BALB/c T CELLS HAVE THE POTENTIAL TO RECOGNIZE THE TEPC 15 PROTOTYPE ANTIBODY AND ITS SOMATIC VARIANTS*

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A number of years ago it was proposed by Jerne (1) that the immune response is regulated through an extended network of interactions based on idiotype-anti-idiotype recognition. In subsequent years, it has been demonstrated (2, 3) that B and T cells share serologically defined idiotypes. Furthermore, both B cells and T cells have been shown to express receptors for idiotype, and there is evidence to suggest that these cells may serve some regulatory function (4–12). The B cell repertoire can be divided into a fixed set of germ line-encoded idiotopes and a variable number of variant idiotopes. Recent structural and genetic analyses of anti-phosphorylcholine myeloma and hybridoma antibodies (13, 14) provided strong evidence that somatic mutational events give rise to variant antibodies of the germ line prototype proteins. Furthermore, the immunoglobulin G (IgG) class anti-phosphorylcholine hybridomas exhibit a strikingly greater diversity than the IgM counterparts. This indicates that somatic variants arise either during a maturation of the B cell repertoire or during an immune response, which in the latter case would be dependent on antigenic stimulation.

As variants arise in the B cell idiotype repertoire, similar somatic events could also take place in the T cell idiotype repertoire. An idiotype network between T cells and B cells based on an equal tendency to mutate for B cell and T cell germ line idiotypes would be highly prone to instability and disregulation. Introducing restriction into T-B collaboration would make network interactions more stable. This could be achieved by assuming that T cells recognize only a limited number of B cell idiotopes and that these idiotopes do not change as variants develop. By this reasoning, idiotopes that remain stable are germ line encoded, and changing idiotopes in variants become unique and individual idiotypes. The concept of auto-anti-idiotype is central for the proposed model of T-B interaction. Recently, it has been demonstrated that anti-idiotypic cells can be induced after immunization with idiotype or with conventional antigens (15–23). These auto-anti-idiotypic responses may represent initial events in the generation of regulatory networks.

Our experiments were conducted to define the specificity of T lymphocytes induced by immunization with a hapten-carrier conjugate for the idiotype of hapten-specific

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antibody. The system studied was the response of BALB/c mice to phosphorylcholine (PC),[1] which is dominated by a single B cell clonotype defined by antisera to the TEPC 15 (T15) myeloma protein (24, 25). Recent studies have shown (26–30) that T cells were involved in the regulation of this predominant idiotype. The splenic fragment culture system was used to determine whether PC-*Limulus polyphemus* hemocyanin (PC-Hy)-immunized BALB/c mice can provide T cell help for 2,4,6 trinitrophenol (TNP)-specific B cells to a series of TNP-conjugated PC-binding myeloma and hybridoma antibodies. Serological analysis of the PC-specific antibodies used in this study indicated that four were positive for the T15 idiotype and the remainder were negative.

This study demonstrates that PC-Hy immunization of BALB/c mice induces T cell recognition of both T15-positive and T15-negative PC-binding antibodies representative of both the prototype germ line-encoded variable region of the heavy chain (V_H) sequence and somatic variants of that sequence. Immunization of BALB/c mice with the T15 myeloma protein also induces recognition of the somatic variants, therefore, at least some of these T cells recognize common determinants between members of this series of PC-binding proteins. It is concluded that PC-Hy immunization of BALB/c mice induces a functional T cell population that recognizes both T15 idiotype-positive and idiotype-negative PC-binding antibodies, suggesting that this T cell may be specific for determinants encoded in the germ line T15 V_H sequence.

Materials and Methods

Animals and Immunizations. BALB/c mice were purchased from both Carworth Farms, Wilmington, Mass., and Cumberland View Farms, Clinton, Ind. Animals to be used as PC-Hy-immunized recipients received two intraperitoneal injections, the first with 0.1 mg Hy in complete Freund's adjuvant (CFA) 8 wk before use, and the second immunization with 0.1 mg PC-Hy in saline 4 wk before use. Mice to be used as T15-immunized recipients received two injections of 0.1 mg i.p., the first in CFA, and the second in saline, 16 wk before use.

