MECHANISMS OF IDIOTYPE SUPPRESSION

IV. Functional Neutralization in Mixtures of Idiotype-specific Suppressor and Hapten-specific Suppressor T Cells*

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Immunological tolerance specific for anti-phosphorylcholine (PC)[1] is induced in BALB/c mice by two methods (1): by injecting immunologically immature mice with either PC-antigen as described for the classical tolerance induction (2), or antibody directed against the idiotype of TEPC15 myeloma protein (T15Id), which is antigenically identical to the major idiotype (Id) of the anti-PC antibody (3). Spleen cells from animals made unresponsive by either method displayed a similar active suppression of anti-PC response of normal spleen cells. The active suppression appeared to be mediated by suppressor T cells (1).

Suppressor T cells (Ts) induced in animals receiving anti-Id antibodies appear to express surface determinants similar to the anti-Id antibody (4–6), whereas Ts induced by antigens exhibit Id-bearing receptors specific for the antigen (7–9). The possible complementarity in the idiotypic-anti-idiotypic recognition signals of antigen-specific and Id-specific Ts led us to examine the functional interaction between the two populations of Ts induced independently by neonatal injection of either PC-antigen or anti-T15Id antibody.

We report here that individual Ts activities can be neutralized when Ts bearing anti-idiotypic receptors are preexposed to Ts bearing idiotypic receptors. Therefore, Jerne's network hypothesis (10), based on Id-anti-Id interactions for stimulation and regulation of immune responses, may also be applied to the regulation of Ts activity.

Materials and Methods

Mice. 6–10-wk-old BALB/c mice were purchased from Cumberland View Farms, Clinton, Tenn. Newborn BALB/c mice were obtained from our breeding colony originating from Cumberland View Farms.

Antigens and Immunization. A rough strain of *Streptococcus pneumoniae*, R36a, was obtained from the American Type Culture Collection, Rockville, Md. A vaccine of R36a was prepared by treatment with 0.5% formalin in 0.15 M NaCl; the vaccine was used as a PC antigen (1, 11, 12). This vaccine can be categorized as a T-independent antigen type 2 (TI-2) (13). 2,4-dinitrophenyl-lysyl-Ficoll (DNP-Lys-Ficoll), prepared by the method of Sharon et al. (14), was used as an immunologically unrelated control TI-2 antigen. Mice were injected intraperitoneally with a mixture containing 2×10^8 R36a and 2 μg of DNP-Lys-Ficoll because no significant

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DNP-Lys-Ficoll, dinitrophenyl-lysyl-Ficoll; HGG, human gamma globulin; M167, MOPC167; PC, phosphorylcholine; PFC, plaque-forming cells; PnC, pneumococcal C-polysaccharide; SRBC, sheep erythrocytes; T15, TEPC15; Ts, suppressor T cells; TI-2, T-independent antigen type 2; Ts-1, antigen-specific Ts; Ts-2, idiotype-specific Ts.

antigenic competition between the antigens is observed in vivo (15, 16). Individual spleen cell cultures were immunized with either 2.5×10^6 R36a or 1 ng of DNP-Lys-Ficoll.

Antibodies. The T15 and MOPC167 (M167) myeloma proteins were purified from the ascites fluid of tumor-bearing mice according to the method of Chesebro and Metzger (17), using a PC-conjugated Sepharose 4B column.

Anti-T15Id antibody was prepared in the ascites of A/He mice by multiple injections of T15 protein (18) and by subsequent absorption of the resulting ascites with immunoabsorbent columns of PC-binding myeloma proteins possessing different Id, as described previously (16). A mock ascites was prepared similarly by injections with a mixture of Freund's complete adjuvant and saline without T15 and used as a control for anti-T15Id ascites. Rabbit anti-T15Id antibodies were prepared by multiple subcutaneous immunization with T15 as described previously (19), and the resulting antiserum was absorbed using a M167-Sepharose column.

