GENERATION OF IDIOTYPE-SPECIFIC T CELL HELP THROUGH NETWORK PERTURBATION*

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Since the first use of anti-idiotypic antibodies as modulators in immune responses (1, 2), the concept of idiotype-specific regulation has received numerous experimental support. Idiotype-specific interactions are not restricted to B cells, as different T cell populations can be characterized by idiotypic or anti-idiotypic reagents (3–5). From these studies, it appears that among the cells that carry complementary idiotypic membrane receptors, there exists, in accordance with Jerne's network theory (6), a delicate balance of idiotypic and anti-idiotypic cellular activities in the immune system. Presumably, this homeostasis can be easily disturbed by introduction of antigen, idiotype, or anti-idiotype. The immune system is a complex network of interacting cells, soluble factors, and antibodies. The vast spectrum of possible connections in this network, based on complementary interacting structures, necessarily dictates that perturbations of the resting state lead to a rippling effect throughout many connecting circuits. The elucidation of the activation pathways in the idiotype network and the characterization of involved cell types is one of the prime interests in experimental immunology.

Antigen, of course, stimulates production of antibody, but the stimulation of accessory cells and subsequent activation of regulatory cells is not fully understood. An early indication that antibody and anti-antibody are produced during the immune response to phosphorylcholine (PC)[1] was provided by Kluskens and Köhler (7). Recent reports show cycling in the idiotypic response to trinitrophenyl (TNP) (8) and PC (9). Presumably, interacting sets of idiotypically related cells regulate each other (10). Exposure to idiotype in vivo either naturally (11) or experimentally as free protein (12) or coupled to autologous spleen cells (13) may generate idiotype-specific or idiotype-bearing suppressor cells. Administration of anti-T15 idiotype to BALB/c mice at birth induces chronic suppression of the T15 idiotype and responsiveness to PC (14). During the recovery of responsiveness, T15 idiotype-negative antibodies predominate in the response to PC (15) and mice so treated may be used to produce

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[1] *Abbreviations used in this paper:* AHS, agammaglobulin horse serum; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DMEM, Dulbecco's modified Eagle medium; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; Hy, *Limulus polyphemus* hemocyanin; IBC, idiotype-binding capacity; M167, MOPC-167; M460, MOPC-460; PBS, phosphate buffered saline; PC, phosphorylcholine; RIA, radioimmunoassay; T15, TEPC-T15; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNP, 2,4,6-trinitrophenyl.

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autologous anti-idiotype antibody by immunization with T15 idiotype (16, 17). Anti-idiotype antibody given to adult mice has different effects, depending on the dose. A high dose induces short-term suppression of idiotype production (1, 2, 18) and may induce idiotype-specific suppressor cells (18, 19). Conversely, a low dose can stimulate idiotype-bearing and -producing cells (20, 21).

In the present work, we studied the perturbation of idiotype networks at the level of clonally defined interactions by utilizing the T15 idiotype in BALB/c mice. The immune response to the hapten PC in BALB/c mice is dominated by antibody of the T15 idiotype (22), which is defined by the antibody product of the myeloma cell line, TEPC-15 (23). Both B and T cells bear receptors identifiable by specific anti-T15 antisera (3). The idiotype homeostasis between B and T cells can only be understood when cell-to-cell interactions are analyzed at the single cell level. Limiting-dilution analysis provides the means to study the interactions of individual cells. The splenic-foci culture technique (24), originally developed for the examination of individual B cells, has recently been adapted for the study of individual T cells (25).

In the present study, we have used the splenic-fragment culture technique to analyze the interaction of an idiotype-defined T cell with hapten-specific B cells under limiting-T cell-dose conditions for the first time. We demonstrate, with this approach, that two different idiotype-specific manipulations of the T cell donor generate idiotype-recognizing helper cells.