Antigens and Immunoadsorbants. PC_8-Hy (8 mol PC/100,000 g Hy) was prepared by reacting *p*-diazophenyl phosphorylcholine (31, 32) with *Limulus polyphemus* hemocyanin (Hy) (Worthington Biochemical Corp., Freehold, N. J.) by standard methods (33). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) was purchased from the Eastman Kodak Co., Rochester, N. Y., bovine serum albumin (BSA) from the Sigma Chemical Co., St. Louis, Mo., and TNP-BSA was prepared by standard methods (33). The PC-binding hybridoma and myeloma antibodies used as in vitro antigens were affinity purified on PC-Sepharose columns and are described in detail elsewhere (13, 34). The myeloma proteins T15, MOPC 167 (M167), and MOPC 460 (M460) were the kind gifts of Dr. Michael Potter, National Cancer Institute, Bethesda, Md. M460, an α, κ 2,4-dinitrophenol-binding antibody, was purified by affinity chromatography on dinitrophenyl-lysine-coupled Sepharose, as described elsewhere (32). Purified antibodies were trinitrophenylated (~15 mol TNP/150,000 g), as previously described (31, 35) and were separated from free hapten by chromatography on Sephadex G25. The PC-binding proteins were trinitrophenylated in the presence of 10^{-2} M PC chloride (Sigma Chemical Co.), and similarly, M460 was trinitrophenylated in the presence of 10^{-1} M dinitrophenyl-glycine (Sigma Chemical Co.) to protect the complementarity-determining sites from chemical modification.

Fragment Cultures and Radioimmunoassay. The methods for obtaining monoclonal B cell antibody responses in in vitro splenic fragment cultures and for carrying out limiting dilutions

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; Hy, *Limulus polyphemus* hemocyanin; M460, MOPC 460; M167, MOPC 167; PC, phosphorylcholine; T15, TEPC 15; TNBS, 2,4,6-trinitrobenzene-sulfonic acid; TNP, 2,4,6-trinitrophenyl; V_H, variable region of the heavy chain; V_L, variable region of the light chain.

of helper T cells in fragment cultures have been described (35, 36). Fragment cultures were stimulated in vitro with TNP-antibodies at concentrations between 10^{-6} and 10^{-8} M TNP. Culture fluids were assayed for the presence of TNP-specific antibody between 9 and 13 d after initial culturing, using a solid-phase radioimmunoassay and rabbit anti-mouse $F(ab')_2$ antibody as a detecting reagent (37, 38). Alternatively, antibody was detected in culture fluids using the ELISA enzyme assay system, described previously (39).

Nylon wool-passaged cells were tested for sensitivity to anti-Thy-1.2 and complement killing and were assayed for the presence of surface Ig-positive cells. Anti-Thy-1.2 antibody from the hybridoma line AT.83A was the kind gift of Dr. Frank Fitch, University of Chicago. The number of surface Ig-positive cells was determined by fluorescent staining with fluorescein-conjugated goat anti-mouse Ig, a kind gift of Dr. Dan Levitt, University of Chicago.

Results

PC-Hy Immunization Induces T15-specific T cells. Experiments were conducted to determine whether immunization of BALB/c mice with the antigen PC induces T cells that recognize the T15 myeloma protein. This particular idiotype, as defined by A/He anti-T15 antibodies, is expressed on >80% of PC-specific BALB/c B cell clones (24, 25) and represents the predominantly secreted antibody in the anti-PC response. It has been demonstrated (27, 30) that T cells with specificity for T15 exist in the BALB/c repertoire and can be induced through perturbation of the network by neonatal suppression of the T15 idiotype or low dose anti-T15 immunization of adult mice. The T15-specific T helper cells promote TNP-specific B cell responses to the in vitro antigen TNP-T15 (30). To directly determine whether immunization with PC induces a T cell population specific for T15 antibody, T cells from PC-Hy-immunized recipients were analyzed for their ability to provide help for TNP-specific B cell responses to the in vitro antigen TNP-T15, using a recent modification of the splenic fragment culture system (36). Graded numbers of nylon wool-passaged donor cells from PC-Hy-immunized, Hy-immunized, or nonimmune BALB/c mice were adoptively transferred to BALB/c nu/nu mice. Fragment cultures were prepared from recipient nude mouse spleens, and stimulated in vitro with TNP-T15. Culture supernatants were then assayed for the presence of TNP-specific antibody. As shown in Table I, spleen cultures prepared from recipients that received PC-Hy-immunized donor cells responded to TNP-T15 by synthesizing TNP-specific antibody. The TNP-specific B cell response of the recipient is dependent on the transfer of donor cells, and the number of responding fragments increases with the numbers of donor cells when between 3×10^5 and 6×10^5 cells are transferred. There were no detectable responses when donor cells were obtained from Hy-immunized or nonimmunized recipients. Thus, PC-Hy immunization appears to induce a population of T cells that has the potential to recognize the T15 antibody.