A hybridoma (SP45) secreting IgM anti-PC antibody bearing T15Id was selected by fusion of SP2/0-Ag14 (20) and spleen cells from BALB/c mice immunized with R36a, as previously described (21). This hybridoma line grew as ascites tumors in BALB/c mice; clarified ascites fluid was used as IgM anti-PC antibody. The presence of T15Id was determined by inhibition of the ^{125}I-T15-Fab-anti-T15Id reaction (16). The isotype of the antibody was identified using gel diffusion and 1% sodium dodecyl sulfate-7.5% polyacrylamide gel electrophoresis of radioimmunoprecipitates with appropriate anti-class antibodies.

Culture supernates of the hybridoma line HO-13-4 (22) were used as a source of monoclonal IgM anti-Thy-1.2 antibody (final dilution 1:400 for 10^7 cells/ml). The IgG fractions of the culture supernates from the 53-77.313 and 53-6.72 hybridoma lines (23) were used as a source of monoclonal anti-Lyt-1 and anti-Lyt-2 antibodies, respectively, as follows: the precipitate from 45% ammonium sulfate was coupled with *p*-azophenylarsonate (24); treatment of spleen cells with the coupled antibody was followed by treatment with rabbit anti-*p*-azophenylarsonate antibody (raised against *p*-azophenylarsonate-keyhole limpet hemocyanin) for complement-mediated cytolysis (23).

Induction of Neonatal Tolerance to PC. Neonates received a single intraperitoneal injection of 50 μl of either pneumococcal C-polysaccharide (PnC) (1 mg/ml) or anti-T15Id ascites within 2 d of birth. Because the injection of mock anti-T15Id or saline did not alter the anti-PC response, untreated littermates were used as controls. The mice were tested for the presence of suppressor cells at 7–10 wk of age.

Selection of Cell Population. The T cell population was enriched by the method of Julius et al. (25) using a nylon wool column with minor modifications, as described previously (26). Greater than 85% of the nylon wool nonadherent spleen cell population was sensitive to treatment with anti-Thy-1.2 and complement.

A two-step (0 and 37°C) method for antibody-dependent complement-mediated cytolysis was used to eliminate cell populations bearing Thy-1.2, Lyt-1, or Lyt-2 markers. A facilitating antibody, rabbit anti-azophenylarsonate antibody, was used to treat the cells exposed to the arsonylated anti-Lyt-1 or anti-Lyt-2 antibody (23). As a source of complement, fresh rabbit serum absorbed with agar (Difco Laboratories, Detroit, Mich.) was used to treat the antibody-sensitized cells at 37°C for 45 min (27). The cells were then washed three times in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 2% fetal calf serum, and readjusted to the original volume.

Separation of splenic cells based on their specific receptors was performed according to the method of Mage et al. (28). Briefly, 2×10^7–3×10^7 spleen cells in RPMI 1640 medium supplemented with 5% fetal calf serum were placed on a 60-mm petri dish previously coated with 5 ml of 200 μg/ml human gamma globulin (HGG), PC-HGG, T15, or M167. The nonadherent cells were then recovered from the dish by gentle rocking after incubation for 1 h at 37°C. After the cells were washed with medium, each aliquot was adjusted to the original volume (2×10^7 cells/ml). For the specific purification of cells adherent to such dishes, 1×10^8 nylon wool-purified splenic T cells were added to a 100-mm petri dish. Adherent cells were harvested with rubber policemen and cold medium, after the nonadherent cells had been removed by gentle washing with warm medium. The harvested cells were washed and the cell number was readjusted (2×10^6 cells/ml).

Spleen Cell Cultures. The Mishell-Dutton technique of immunizing mouse spleen cells (29)

was used, with the exception that the culture medium used was RPMI 1640 with 25 mM Hepes buffer (Grand Island Biological Co.). Individual cultures contained 10^7 normal spleen cells plus additional cells as indicated in the individual experiments. Individual cultures were immunized with either 2.5×10^6 R36a or 1 ng of DNP-Lys-Ficoll in 25 μl. The final volume of each individual culture was adjusted to 1 ml and the cultures were incubated for 4 d to elicit immune responses in vitro.