Materials and Methods

Mice. A/He females, 6–8 wks old, were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c females and males, 6–8 wk old, were obtained from Cumberland View Farms, Clinton, Tenn. BALB/c neonates were born in our breeding colony at the La Rabida-University of Chicago Institute, Chicago, Ill. Athymic, nu/nu BALB/c were obtained from Harlan Sprague-Dawley, Madison, Wis. and from our own breeding colony.

Myeloma Proteins. The plasma cell tumors, TEPC-15, MOPC-167 and MOPC-460 were obtained from Dr. M. Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md. and maintained by serial passage as ascites in BALB/c mice. The myeloma proteins from TEPC-15 (T15), MOPC-167 (M167), and MOPC-460 (M460) were purified from the ascitic fluid by antigen affinity-column purification (26).

Antigens. Limulus polyphemus hemocyanin (Hy) was purchased from the Millipore Corp., Bedford, Mass. Trinitrophenylated hemocyanin (TNP-Hy) was prepared by the reaction of 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma Chemical Co., St. Louis, Mo.) and Hy by the method of Klinman (24). TNP-T15, TNP-M167, and TNP-M460 were prepared by the reaction of TNBS with purified T15, M167, or M460 myeloma proteins, which had their hapten-binding sites blocked by 0.1 M phosphorylcholine chloride (Sigma Chemical Co.) or 0.1 M dinitrophenyl-glycine (Sigma Chemical Co.). The hapten was removed after trinitrophenylation by dialysis against borate-buffered saline. TNP-conjugated bovine serum albumin (TNP-BSA) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) was prepared by reaction of BSA with TNBS.

Preparation of Anti-T15 Antiserum. Homologous anti-T15 antiserum was raised in A/He female mice according to the method of Potter and Lieberman (23). Allotype-specific antibodies were removed by adsorption to Sepharose-immobilized BALB/c immunoglobulins, which had been depleted of T15 antibodies by prior passage over a PC-Sepharose column. The antiserum, after adsorption, was shown to be specific for T15 by radioimmunoassay (RIA). The idiotype-binding capacity (IBC) was determined by the precipitation of ^{125}I-T15 myeloma protein by serial dilutions of the adsorbed antiserum. The IBC was determined to be 140 μg/ml T15-specific antiserum.

Neonatal Suppression of the T15 Idiotype of Donor Mice. Neonatal BALB/c mice, <3 d old, were given A/He anti-T15 antiserum, equivalent to 5 μg IBC, i.p. At 8 wk of age, the mice were eye-

bled and their sera individually tested for T15 idiotype by RIA. None of the neonatally T15 suppressed mice had >5 μg T15/ml serum, which is ~10% of the normal level in adult BALB/c mice.

Priming of Mice. Adult BALB/c mice, 6–8 wk of age, were given 0.1 μg (IBC) A/He anti-T15 antiserum i.v.; 6–10 wk later, the mice were eye-bled and their sera individually tested for T15 idiotype by RIA. The serum levels of T15 were within the normal range (50–100 μg/ml) for BALB/c mice. Mice to be used as Hy-primed donors or recipients in the splenic-fragment culture system received 200 μg Hy in complete Freund's adjuvant (CFA) i.p. 6 wk before use.

Anti-Thy-1.2 Treatment of Donor Spleen Cells. Monoclonal heterologous anti-Thy-1.2 supernatant antibody from the hybridoma cell line AT83A.3, and rabbit complement were gifts of Dr. F. Fitch, University of Chicago. 1.0×10^6 nylon wool-passed cells were suspended in 1.0 ml Dulbecco's modified Eagle medium (DMEM) (Grand Island Biological Co., Grand Island, N. Y.) with 10% aggammaglobulin horse serum (AHS) (Grand Island Biological Co.). 0.025 ml AT83A.3 supernate was added and the suspension was incubated at 0°C for 30 min. The cells were washed twice, resuspended in 0.8 ml DMEM-10% AHS, and incubated with 0.2 ml rabbit complement at 37°C for 45 min. Viability was determined by trypan blue dye exclusion. 90% or more of the nylon wool-passed cells were sensitive to the anti-Thy-1.2 supernate and complement.

