotypically defined anti-PC antibodies in the BALB/c mouse, which are used as carriers for an anti-TNP B cell response. This system makes it possible to probe the specificity of T helper cells recognizing defined idiotypic carrier determinants.

Helper T cells for idiotype carriers can be induced by anti-idiotypic antibody or by priming with antigen (4, 6). Because priming with antigen resembles a common situation in nature where exposure to the same antigen occurs repeatedly, this antigen-induced helper cell was studied further. In particular, we were interested in the question of whether different idiotype carriers are recognized by the same or different T cell clones.

One approach to determining the number of clones is to measure their precursor frequencies, as different clones would most likely have different precursor frequencies. Accordingly, we determined the frequencies of helper cells, induced by PC-Hy priming, that recognize TNP-T15 and TNP-167. We also established the homing efficiency of T helper cells after transfer to athymic mice. Because the homing efficiency appears to be several-fold higher for T cells than for B cells (22), the calculated frequencies for T15- and M167-recognizing T cells were rather low. The frequencies of T helper cells responding to TNP-T15 and TNP-M167 are identical, within the error of method. Thus, this finding supports the notion that T15 and M167 are recognized by the same population of T cells, although identical frequencies give no formal proof for this conclusion.

Further evidence for one population of T helpers for T15 and M167 would emerge if a common idiotypic determinant on T15 and M167 is the target for the T help. To localize the epitope recognized by the T helper cells, the interaction of the idiotype carrier and helper cells was inhibited by several specific compounds. First, if the common epitope of T15 and M167 is the binding site for PC, free hapten or PC-antigen should inhibit the T helper function. Because PC does block the help for TNP-T15 and TNP-M167, we can conclude that the PC binding site is at least a major determinant recognized by T helper cells on T15 and M167.

Next, we conducted experiments to determine if unhaptenated homologous and heterologous proteins could equally inhibit the recognition of TNP-T15 in vitro. We show in Table V that a heterologous protein (M167) inhibited T cell recognition of TNP-T15 as well as, if not better than, the homologous protein (T15). Therefore, we conclude that a common structure, recognized by T helper cells, must be shared between the PC-binding myeloma proteins.

If a shared determinant on T15 and M167 is the target for T help, then this common determinant would more likely be determined by the heavy chain than by the light chain, as the heavy chains in T15 and M167 are almost identical, whereas the light chains are very different (7). Using isolated heavy and light chains from T15 and M167 as inhibitors of T help in vitro, we observed that only the heavy chains of both myeloma proteins had inhibitory activities. As the inhibition by heavy chains is incomplete, the antigenic determinant may require an intact secondary structure,

which can be altered by reduction and alkylation. In addition, the epitopic PC site on T15 and 167 could require the presence of the light chain for full expression of its T cell target determinant.

The function of the described idiotope-recognizing T helper cell in a "normal" immune response might be several-fold. Idiotype-specific T helper cells might be needed for clonal dominance (1, 18) and are involved in the cyclical appearance of idiotype and anti-idiotype during an immune response (23). The fact that these idiotype-specific helper cells are induced by priming with antigen implies strongly that this cell has a role in the regulation of the immune response against the same antigen. It would be economical for an antigen-induced regulatory mechanism to affect the entire clonal response spectrum, which includes several different idiotypic specificities. Bona et al. (24) have postulated that although antibodies may have a number of individual V region antigenic determinants (idiotopes), only one or a few of these are involved in regulatory activities. The regulatory idiotope should be conserved throughout antibody diversification to provide for continuity in immune regulation. Thus, the shared determinant exhibited by T15 and M167 may be such a regulatory idiotope.

The fact that the regulatory idiotypic determinant is found on the heavy chain may be significant with respect to the nature of T cell receptors and the cellular induction loop for the idiotype-recognizing helper T cell. We have evidence (M. McNamara, K. Gleason, and H. Köhler, unpublished data) that the generation of idiotype-recognizing T helpers occurs via a T-T cell interaction scheme. Thus, the recognition and interaction sites in such an antigen-idiotype T cell loop are T cell receptors and T cell factors known to express V_H gene-encoded determinants (25) that do not possess light chain related structures. Therefore, it is quite likely that the T helper cell circuit proposed here parallels the T suppressor cell circuitries described in other systems (26, 27).

Summary

Priming of BALB/c mice with phosphorylcholine-hemocyanin (PC-Hy) induces T helper cells that are detected in splenic fragment cultures responding to immunization with trinitrophenylated PC-binding myeloma proteins, TEPC 15 (TNP-T15) and MOPC 167 (TNP-M167). Trinitrophenylation did not alter the binding site, idiotype, or isotype of the antibodies as demonstrated by binding studies. To assay idiotype-recognizing helper cells, Ly-2.2-depleted T cells from PC-Hy-primed donor mice were transferred to syngeneic athymic mice. Splenic anti-trinitrophenol fragment cultures were prepared from the nude recipients, and the response to TNP-T15 and TNP-M167 was measured by enzyme-linked immunosorbent assay. The number of responding fragments is dependent on the number of transferred primed T cells. The homing efficiency of ^{51}Cr-labeled helper cells into the spleen of nude recipients was determined. The frequencies of T helper cells taken from PC-Hy-primed donors required for a B cell response to TNP-T15 or TNP-M167 were indistinguishable. The fine specificity of the anti-PC idiotype-recognizing T helper cells was studied by adding hapten (PC) or unconjugated myeloma proteins to fragment cultures as inhibitors at the time of immunization. PC and PC-bovine serum albumin, as well as T15 and M167, inhibited the helper function in vitro. Furthermore, free heavy chains of T15 and M167 partially inhibited T help, but free light chains of both idiotypes had no effect. These

findings collectively show that T helper cells, induced by priming with antigen, recognize a shared idiotypic determinant on T15 and M167 that is part of the PC binding site. The heavy chains of T15 and M167 appears to be the major structural component of this determinant. Evidently, T helper cells can recognize a shared determinant that is present on idiotypically different myeloma proteins. This determinant appears to be conserved throughout evolutionary and somatic mutations. The role of this shared, binding site-related idiotypic determinant as a regulatory idiotype in T-B cell interaction is discussed.

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