Hemolytic Plaque Assay. The number of plaque-forming cells (PFC) was determined by a slide modification of the Jerne-Nordin hemolytic plaque technique (30). To detect PFC to PC, sheep erythrocytes (SRBC) were coated with PnC, using chromic chloride (31), and were used as the specific target cells (12). For target cells of DNP-specific PFC, SRBC conjugated with trinitrobenzene sulfonic acid (32) were used.

The number of PFC secreting anti-PC antibodies with T15Id was determined by the method of anti-T15Id-mediated inhibition of plaque formation (33), as adapted previously (16).

Results

Suppression of Anti-PC Response by Neonatal Treatment with Either PnC or Anti-T15Id Antibody. A single injection of BALB/c neonates within 48 h after birth with either PnC or anti-T15Id antibody rendered them completely unresponsive to R36a, a TI-2 PC antigen, at 8 wk of age (Table I). This unresponsiveness appears to be restricted to anti-PC response, because these tolerant animals were able to respond as well to an unrelated TI-2 antigen (DNP-Lys-Ficoll), as were control littermates.

The profile of PFC Id was analyzed using anti-T15Id antibody. The result indicated that the dominance of T15Id is maintained in mice tolerized by neonatal injection with PnC but not in mice suppressed by anti-T15Id antibody injection. These results suggest that suppression by neonatal injection with antigen does not alter the T15Id dominance, in contrast to suppression by neonatal injection with anti-T15Id antibody where that particular Id is suppressed.

Characterization of Suppressor Cell Types. Spleen cells from mice tolerized by neonatal injection with anti-T15Id antibody were capable of suppressing 64–94% of the anti-PC response of normal spleen cells when these cells were co-cultured throughout the period (4 d) of in vitro anti-PC antibody induction with R36a (Table II). To confirm the T cell nature of suppressor cells in those tolerant mice (1), aliquots of spleen cells

TABLE I

Response to a Mixture of R36a and DNP-Lys-Ficoll in Mice Suppressed Neonatally by Treatment with Either PnC or Anti-T15Id Antibody

Neonatal treatments*	Number of mice	PFC/spleen ($\times 10^{-2}$)‡		
		PC	T15Id+§	DNP
			%	
Control	5	215 ± 53	93	1663 ± 84
PnC	5	17 ± 5	85	1874 ± 57
Anti-T15Id antibody	4	2 ± 1	0	2004 ± 165

* Individual neonates were injected intraperitoneally with 50 μl of either PnC (1 mg/ml) or anti-T15Id ascites. As a control group, neonates were injected with saline.

‡ Mice at 8 wk of age were intravenously injected with a mixture of R36a and DNP-Lys-Ficoll and 4 d later their PFC specific for PC and DNP were assayed using PnC- and TNP-coated SRBC after subtracting the background PFC to SRBC. The arithmetic mean and the standard error of triplicate measurements are shown.

§ The number of PFC producing anti-PC antibody with T15Id was determined by specific inhibition of plaque formation by treatment with anti-T15Id antibody.

TABLE II

Presence of Ts Cells from Mice Neonatally Injected with Either PnC or Anti-T15Id Antibody

Spleen cells/culture*		PFC/culture‡		
Suppressed	Normal	Experiment 1	Experiment 2	T15Id$^+$
				%
(1×10^7)§ +	1×10^7	1,660 ± 170	1,358 ± 8	95
Cells from mice suppressed with anti-T15Id				
1×10^7	1×10^7§	3 ± 2	18 ± 9	0
1×10^7	1×10^7	763 ± 128	295 ± 90	52
1×10^7 anti-Thy-1.2 + C	1×10^7	1,578 ± 18	1,188 ± 18	
1×10^7 anti-Lyt-1 + C	1×10^7	NT	230 ± 80	
1×10^7 anti-Lyt-2 + C	1×10^7	NT	1,080 ± 33	
1×10^7 C	1×10^7	887 ± 68	180 ± 70	
Cells from mice suppressed with PnC				
1×10^7	1×10^7§	0 ± 0	38 ± 19	75
1×10^7	1×10^7	435 ± 60	85 ± 15	21
1×10^7 anti-Thy-1.2 + C	1×10^7	1,093 ± 73	1,198 ± 53	
1×10^7 anti-Lyt-1 + C	1×10^7	NT	600 ± 75	
1×10^7 anti-Lyt-2 + C	1×10^7	NT	520 ± 30	
1×10^7 C	1×10^7	482 ± 38	183 ± 58	