Antibody Panning of Donor Spleen Cells. 100- × 15-mm polystyrene bacteriological petri dishes (Fisher Scientific Co., Pittsburgh, Pa.) were coated with purified myeloma proteins by the method of Wysocki and Sato (27). 250 μg purified T15 or M460 myeloma protein in 10 ml 0.1 M Tris, pH 8.6, was applied to plastic dishes and incubated at 37°C for 60 min. The fluid was decanted and saved and the dish was washed with phosphate-buffered saline (PBS). 1.5×10^7 spleen cells were suspended in duplicate 8-ml aliquots of Hanks' balanced salt solution (HBSS) (Grand Island Biological Co.) with 10% fetal calf serum (FCS) (Grand Island Biological Co.). The suspensions were poured onto the dishes and incubated at 37°C for 45 min. Nonadherent cells were collected, and viable cells counted by trypan blue dye exclusion.

Cell Transfers and Splenic-Fragment Cultures. Two variations of the original splenic-fragment culture system were employed to study the interactions of T and B cell populations. The general protocol is shown in Fig. 1.

The first method we used to analyze individual T helper cells has been previously described (25). Briefly, T cells prepared by passage of spleen cells over nylon wool columns (28). Graded numbers of purified T cells were transferred to nu/nu BALB/c mice. 48 h later, the spleens of recipient mice were removed asceptically and diced into 1-mm cubes. The cubes were cultured separately in sterile 96-well microtiter plates (Costar Data Packaging, Cambridge, Mass.) in DMEM-10% AHS, which contained the antigen. The plates were maintained in humidified culture boxes at 37°C in an 8% CO_2-92% O_2 atmosphere. After 3–4 d of culture, the supernates were removed and the cultures replenished with fresh medium without antigen. Subsequently, every 3–4 d, the supernates were collected and assayed for antibody by radioimmunoassay throughout 13 d of culture. The antigens, TNP_5-T15, TNP_9-M167, and TNP_6-M460, were used in vitro at a concentration of 10^{-8} M TNP.

The second variation of the splenic-fragment culture system was employed to study helper T cell populations in nonlimiting numbers in collaboration with their own B cell populations. Briefly, spleen cell suspensions containing T and B lymphocytes were prepared in HBSS and the cells transferred to recipient mice which were x irradiated with 1400 rad 6 h before cell transfer to eliminate host B and T cell responses. 48 h later, the spleens of recipient mice were diced into 1-mm cubes and the cubes cultured individually in 96-well microtiter plates. Cultures were maintained as described above. The induction of monofocal antibody responses in splenic fragment cultures has been described elsewhere (24, 29). The antigens TNP-T15, TNP-M167, and TNP-M460 were used in vitro as above. Additionally, the antigen TNP_{29}-Hy was used in vitro at a concentration of 5×10^{-7} M TNP.

RIA. Anti-hapten RIA was modified for use in 96-well polyvinyl microtiter plates (Cooke Engineering Co., Alexandria, Va.) as reported elsewhere (29). Idiotype analysis was performed as previously described (29). The idiotype specificity of the anti-T15 antiserum was demonstrated by the inability of other, non-T15 idiotype anti-PC myeloma proteins to inhibit the binding of ^{125}I-T15 to anti-T15.