PC-Hy Immunization Induces Help Specific for PC-binding Antibodies That Are Both T15 Positive and Negative. To further characterize the PC-Hy-immunized T cell population with respect to its ability to recognize BALB/c PC-binding antibodies other than the T15 antibody, a series of trinitrophenylated PC-binding myeloma and hybridoma antibodies were used as in vitro antigens. These proteins are listed in Table II along with the previously published (13, 40) light chain variable region (V_L) and V_H amino terminal sequences, the serologically defined idiotype, heavy chain isotype, and light chain group of each. The ability of T cells to recognize these proteins is of interest because both T15-positive and T15-negative antibodies are included in this group. The T15 V_H chain is found associated with three distinct groups of light chains; the

TABLE I

T Cells from PC-Hy-primed Mice Provide Help in a Response to TNP-T15

T cell donor	Donor treatment*	Number of donor cells injected $\times 10^5$‡	In vitro antigen§	Percent positive cultures producing TNP-specific antibody‖
BALB/c	Hy-primed	6.0	TNP-T15	3
		9.0	TNP-T15	2
BALB/c	PC-Hy-primed	0.0	TNP-T15	<1
		3.0	TNP-T15	13
		6.0	TNP-T15	22
		9.0	TNP-T15	20

* Mice to be used as Hy-primed donors received 200 μg Hy in CFA 6 wk before use. Mice to be used as PC-Hy-primed donors received 200 μg Hy in CFA 8 wk before use and 100 μg PC-Hy in incomplete Freund's adjuvant or saline 3–4 wk before use.

‡ Nylon wool-passaged T cells were injected into BALB/c nu/nu mice. More than 90% of the cells purified by this method were demonstrated to be anti-Thy-1.2 plus complement sensitive, and no surface Ig-positive cells were detected.

§ Splenic fragment cultures were stimulated in vitro with TNP_7-T15 at 5×10^{-7} to 5×10^{-8} M TNP.

‖ Using a radioimmunoassay, 288 cultures were assayed for the presence of anti-TNP antibodies at days 10 and 13.

TABLE II

*The Amino Acid Sequence, Isotype, and Idiotype of Myeloma and Hybridoma Antibodies Used as In Vitro Antigens**

	V_L subgroup	V_L	T15 idiotype
		10 20 30	
T15	T15	D I V M T Q S P T F L A V T A S K K V T I S C T A S E S L Y S S K H K V H Y	+
HPCM 2	T15	– –	+
HPCG 8	T15	– – – – – – – – – – – – – – – – E – – – – – – – – – – – – – – – – – – –	+
HPCG 11	T15	– –	+
HPCG 15	M603	– – – – S – – – S S – – – S – G E – – – M – – K S – Q – – L N – R T R K N –	–
HPCG 9	M167	– – – I – – D E L S N P – – S G E S – S – – – R S – K – – L Y K S G – T Y L	–
HPCG 13	M167	– – – I – – N E L S N P – – S G E S – S – – – R S – K – – L Y K D G – T Y L	–
M167	M167	– – – I – – D E L S N P – – S G E S – S – – – R S – K – – L Y K D G – T Y L	–
	Isotype	V_H	
		10 20 30	
T15	IgA	E V K L V E S G G G L V Q P G G S L R L S C A T S G F T F S D F Y M E W	+
HPCM 2	IgM	– –	+
HPCG 8	IgG_3	– –	+
HPCG 11	IgG_3	– I – – – – – – – – – – –	+
HPCG 15	IgG_1	– E I – – – – S T – Y – – S –	–
HPCG 9	IgG_3	– –	–
HPCG 13	IgG_1	– L – – – – – – – – – – –	–
M167	IgA	– – – V –	–

* From Gearhart et al. (14).

T15-V_L, M603-V_L, and M167-V_L groups. The T15-positive antibodies chosen for this study all express the T15 light chain. Although the T15 light chain is necessary for the expression of the T15 idiotype, it is not sufficient. Although it is not apparent from this table, not all antibodies with the T15 V_H and T15 V_L are serologically T15

TABLE III

Helper Potential of PC-Hy- or T15-immunized Recipients for TNP-coupled PC-binding Antibodies

	Recipient priming‡					
	PC-Hy		Hy		T15	
In vitro antigens*	Total number of donor cells analyzed × 10^{-6}	Frequency of TNP-specific foci‖	Total number of donor cells analyzed × 10^{-6}	Frequency of TNP-specific foci	Total number of donor cells analyzed × 10^{-6}	Frequency of TNP-specific foci
TNP-T15	115	0.67	14	<0.07§	8	0.67
TNP-M460	12	0.07	ND¶	ND	ND	ND
TNP-M167	31	0.77	14	<0.07	ND	ND
TNP-HPCG 8	26	0.66	9	<0.11	ND	ND
TNP-HPCG 9	26	0.69	9	<0.11	8	0.47
TNP-HPCM 2	26	0.43	9	<0.11	8	0.54
TNP-HPCG 11	26	0.51	9	<0.11	8	0.60
TNP-HPCG 13	26	0.66	9	<0.11	ND	ND
TNP-HPCG 15	26	0.70	9	<0.11	8	0.73

* In vitro antigens were used at a concentration of 5×10^{-7} M TNP.