* Spleen cells from mice (8–10 wk of age) injected neonatally with either PnC or anti-T15Id antibody were treated with monoclonal antibodies of anti-Thy-1.2 (HO-13-4), anti-Lyt-1 (53-7.313), and anti-Lyt-2 (53-6.72), followed by treatment with rabbit complement. The treated spleen cells, along with untreated cells (1×10^7 cells/culture) were then co-cultured with 1×10^7 normal syngeneic spleen cells in the presence of R36a for 4 d before their PFC were assayed against PC-SRBC.

‡ The number of PFC per culture represents the arithmetic mean and standard error of determinations for individual triplicate cultures.

§ Nucleated normal spleen cells were gamma-irradiated (2,000 rad) and used as filler cells to adjust the cell number of cultures.

were treated with monoclonal anti-Thy-1.2 antibody plus rabbit complement. Such treatment completely eliminated the suppressor activity from both types of suppressor cells: those induced by antigen injection and those induced by anti-Id antibody injection. These data indicate that suppression of anti-PC response involves T cells.

The Ts populations were further analyzed for the expression of Lyt antigens on the cell surface (Table II). The suppressor cell activity of anti-Id-induced Ts was completely abrogated by treatment with anti-Lyt-2 (but not with anti-Lyt-1) antibody plus complement. In contrast, the suppressor cell activity of PnC-induced Ts was partly reduced by treatment with either anti-Lyt-1 (50%) or anti-Lyt-2 (43%) antibody (Table II): treatment with both antibodies totally eliminated suppressor cell activity (data not shown). Therefore, anti-Id-induced Ts appear to bear Lyt-2 antigens, whereas PnC-induced Ts bear Lyt-1 or Lyt-2 antigens.

Identification of Specific Receptors of Ts Cells. To identify the specific receptors of anti-Id-induced Ts, the suppressive activities of spleen cell aliquots depleted of specific cells were examined (Table III). After removal of cells bound to T15-coated petri dishes, the suppressor cell activity of spleen cells from mice neonatally injected with anti-T15Id antibody was completely eliminated, whereas an aliquot of spleen cells lacking cells adherent to PC-HGG-coated dishes continued to exhibit unaltered suppressor activity. In addition, the suppressor cell activity could not be removed by

TABLE III

Characterization of Specific Signals of PnC- and Anti-T15Id-induced Ts Cells

Cells added to normal spleen cell culture*	PFC/culture			
	Experiment 1	Percent suppression‡	Experiment 2	Percent suppression
Normal irradiated	1,288 ± 76	0	1,358 ± 8	0
Suppressed with anti-T15Id				
Untreated	417 ± 113	68	295 ± 90	78
Nonadherent to PC-HGG	470 ± 48	64	NT	
Nonadherent to T15	1,028 ± 58	20	NT	
Nonadherent to M167	513 ± 62	60	NT	
SP45 + C§	NT		1,145 ± 170	16
Rabbit anti-T15Id + C	NT		214 ± 32	84
C	NT		180 ± 70	87
Suppressed with PnC				
Untreated	742 ± 18	42	85 ± 15	94
Nonadherent to PC-HGG	1,380 ± 90	0	NT	
Nonadherent to HGG	687 ± 26	46	NT	
Nonadherent to T15	657 ± 9	48	NT	
SP45 + C	NT		210 ± 2	85
Rabbit anti-T15Id + C	NT		1,085 ± 24	20
C	NT		183 ± 58	87

* 1×10^7 cells treated as indicated were cocultured with 1×10^7 normal BALB/c spleen cells per culture in the presence of R36a for 4 d before PFC assay for anti-PC production. Plastic petri dishes coated with PC-HGG, HGG, T15, or M167 were used to eliminate specific cells; the nonadherent cells were cocultured with normal spleen cells.