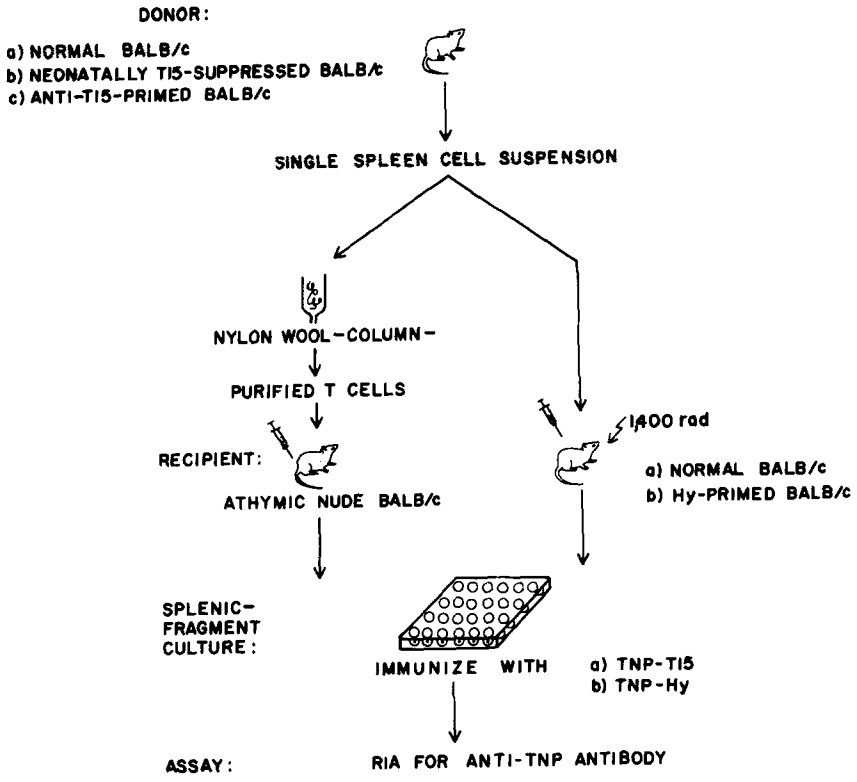

FIG. 1

Results

Response of Spleen Cell Populations to TNP-T15 in Fragment Cultures. Spleen cells from neonatally T15 suppressed or anti-T15-primed BALB/c mice were analyzed for their ability to provide help for primary TNP-specific B cell responses in vitro for the antigen TNP-T15. To accomplish this, spleen cell populations containing both T and B lymphocytes were transferred to unimmunized, irradiated recipients. A summary of these experiments is shown in Table I. TNP-specific B cell responses to TNP-T15 or TNP-Hy were not observed when no donor cells were transferred or when donor cell populations were obtained from untreated animals. When spleen cells from anti-T15-treated donors were transferred, responses to TNP-15 were observed. The responses appear to be dependent on the recognition of the T15 idiotype, as when cultures are stimulated with TNP-Hy, no responses are observed. TNP-specific B cell responses to TNP-T15 or TNP-Hy were not observed when no donor cells were transferred to Hy-primed recipients. The transfer of spleen cells from untreated mice resulted in a TNP-specific B cell response when the cultures were stimulated with TNP-Hy, but not with TNP-T15. This indicates that Hy priming is not sufficient to induce T15-specific helper cells.

The transfer of spleen cells from anti-T15-treated donors to Hy-primed recipients resulted in TNP-specific B cell responses to both TNP-T15 and TNP-Hy. The percent of spleen fragments that synthesized TNP-specific antibody was somewhat higher when donor cells were transferred to Hy-primed recipients and stimulated with TNP-

TABLE I

Anti-T15-treated BALB/c Have T15 Idiotype-specific Help

Donor*	Donor treatment‡	BALB/c recipient immunization§	In vitro TNP-carrier‖	Anti-TNP-positive wells¶
				%
—	—	—	TNP-T15	3
			TNP-Hy	0
BALB/c	—	—	TNP-T15	2
			TNP-Hy	2
BALB/c	Neonatally T15 suppressed	—	TNP-T15	18
			TNP-Hy	3
BALB/c	Anti-T15 primed	—	TNP-T15	17
			TNP-Hy	3
—	—	Hy-primed	TNP-T15	7
			TNP-Hy	5
BALB/c	—	Hy-primed	TNP-T15	6
			TNP-Hy	28
BALB/c	Neonatally T15 suppressed	Hy-primed	TNP-T15	15
			TNP-Hy	31
BALB/c	Anti-T15 primed	Hy-primed	TNP-T15	19
			TNP-Hy	29

* No or 1×10^7 spleen cells were transferred to each recipient.