‡ Recipients received between 4×10^6 and 8×10^6 donor spleen cells. Recipient fragment cultures were pooled or cultured individually with the antigen indicated.

§ When no positive cultures were detected the frequency is indicated as <1 per the total number of cells analyzed.

‖ Frequency represents the number of TNP-specific foci per 10^6 cells transferred. When nonimmune recipients were examined, the frequency of TNP-specific foci was <0.04 for a total of 30×10^6 cells analyzed, for all antigens tested.

¶ Not done.

positive because antibodies having several amino acid substitutions in V_H are idiotype negative (13).

Analysis of the amino terminal V_H sequences indicates that there are more V_H regions participating in the response to PC than can be directly encoded in the germ line sequence (13). The myeloma protein T15 and hybridoma antibody HPCM 2 are representative of the germ line-encoded V_H sequence, whereas HPCG 9, 11, 8, 13, and 15 may represent V_H that have somatically diversified from this prototype sequence.

To assess the potential of PC-Hy immunization to induce T cells that recognize the PC-binding proteins listed in Table II, the splenic fragment culture system was used to determine whether PC-Hy-immunized irradiated mice can promote TNP-specific nonimmune donor B cell antibody responses to the trinitrophenylated PC-binding antibodies. It has been previously demonstrated (36, 37, 41)[2] that in this experimental system, donor B cell responses are absolutely dependent on the appropriate carrier-priming of the recipient and thus the splenic fragment culture system has been successfully used to study the specificity of helper T cell responses. Nonimmune B cells were transferred to PC-Hy-immunized BALB/c recipients, and fragment cultures were challenged in vitro with the trinitrophenylated PC-binding proteins. The results of these experiments are shown in Table III. PC-Hy-immunized recipients are able to promote TNP-specific B cell responses to the in vitro antigen TNP-T15 but not to the antigen TNP-M460. Nonimmune or Hy-immunized recipients did not promote B cell

[2] Speck, N. A., P. H. Maurer, and S. K. Pierce. The functional T cell repertoire specific for L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). J. Immunol. In press.

responses to TNP-T15. As shown, PC-Hy immunization also appears to induce the recognition of all PC-binding hybridoma and myeloma proteins tested. The ability of PC-Hy immunized recipients to promote B cell responses to these antigens appears to be independent of the idiotype or the heavy chain isotype of the antibody. The only common feature of these antibodies is their ability to bind PC. The observed frequencies of the B cell responses are somewhat variable and might be dependent on the relative abundance of T cells that recognize these proteins, or alternatively, on the relative immunogenicity of the trinitrophenylated antibodies as prepared.

The promotion of B cell responses by PC-Hy-immunized recipients to the series of trinitrophenylated PC-binding myeloma and hybridoma antibodies may be accounted for in one of two ways. As shown in Table II, the V_H of the antibodies tested have extensive amino acid homology. Amino acid sequences that are common to the T15 prototype and its variants may be recognized by individual T cell clones. Alternatively, distinct T cell clones may recognize the amino acid sequences that are unique to the individual proteins. These alternatives are not mutually exclusive, and both T cell recognitions might exist simultaneously in the population. To test the first possibility, experiments were conducted to determine whether immunization with T15 induced helper T cells that recognized four of the PC-binding proteins listed in Table II in addition to T15. The results of these studies, also summarized in Table III, demonstrate that T15 immunization induces recognition of T15-positive and T15-negative PC-binding proteins, which were also recognized by recipient T cells after PC-Hy immunization. Thus, a population of T15-specific T cells exists that recognizes the germ line T15 prototype and variants that have diversified from the T15 prototype amino acid sequence.

Discussion

Immunization of BALB/c mice with PC-Hy induces T cell recognition of the T15 antibody as well as a series of somatic variants of the T15 prototype sequence. This can be interpreted as a T cell response to PC-specific antibody that is induced as a result of PC-Hy immunization because T15-specific T cells are not found in Hy-primed or nonimmune mice. Several variants of the prototype T15 antibody that are recognized by PC-Hy-induced T cells were negative for the serologically defined T15 idiotype. Thus, the initial T cell response to antibody synthesized in response to PC-Hy immunization in BALB/c mice, which is predominantly of the T15 idiotype, might not be restricted to recognizing what has been serologically defined as idiotype.

