REGULATION BY COMPLEMENTARY IDIOTYPES

Ig Protects the Clone Producing It*

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The concept of regulation through complementary idiotypes in an immunologic network includes two essential ideas: (a) complementary responses may be stimulated by a single epitope because the specific products of the idiotypic and anti-idiotypic responses are potentially immunogenic, and (b) the products of either complementary response can potentially regulate the other response (1, 2).

Idiotypic and anti-idiotypic antibody, and T cells (both suppressor and helper) have been shown to regulate both humoral and cellular immune responses in vivo and in vitro (3–40). Because these reactants can be produced autogenously during the course of an immune response, it has been suggested that phenomena such as the "self-limitation" of individual antibody responses, the progressive decline in the antibody response after repeated antigen injections, and the oscillation of responses after immunization with some kinds of antigens may be due to autoregulation through an anti-idiotypic network (5, 7, 19, 31–36, 40).

Undoubtedly, the repertoire of specificities and the multiplicity of functionally distinct lymphocyte subpopulations are sufficient to provide the necessary reactants for a complex network. However, our studies cast doubt on the simple assumption that because anti-idiotypic reactants are demonstrable, they are necessarily effective mediators of regulation once an antibody response has been stimulated. The reasons for doubt are that antibody produced early and rapidly may "tolerize" complementary clones, or if complementary reactants do coexist, then the Ig produced may shield or protect the clones producing it from the complementary reactants.

In our model, specific suppression is produced by immunization of A/He mice with the phosphorylcholine (PC)[1]-binding IgA myeloma protein (T15 idiotype) secreted by the plasmocytoma HOPC8 (H8). Such actively immunized mice are unresponsive to immunization with PC-containing antigens. Suppression caused by active immunization can be reproduced by giving anti-H8 antibody (αH8) passively to normal A/He mice. Conversely, A/He mice actively immunized with PC are unresponsive to immunization with H8 and this unresponsiveness can be reproduced by giving anti-PC antibody (αPC) passively to normal A/He mice (12). Because the antibodies αH8 and αPC are directed at least partially against a combining site structure of the other,

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[1] *Abbreviations used in this paper:* αH8, anti-HOPC8; αPC, anti-phosphorylcholine; DNFB, dinitrofluorobenzene; NWP, nylon wool purified; PC, phosphorylcholine; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TNP, trinitrophenyl.

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and because each response can cause reciprocal regulation of the other response, the antibodies and corresponding responses are referred to as complementary.

Although a prior αH8 response suppressed the complementary response and vice versa, neither complementary response was diminished when mice were actively immunized to have simultaneous complementary responses (12, 24). This absence of reciprocal regulation could not be accounted for by a shift in idiotypes or to formation of circulating complexes of complementary antibodies (24). Possibly, the complementary antibodies that were produced simultaneously protected the clones producing them from reciprocal regulation. Here we report findings consistent with this possibility; furthermore, we show that antibody of the specificity produced by the suppressed clone can be given passively to rescue the suppressed clone.

Materials and Methods

Mice. A/He females, 8–12 wk old, were purchased from Cumberland View Farms, Clinton, Tenn.

Antigens. The same preparation of formalin-killed vaccine of *Diplococcus pneumoniae*, R36A, was used as in previous studies (12, 24); the vaccine has PC as a major antigenic determinant to which mice respond. 2,4,6-trinitrophenyl (TNP)-conjugated N-(2-aminoethyl) carbamylinethyl Ficoll was prepared and kindly given to us by Dr. Jose Quintans, La Rabida-University of Chicago Institute. Mice were injected intravenously with 0.2 ml of a mixture containing 10^9 R36A organisms and 10 μg TNP (PC-TNP).

A single batch of myeloma protein (H8) produced by the plasmacytoma HPOC8 in BALB/c mice and purified from ascitic fluid (12) was used to immunize mice to produce αH8, an antibody that is complementary to αPC. H8 is of the same idiotype (T15) as the protein produced by the plasmacytoma TEPC15. For active immunization, one part H8, 1.2 mg/ml, was homogenized with one part methylated bovine serum albumin and two parts Freund's complete adjuvant; 0.05 ml of the mixture was injected into each hind footpad. Injections of H8 in incomplete adjuvant were repeated 1 and 2 wk later. Times reported after immunization with H8 were measured after the third injection.

Plaque-forming Cells (PFC) and Antibody Titers. PFC assays and antibody titers ($\log_2$) were performed as previously described (12, 24), except for the experiment recorded in Table V. In this experiment, PFC against PC were assayed in the presence and absence of a monoclonal αT15 reagent (kindly provided by Dr. John Kearney, University of Alabama, Birmingham, Ala.) to determine the percentage of PFC of T15 idiotype (39). Data were calculated and results were recorded as previously described (24).