‡ Mice to be used as neonatally T15-suppressed donors received 5 μg A/He anti-T15 antiserum i.p. at birth. Mice to be used as anti-T15 primed donors received 0.1 μg A/He antiserum i.v. 6–10 wk before use.

§ Recipients were x-irradiated with 1,400 rad 6 h before cell transfer. Mice used as Hy-primed recipients received 200 μg Hy in CFA 6 wk before use.

‖ Splenic-fragment cultures were stimulated with TNP_5-T15 at 10^{-8} M TNP or TNP_{29}-Hy at 5×10^{-7} M TNP.

¶ 48–96 culture supernates were assayed for anti-TNP antibodies at days 10 and 13 by RIA.

Hy than when stimulated with TNP-T15. Thus, it appears that the donor T cells from neonatally T15-suppressed or anti-T15-primed mice are not able to promote B cell responses of all TNP-specific B cells present in the fragment cultures when stimulated with TNP-T15. This may indicate that the donor helper cells responsible for TNP-specific B cell stimulation are in limiting numbers as compared with the Hy-specific T cells present in Hy-primed, irradiated recipients.

Idiotype Specificity of T15 Help. An analysis of the specificity of the helper cells observed in neonatally T15 suppressed mice was performed using a cell-panning technique. Plastic petri dishes, coated with either purified T15 or M460 myeloma protein, were used to selectively remove T15-recognizing cells from the neonatally T15-suppressed spleen cell population. Nonadherent cells were collected and injected into unimmunized, irradiated recipients. Splenic fragment cultures were stimulated with TNP-T15. The results are shown in Table II.

TABLE II

Elimination of T15 Antibody-specific Help on T15 Antibody-coated Dishes

Donor BALB/c*	Donor cell treatment‡	Irradiated recipient BALB/c§	Anti-TNP positive wells‖
			%
Normal	—	Normal	1
Neonatally T15 suppressed	—	Normal	14
Neonatally T15 suppressed	Panned on T15-dish	Normal	0
Neonatally T15 suppressed	Panned on M460-dish	Normal	15

* Mice to be used as neonatally T15-suppressed donors received 5 μg A/He anti-T15 antiserum i.p. at birth.

‡ Spleen cells (1.5×10^8) were suspended in 8 ml HBSS-10% FCS and poured onto plastic dishes coated with the purified PC-binding myeloma protein T15 (α,κ) or the purified TNP-binding myeloma protein M460 (α,κ). After incubation for 45 min at 37°C, the cells were collected. 1.0×10^7 untreated or treated spleen cells were given to each recipient.

§ Recipients were x-irradiated with 1,400 rad 6 h before cell transfer.

‖ Splenic-fragment cultures (72) were immunized with TNP_5-T15 at 10^{-8} M TNP and assayed on days 10 and 13 for anti-TNP antibodies by RIA.

Spleen cells from untreated donor BALB/c mice did not provide help for a TNP-specific B cell response to TNP-T15. Unpanned spleen cells from neonatally T15-suppressed mice show measurable help for a TNP-specific B cell response to TNP-T15. However, the helper cell activity can be adsorbed on dishes coated with T15. Panning on dishes coated with M460 did not remove the helper cell activity. As both M460 and T15 are BALB/c α,κ myeloma proteins, the helper cell population does not appear to be specific for the α or κ chain determinants of BALB/c antibodies.