‡ Percent suppression was calculated based on the response of cultures containing 1×10^7 irradiated normal spleen cells and 1×10^7 untreated normal spleen cells.

§ Ascites of a hybridoma line secreting IgM anti-PC antibody bearing T15Id.

using petri dishes coated with M167 protein (a PC-binding protein that does not bear the T15Id). Therefore, Ts from mice tolerized with anti-T15Id antibody appear to specifically recognize T15Id.

This property of anti-T15Id-induced Ts was further confirmed by an alternative approach: treatment with T15Id-bearing IgM anti-PC hybridoma protein plus complement. This treatment abrogated the suppressor cell activity in the spleen cell population and resulted in at least 84% of the response seen in the absence of suppressor cells, whereas treatment with either rabbit anti-T15Id plus complement or complement alone did not significantly alter the suppressor cell activity. These results strongly suggest that anti-T15Id-induced Ts bear anti-idiotypic receptors recognizing T15Id.

The recognition signals of Ts from mice tolerized by neonatal injection with PnC were similarly characterized (Table III). The tolerogen-induced Ts appear to recognize PC, but not T15Id, in contrast to anti-T15Id-induced Ts; the suppressor cell activity was completely eliminated after removal of cells adherent to PC-HGG-coated petri dishes, but not after removal of either HGG- or T15-adherent cells. In addition, treatment of the spleen cells with IgM anti-PC antibody plus complement did not reduce the suppressor activity, whereas treatment with rabbit anti-T15Id antibody plus complement eliminated most of the suppressor activity. These results indicate that the majority of PnC-induced Ts exhibit T15Id-bearing receptors specific for PC.

In Vitro Interactions between PnC-induced and Anti-Id-induced Suppressor Cells. The presence of active suppressor cell activity was determined by co-culturing various numbers (0×10^6–20×10^6 cells/culture) of spleen cells from suppressed mice with compensating numbers (20×10^6–0×10^6) of normal spleen cells for a total of 2×10^7 cells per culture in the presence of PC antigen (Fig. 1A). A mixture of 1×10^7 spleen cells from mice neonatally tolerized by either PnC or anti-T15Id antibody and 1×10^7 normal spleen cells gave ~20% of the anti-PC response shown by normal spleen cells alone, which is significantly less than the expected value of at least 50% in the absence of active suppression.

To examine the effect of possible interaction between the PnC-induced Ts and the anti-Id-induced Ts, a mixture (1×10^7) of these spleen cells in various ratios was co-cultured with 1×10^7 normal spleen cells (Fig. 1B). The result clearly indicates that suppressor cells from PnC-treated mice function antagonistically against suppressor cells from anti-Id-treated mice, becasue the mixtures of these suppressor cells (separately displaying strong suppressor activity) no longer suppress the anti-PC response of normal spleen cells. Maximum neutralization of suppressor cell activity depended on an optimal ratio of PnC-induced suppressor cells to anti-Id-induced suppressor cells. Suppression by the individual suppressor cell types and neutralization of their suppressor cell activity when mixed appear to be specific for anti-PC response because suppression of anti-PC response was independent of an unrelated immune response to DNP-Lys-Ficoll (Fig. 1B).