It appears that the helper T cell population in the BALB/c mouse has the potential to recognize common antigenic determinants shared by the T15 myeloma antibody and the other antibodies tested. This is indicated by the fact that immunization with the T15 myeloma protein itself induces the recognition of the entire series of PC-binding proteins analyzed. It remains to be determined whether PC-Hy immunization also induces T cells that recognize unique amino acid determinants on the individual proteins that have diversified from the prototype sequence. If T cells are able to recognize a common antigenic determinant shared by the prototype T15 sequence as well as somatic variants of this sequence, it would suggest that a common "regulatory idiotope" is expressed by this series of anti-PC antibodies. Both amino terminal sequence analysis of the V_H of a large number of PC-binding hybridoma and myeloma proteins and nucleotide sequencing of the T15 V_H germ line gene suggest that V_H

that have diversified from the T15 germ line sequence accomplish this via somatic mutation (13, 14). Common regulatory idiotopes expressed by these variants have remained unchanged and thus might be encoded in the germ line. These idiotopes may not necessarily be related to serologically defined idiotypes. The common idiotope expressed by a group of antibodies might induce idiotope-specific T cells that are able to regulate B cell responses to a particular antigen through the recognition of this common idiotopic determinant.

Bona et al. (16) have recently proposed that anti-idiotypic antibodies that develop in autologous systems might be elicited against only a limited number of idiotopic determinants expressed by the antigen-specific antibodies in the initial response to antigen. Thus, a population of antigen-specific cells, although expressing a variety of idiotypes contributing to a heterogeneous response, might express a limited number of regulatory idiotopes that function to elicit auto-anti-idiotope-related responses. The results presented in this report demonstrate that helper T cells recognize common determinants shared by the T15 prototype sequence and its somatic variants. A logical extension of this hypothesis is the prediction that these regulatory idiotopes are encoded in the germ line.

Amino terminal sequence analysis of the PC-binding myeloma and hybridoma proteins indicated that there is more variability in the IgG antibodies than in their IgM counterparts (13). It was suggested that the selection of V_H variants in the maturing secondary B cell repertoire might occur as a result of idiotype-specific regulation. Thus, B cells with somatically diversified sequences might escape an idiotype-specific network generated against the prototype, or germ line sequence. However, our results demonstrate that the PC-Hy-immunized T cell population maintains the ability to recognize these variant sequences. This recognition is measured as the ability to promote TNP-specific B cell responses to a trinitrophenylated antibody and does not address the possible regulatory function of this T cell population. Therefore, there is no direct evidence that T cells that recognize the variant antibodies function in the humoral immune response to PC-Hy. Nevertheless, T cells that recognize these antibodies can be observed only after PC-Hy immunization. Our data does not exclude the possibility that T cells can recognize and functionally interact with unique idiotopic determinants on variant idiotypes that would not be encoded by germ line genes. Experiments are in progress to probe the interaction of T cells with variant derived idiotopes. The panel of different defined T15 variants (13) used as carrier targets for T cell help or cellular adsorbants is crucial for these experiments. Furthermore, hybridoma anti-T15 antibodies directed against different idiotopes on the T15 molecule will become instrumental in these studies.[3]

Summary

Immunization of BALB/c mice with phosphorylcholine-*Limulus polyphemus* hemocyanin (PC-Hy) induces a population of T cells that recognize the predominant PC-binding antibody, TEPC15 (T15). The splenic fragment culture system was used to examine the specificity of these T cells for a series of PC-binding myeloma and hybridoma antibodies representing the prototype variable region of the heavy chain (V_H)T15 sequence as well as somatic variants of the T15 germ line-encoded sequence.

[3] Wittner, M. K., M. A. Bach, and H. Kohler. Immune response to phosphorylcholine. IX. Characterization of hybridoma anti-TEPC 15 antibodies. Manuscript submitted for publication.

Included in this group of PC-binding proteins were both T15-positive and T15-negative antibodies, as defined by anti-idiotypic antibody. T cell help was identified by the ability to promote TNP-specific B cell responses to trinitrophenylated PC-binding proteins. It was found that T cells generated by immunization with PC-Hy recognize both antibodies with the T15 prototype sequence and the putitive somatic variants of this sequence. A population of these T cells appear to recognize common determinants shared by these proteins because immunization with T15 itself also induces the recognition of the somatic variants. This suggests that idiotopes encoded in the T15 germ line gene expressed by the T15 prototype idiotype and the somatic variants can function as targets for T cell recognition and are thus regulatory idiotopes.

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