The idiotype-specific recognition of helper T cells was further seen in experiments using trinitrophenylated myeloma proteins as antigens in fragment culture which differ in hapten binding or idiotype. T15 and M167 are both PC-myeloma proteins but differ in idiotype (23). As seen in Table III, T cells from neonatally suppressed and anti-T15-primed animals preferentially recognized the T15 idiotype as carrier for help in the anti-TNP B-cell response.

The data presented here demonstrate the specificity in the recognition of an idiotype by T helper cells and thus indicate that the two manipulations of the T cell donors with anti-idiotype induce selectively a specific idiotype anti-idiotype cascade.

Response of Individual T cells to TNP-T15 in Splenic Fragment Cultures. Graded numbers of nylon wool-purified T cells from neonatally T15-suppressed mice were transferred to nu/nu BALB/c recipients and the in vitro fragments were stimulated with TNP-T15.

As seen in Table IV, T cells from untreated BALB/c donors did not provide help for a TNP-specific B cell response to TNP-T15 over a wide range of cell doses. However, the relationship between the numbers of donor T cells from neonatally T15-suppressed BALB/c donors transferred to each nu/nu BALB/c recipient and the percent of spleen fragments that were positive for the synthesis of TNP-specific antibody is linear. The linear relationship offers strong evidence that the observed antibody responses are dependent on a single donor cell. The antibody response is

TABLE III

Anti-T15 Treatment Generates T15 Idiotype-specific T Helper Cells

BALB/c T cell donor*	In vitro TNP-antigen‡	Anti-TNP-positive wells§
		%
Neonatally T15 suppressed	TNP-T15	11
	TNP-M167	0
	TNP-M460	3
Anti-T15 primed	TNP-T15	9
	TNP-M167	4
	TNP-M460	1

* Neonatally T15-suppressed BALB/c mice were given 5 μg A/He anti-T15 antiserum i.p. at birth and used at 8 wk of age. Anti-T15-primed BALB/c were given 0.1 μg A/He anti-T15 antiserum i.v. and used 6–8 wk later. 5×10^5 nylon wool-purified T cells were given to BALB/c nu/nu recipients.

‡ Splenic-fragment cultures were immunized with TNP_5-T15, TNP_9-M167, or TNP_6-M460 at 10^{-8} M TNP.

§ 96 splenic-fragment culture supernates were assayed for anti-TNP antibodies at days 10 and 13 by RIA.

dependent on the transfer of T cells, as when the donor cells are treated with anti-Thy-1.2 and complement, the response disappears.

Similar experiments were carried out to study the T cells obtained from anti-T15-primed mice. The TNP-specific antibody responses could be detected when 1.0×10^6 donor cells were transferred to each nu/nu BALB/c recipient. The helper activity is eliminated by anti-Thy-1.2 and complement treatment of the donor cells.

Thus, the TNP-specific antibody response to TNP-T15 appears to be dependent on the number of transferred T cells in the inoculum of anti-T15-treated donor mice, indicating that the interaction of single idiotypic T cells with B cells is observed here.

Discussion

In the present study, an in vitro response system was used to demonstrate the presence and specificity of idiotype-specific helper T cells. The splenic-fragment culture technique, originally described by Klinman (24) for the analysis of B cell precursors, had been recently adapted by Pierce et al. (25) to study T cells under limiting-cell-dilution conditions. The T cells in this report recognize the T15 idiotype as carrier for the hapten TNP, and the B cell response to TNP is used as indicator for T cell help. In these experiments, we transferred T cells or whole spleen cells from anti-T15-idiotype-treated mice to nu/nu or lethally irradiated conventional BALB/c, respectively. The response to TNP-T15 is linearly dependent on the number of transferred T cells and is abolished if the T donor cells are first treated with anti-Thy 1.2 and complement. The idiotype specificity of the T helper cell was established by specific removal of help through panning on T15-coated dishes and the selective response to TNP-T15. Although the T15-adherent T cells were not used as donor cells, these data demonstrate that T15 idiotype-specific helper T cells are required for the response to trinitrophenylated T15.