Mutal Inactivation of Suppressor Activities of Purified PC- and T15Id-specific Ts by Preincubation of the Mixtures. To confirm the occurrence of neutralization of suppressor cell activity, splenic T cell populations from either normal mice or mice tolerized with PnC or anti-T15Id antibody were purified using nylon wool columns. Over 90% of the purified T cell population was sensitive to treatment with monoclonal anti-Thy-1.2 antibody and complement. The nylon wool-purified splenic T cell population

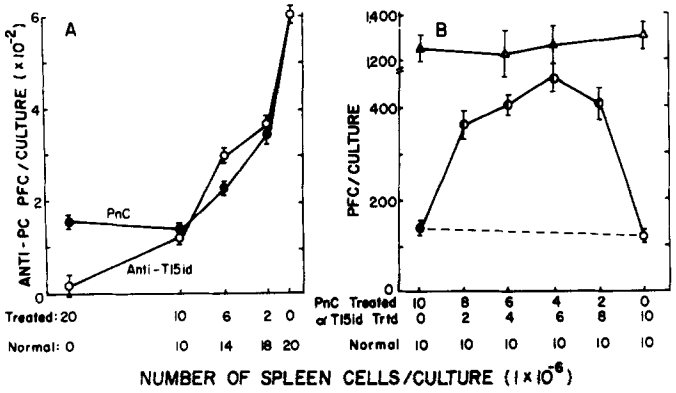

FIG. 1. Neutralization of suppressor cell function after co-culturing spleen cells from mice neonatally treated with PnC and those from mice treated with anti-T15Id antibody. The mixture of the two types of spleen cells (1×10^7) was preincubated for 30 min and then normal spleen cells (1×10^7) were added along with R36a or DNP-Lys-Ficoll. Circles represent the anti-PC response of cultures containing suppressor cells induced by PnC (●) or anti-T15Id (○), or those containing both types of suppressor cells (◐). Triangles represent the anti-DNP response of cultures containing suppressor cells induced by either PnC (▲) or anti-T15Id (△), or those containing mixtures of both types of suppressor cells (◭). The dotted line indicates the value expected when no synergism or antagonism is observed.

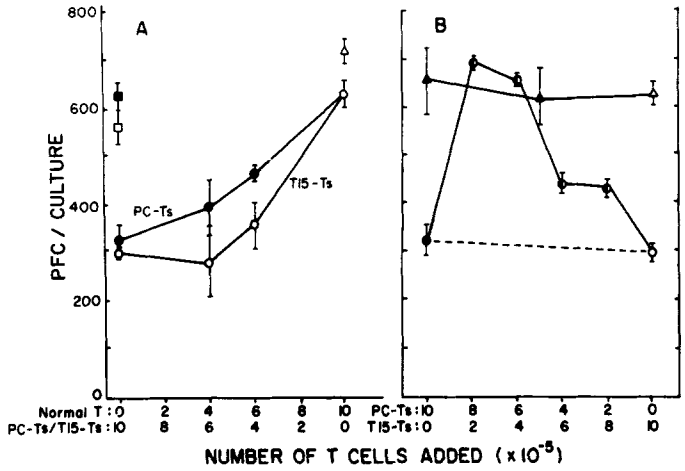

FIG. 2. Loss of suppressor cell function after mixing purified PC-specific Ts with T15Id-specific Ts. Splenic T cell populations from mice suppressed by neonatal injection of either PnC or anti-T15Id antibody (and from control littermates) were purified using nylon wool columns. The nylon wool-purified T cells from mice neonatally treated with PnC and anti-T15Id antibody were further subjected to PC-BSA- (or BSA-) and T15-coated (or plain) petri dishes, respectively. Specific T cells adherent to the dishes were harvested and preincubated for 1 hr at various ratios before addition to cultures containing 1.2×10^7 normal BALB/c spleen cells. The cell mixture was incubated for 4 d with either R36a or DNP-Lys-Ficoll. Circles represent the anti-PC response of cultures containing T cells adherent to PC-BSA (●), T15 (○), or those containing both types of T cells (◐). Squares represent anti-PC response of cultures containing T cells adherent to BSA-coated (■) or plain (□) petri dishes. Triangles represent anti-DNP responses by cultures containing PC-BSA-adherent (▲), T15-adherent (△), or both (◭) of T cells.