The different induction modes to generate T15 carrier help are familiar and have been used to elicit alterations primarily in the B cell compartment. Neonatal admin-

TABLE IV

T Cells from Anti-T15 Treated BALB/c Mice Provided T15 Idiotype-specific Help for a Response to TNP-T15

T cell donor	Donor treatment*	T cells injected‡	Treatment of transferred cells§	Anti-TNP positive wells‖
				%
BALB/c	—	1.0×10^5	—	5
		2.0×10^5	—	0
		4.0×10^5	—	0
		8.0×10^6	—	0
		1.0×10^6	—	0
		1.6×10^6	—	0
BALB/c	Neonatally T15 suppressed	1.0×10^5	—	6
		2.0×10^5	—	9
		4.0×10^5	—	11
		6.0×10^5	—	13
		8.0×10^5	—	14
		1.0×10^6	—	5
		1.6×10^6	—	2
		1.0×10^6	Anti-Thy-1.2 + C′	0
BALB/c	Anti-T15 primed	1.0×10^6	—	13
		1.0×10^6	Anti-Thy-1.2 + C′	0

* Mice to be used as neonatally T15-suppressed donors were given 5 μg A/He anti-T15 antiserum i.p. at birth. Mice to be used as anti-T15-primed donors received 0.1 μg A/He anti-T15 antiserum i.v. 6–8 wk before use.

‡ Nylon wool-purified T cells were injected into nu/nu BALB/c mice.

§ Some T cell preparations were treated with hybridoma cell line AT83A.3 supernate (anti-Thy-1.2) and rabbit complement (C′).

‖ Splenic-fragment cultures were immunized with TNP_5-T15 at 10^{-8} M TNP. 48–192 cultures were assayed for anti-TNP antibodies at days 10 and 13 by RIA.

istration of anti-T15 serum induces chronic suppression of the T15 idiotype (14). In contrast, administration of a small amount of anti-T15 to adult mice primes B cells to recognize PC as hapten (3).

The data presented here demonstrate that manipulations using anti-idiotypic antibodies also have profound effects on T cells, specifically on T helper cells, which recognize the idiotype against which the anti-idiotypic serum was prepared. The mechanisms that induce T15-specific help cannot be accounted for with direct stimulation of idiotype-recognizing T-helper cells by anti-idiotypic antibody; rather, one must take into consideration some indirect stimulation routes involving additional elements of an idiotype network. It should be noted that these indirect stimulatory modes are still highly specific because only the T15 idiotype can function as carrier for T cells from anti-T15 suppressed or primed mice.

Administration of anti-idiotype antibodies could act either at the level of circulating immunoglobulins or on certain idiotype-bearing cells. As neonatal administration of anti-idiotype antibody results in chronic reduction of circulating T15-idiotype antibody, a normally inactive T15-recognizing T helper cell may be freed. This could occur by reduction of circulating T15, as a result of suppression of T15-producing B

cells (14, 30). A low dose of anti-idiotype antibody given to adult mice stimulates idiotype-bearing and -producing cells (3, 5); this may induce cycling of idiotypic and anti-idiotypic cells because it occurs after administration of antigen (9). Regulatory cells bearing receptors and producing factors complementary to receptors of the initially activated cells may thus be activated.

Alternatively, the findings in this study could be explained by assuming that anti-idiotypic antibody suppresses a naturally occurring, idiotype-bearing suppressor cell, analogous to the naturally occurring, idiotype-specific suppressor cell (11, 31), or activates an idiotype-bearing helper cell. The target for both cells would be the T15-recognizing T-helper cell needed by the B cell responding to the TNP-T15 idiotype.