from the tolerized mice was further purified, based on specific binding to PC-bovine serum albumin (BSA)- and T15-coated petri dishes; T cells from PnC- and anti-T15Id-tolerized mice were incubated in PC-BSA- and T15-coated petri dishes, respectively. As controls for specific adherence, aliquots of the T cells were incubated in BSA-coated or plain dishes. To examine the suppressor cell activity of those PC- and T15-adherent Ts, various numbers of the purified Ts were co-cultured with 1×10^7 normal spleen cells in the presence of antigen; the total T cell number (1×10^6) added to normal cultures was compensated for by adding nylon wool-purified normal T cells. T cells adherent to either PC-BSA- or T15-coated petri dishes displayed significant suppressor cell activity (>50% suppression with 1×10^6 cells) against the anti-PC response of normal spleen cell cultures. However, the same number of cells adherent to either BSA-coated or plain petri dishes did not result in significant suppression (Fig. 2A). These results indicate that specific Ts can be enriched by selecting cells adherent to PC or T15 from PnC- and anti-Id-induced tolerant mice, respectively.

The possibility that these PC-specific and T15Id-specific suppressor cells interact through their complementary receptors, resulting in alteration of their suppressor function, has been examined. Various mixtures of the Ts isolated according to recognition signals were incubated for 1 h to allow possible interactions. The mixtures of the Ts (1×10^6 cells/culture) were then co-cultured with normal spleen cells (1×10^7) in the presence of either R36a or a control antigen (DNP-Lys-Ficoll) to confirm that the neutralization of suppressor cell activity seen with whole spleen cell popula-

tions is due to interactions between the Ts populations (Fig. 2B). The result demonstrates that preincubation of purified PC-specific and T15Id-specific Ts could also result in complete neutralization of their specific suppressor cell activity against anti-PC response.

Discussion

We have demonstrated here that Ts specific for hapten and the major Id can be detected in BALB/c mice tolerized to PC by a single neonatal injection of PnC or anti-Id antibody, respectively. These results are comparable to previous reports demonstrating that the antigen-specific Ts are generally induced in vivo at various ages (2, 6–9), as well as in vitro (34, 35) by antigen treatment, including hapten-conjugated isogeneic lymphocytes (36–38). Similarly, Id-specific (anti-idiotypic) Ts are induced by injection of anti-Id antibody (4–6) or Id-conjugated isogeneic lymphocytes (39, 40).

Addition of either PC-specific or T15Id-specific Ts to normal spleen cells resulted in drastic suppression of the anti-PC response (Table III). This significant suppression by T15Id-specific Ts may reflect the clonal dominance of T15Id-bearing B cells (>95%) in BALB/c mice (12). Because the anti-PC response to R36a is T independent (13, 15), the T15Id-specific and PC-specific Ts may act on the T15Id-bearing B cells through direct receptor interaction, as shown in the DNP-binding myeloma systems (40, 41) or through an antigen bridge, respectively.

The majority of PC-specific Ts induced in BALB/c mice by neonatal injection with PnC appear to bear Id cross-reactive with T15Id, because essentially all the PC-specific suppressor cell activity could be eliminated by treatment with rabbit anti-T15Id antibody and complement. Similar dominance of T15Id-like receptors has also been demonstrated in PC-specific helper T cell populations in PC-primed BALB/c mice (42). Receptors of some antigen-specific Ts (bearing an Id) are expected to be complementary to those of Id-specific Ts. This interaction is likely to be amplified in the PC-system because the majority of PC-specific Ts appear to bear T15Id, and T15Id-specific Ts can be exclusively induced by neonatal treatment with anti-T15Id antibody (Table III).

Two types of Id-mediated interaction between the two types of Ts are anticipated by the network hypothesis (10): synergistic and antagonistic effects. Although no evidence for direct functional synergism has yet been reported, it has been well documented that antigen-specific Ts (Ts-1) induce Id-specific Ts (Ts-2), perhaps through soluble mediators (43, 44). On the other hand, our results clearly indicate that hapten-specific and Id-specific Ts induced independently can interact antagonistically, resulting in neutralization of Ts function (Figs. 1 and 2). Although the physiological significance of such neutralization for immune regulation is not yet clear, similar neutralization of Ts function has been observed in transferring suppression of dinitrofluorobenzene-specific contact sensitivity when mixtures of hapten-specific Ts (Ts-1) and anti-idiotypic Ts (Ts-2) are used for adoptive transfer (Dr. Stephen D. Miller, personal communication). These results suggest that mutual neutralization of Ts may play an important role in the regulation of immune responses. Either induction or neutralization of Ts-2 by Ts-1 (or vice versa) may depend on the balance between the two Ts populations, analogous to stimulation or

suppression of B cell response by anti-Id antibody depending on the concentration or subclass of the antibody (45–47).

No direct evidence for persistent elevation of both functional, antigen-specific and Id-specific Ts in the same experimental animals has yet been reported; predominance of one type of Ts has been displayed (43, 44). However, recently Hirai and Nisonoff (48) demonstrated that both hapten-specific and Id-specific Ts factors are present in the early stage of anti-arsonate cross-reactive Id suppression in A/J mice, whereas an Id-specific Ts population predominates in such cross-reactive Id-suppressed mice at a later period (4). In addition, under some experimental systems, antigen alone (49) or hapten-spleen cells (50) may result in Id-specific suppression. These results support the possibility that Ts-2 may be induced after generation of Id-bearing Ts (Ts-1) within the same individual during the course of specific Ts-mediated regulation. Because we tested the Ts activity 8–10 wk after neonatal treatment with anti-Id antibody, the dominance of Ts-2 may have already been established in the Id-suppressed mice. However, we cannot yet rule out the possibility that the method of neonatal T15Id suppression, which does not require antigen administration after anti-Id antibody treatment (in contrast to generation of Ts specific for the cross-reactive Id of anti-arsonate antibody [4]) may result in the generation of Id-specific Ts through a pathway other than Ts-1 involvement.

Nevertheless, we have demonstrated here that a brief preincubation of independently induced hapten-specific Ts and Id-specific Ts results in neutralization of their Ts function, probably after interaction through their Id-anti-Id recognition signals. Because it has been well established that hapten-specific Ts induce effector-phase Ts (Ts-2), induction (or stimulation) or inhibition of either type of Ts by the other may be based on a delicate balance between the Ts populations; i.e., low quantities may be stimulatory and high quantities inhibitory, as has been established for the anti-Id antibody effect on B cell response (45–47).

Summary

Specific tolerance to phosphorylcholine (PC) can be induced in BALB/c mice by neonatal injection with either pneumococcal C-polysaccharide (PnC) or anti-TEPC15 idiotype (T15Id) antibody specific for the major idiotype (Id) of anti-PC antibody. Spleen cells from these tolerant mice exhibited T cell-mediated active suppression of anti-PC response when they were co-cultured with normal spleen cells. Suppressor cells from the PnC-injected mice appeared to bear either Lyt-1 or Lyt-2 antigens, whereas suppressor cells from anti-Id-treated mice expressed Lyt-2 antigens. Analyses of the specific receptors of these suppressor T cells, based on either adherence to PC- and T15-coated petri dishes or cytolysis by rabbit anti-T15Id and monoclonal IgM anti-PC antibody with complement, revealed that receptors of PnC-induced suppressor T cells recognize PC, whereas receptors of anti-Id-induced suppressor T cells react with the T15Id. The possible interaction of the two different types of suppressor T cells was examined by co-culturing normal spleen cells with mixtures of the different suppressor cell types in various cell ratios in the presence of the T-independent PC-antigen, R36a. A brief incubation of anti-Id-induced, T15Id-specific suppressor T cells with PnC-induced, hapten-specific, and T15Id-bearing suppressor T cells resulted

in complete cancellation of their suppressor function. These results suggest that idiotype network regulation may also occur among suppressor T cell populations.

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