The data reported here, together with earlier work on anti-idiotypic antibodies (1, 5), underscore the profound immunological effects of antibodies that recognize idiotypic determinants. Depending on whether a B or T cell function is tested, different alterations (suppression or stimulation) in the homeostasis of the immune network can be detected. The experimental demonstration of induced idiotype-specific alterations is important for the understanding of the normal interaction of B and T cells during an immune response. The physiological significance of cellular interactions within an idiotype network is emphasized by several observations of reciprocal changes of idiotypes and anti-idiotypes during an immune response. Kluskens and Köhler (7) demonstrated the appearance of anti-idiotypic antibodies during a prolonged anti-PC response. This finding was later confirmed by Cosenza (32) in plaque-forming cells. Similar observations were made by Goidl et al. (8) in the anti-TNP response. Kelsoe and Cerny (9) observed a cyclic change of T15-positive and T15-binding cells after stimulation with PC and demonstrated that this cycling is T cell dependent (33). Brown and Rodkey (34) also found auto-anti-idiotypic antibodies in rabbits after immunization with *Micrococcus lysodeikticus* and could purify them.

The collaboration of B and T cells is in the central focus of research on mechanisms in the response against T-dependent antigens. Classically, a carrier molecule provides the molecular bridge between both kinds of cells. More recently (35, 36), an additional mechanism of T-B interaction has been proposed; the role of this mode of collaboration could be the selective recruitment of certain B cell clones in T cell-dependent responses or an increase in the magnitude of B cell activation. In our experiments, the activity of the classical T helper cell was examined. Priming with Hy did not induce carrier-specific help for idiotype (see Table I). Other authors (36, 37) have found that priming induces helper cells that evidently can interact with B cells without an antigen bridge. It remains to be determined if the T15-specific T helper cells we describe can also perform the idiotype-inducing function in an anti-PC response as presented in those experiments. The intent of our future studies is to address these and other related questions in the interaction of B and T cells. The experimental system described here allows the dissection of the T-B interaction at the level of single cells because each cell type can be studied under limiting-cell-dilution conditions in the splenic-fragment culture system. Furthermore, the availability of idiotype markers for T and B cells provides for the necessary accuracy in the analysis of T-B interactions. The occurrence of naturally predominant idiotypes on B and T cells is aiding such studies. In BALB/c mice, the response to PC antigens is idiotypically restricted to the expression of the TEPC-15 idiotype at the B and T cell level (3, 38). In such a system, certain key questions can be answered with great precision: How many B cells can be served

by a single T cell within a given time span? What is the contribution of the idiotype-specific T cell in the stimulation of B cells compared with the conventional carrier-specific T helper cell? Is the selective suppression of secondary B cells in a syngeneic T cell environment caused by idiotypic regulation within the V-gene repertoire of B and T cells? Although in the present study, only the carrier-specific helper T cell has been studied, the experimental protocol can be easily modified to investigate these questions and, in particular, the role of the anti-idiotypic T cell that acts directly on the B cell idiotype (36).

Summary

Different manipulations of BALB/c mice were used to generate idiotype-specific help: neonatally induced suppression of the T15 idiotype and low-dose priming with anti-T15 antibody. The splenic foci culture system was used to study T15-idiotype-recognizing helper T cells under limiting-cell-dose conditions. These treatments induced T15 idiotype-specific help for B cells responding to TNP-T15. Normal or hemocyanin-primed BALB/c mice did not supply T15 idiotype-specific help. The helper cells were sensitive to anti-Thy-1.2 and complement treatment and can distinguish T15 from an idiotype-different, PC-binding myeloma protein, M167, and the TNP-binding myeloma protein, M460.

These data show that idiotype-specific T helper cells can be induced by at least two different manipulations of the idiotype network. These manipulations presumably do not act directly on the T15-recognizing T cells, but must involve complementary idiotypic circuits that stimulate anti-T15 specific T cells. Furthermore, this study demonstrates that the splenic-fragment culture technique provides a general method to investigate, at the single cell level, idiotypic T-B cell interactions induced by perturbations of the immune network.

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