Cultures. Spleen cells, either unseparated or purified, were cultured as previously reported using the modification of the Mishell-Dutton procedure (24). Unseparated spleen cells treated with αThy-1.2 serum were designated as B cells in Table I. 2.5 × 10^7 whole spleen cells/ml were mixed with 20 μl anti-Thy-1.2 (Litton Bionetics Inc., Kensington, Md.) per ml of cells. The cells were held on ice for 30 min and were then washed once. 100 μl of complement (Low Tox-M rabbit complement; Accurate Chemical and Scientific Corp., Hicksville, N. Y.) was added per 0.9 ml cells; the mixture was incubated at 37°C for 45 min, and the cells were washed once. Spleen cells were separated in nylon wool (Fenwal Laboratories, Morton Grove, Ill.) as described by Julius et al. (41). Columns were prepared in 10-ml syringe barrels. 2.0 ml of medium containing 10^8 spleen cells was added slowly to the columns, followed by 1.0 ml of medium. The columns were incubated for 45 min at 37°C. Cells were washed off the columns by adding 20 ml of medium drop by drop. The medium was Dulbecco's phosphate-buffered saline containing 5% heat-inactivated fetal calf serum. The purity and functional characteristics of the cell preparations were the same as those reported previously from this Institution using identical materials and procedures (14, 39).

Irradiation. Mice received 300 rad total body irradiation from a ^{137}Cs source at a rate of 200 rad/min. When irradiated mice received nylon wool-purified (NWP) cells, the cells were prepared in the same way as for culture.

Passive Immunization. A single pool of serum was prepared from bleedings of 18 A/He mice

injected in multiple intradermal sites four or five times at weekly intervals with 0.2 ml of H8 in adjuvants as previously described (12). The serum (αH8) had an agglutinin titer of 11 to H8-sheep erythrocytes (SRBC). Normal mice injected with 0.2 ml αH8 had titers of 6–8 the next day, titers of 3–5 2 wk later, and 1–3 4 wk later.

A pool of primary response serum was prepared from 24 A/He mice immunized with PC and bled at 6 d; the serum had an agglutinin titer against PC-coated SRBC of 7. This serum was compared with αPC and was used in experiments that are discussed below.

A culture line of hybridoma cells, designated M2, that secretes IgM αPC of T15 idiotype, was kindly supplied by Dr. Patricia Gearhart, Carnegie Institute of Washington, Baltimore, Md. The hybridoma was produced by fusion between spleen cells from a BALB/c mouse and cells from the SP 2/0 line (nonsecreting myeloma cells). 10^6 hybridoma cells grown in culture were injected intraperitoneally into each BALB/c mouse that had been injected intraperitoneally 2 wk earlier with 0.5 ml Pristine. Ascitic fluid was tapped 10–14 d later and again one or two more times at 3–4-d intervals. The idiotype was confirmed using monoclonal αT15, which completely inhibited plaque formation against PC-SRBC using hybridoma cells recovered from ascitic fluid. A pool of 100 ml ascitic fluid was prepared that had an agglutinin titer against PC-SRBC of 11. Ascitic fluid, referred to in the text as αPC, was used for passive immunization in all experiments reported here in detail, but before its use, the biological activity was compared with primary response serum obtained from A/He mice immunized with PC, and with αPC purified from the ascitic fluid by absorption and elution from PC-Sepharose. The αPC and purified aPC were diluted to have titers of 7, i.e., equivalent to the titer of the primary response antisera. The three preparations all caused >50% and <90% suppression to immunization with PC when given to mice (0.2 ml intravenously/mouse) or added to cultures (50 μl, 1:10) at the time of immunization with PC-TNP; none of the three preparations affected the response to TNP.

Normal mice and irradiated normal mice were injected intraperitoneally with 0.5 ml αPC every other day three times. The mice were bled serially. αPC titers of 6–8 were sustained by the injections; when injections were stopped, titers fell to baseline levels by 4 wk after the last injection. Titers were equivalent in normal and irradiated mice. 0.5 ml αPC given intraperitoneally every other day three or four times produced levels of serum antibody that approximated levels resulting from active immunization of A/He mice with PC in adjuvants as had been done in previous experiments demonstrating simultaneous complementary responses (12).

Results

Preliminary Observations.   Normal young adult A/He mice have no or very low (1–3) titers of αPC and <100 background PFC/spleen to PC-SRBC. In contrast, BALB/c mice usually have background serum titers of 4–7 and as many as 1,000 background PFC/spleen. The response of A/He mice or their spleen cells in culture to immunization with PC is quite low; e.g., responses are usually 10–20% as high as for BALB/c mice. Approximately 50% (individual variation, 30–70%) of PFC from A/He mice immunized with PC are of T15 idiotype, compared with 90% or greater for BALB/c mice. A/He mice respond to immunization with T15 or H8 by producing high titers of αH8, whereas BALB/c mice do not respond unless they have been made neonatally tolerant to PC (42).

A/He mice that have been actively immunized with H8 (but not normal A/He mice) develop shock when injected with either 0.2 ml αPC or 0.2 ml A/He antiserum to PC. Interestingly, in previous studies, αT15 given intravenously to BALB/c mice caused an identical reaction that was considered to be anaphylaxis resulting from interaction of αT15 with normally circulating αPC antibodies occurring in this mouse strain. The reaction in BALB/c mice was circumvented by giving αT15 intraperitoneally (3); the reaction in A/He mice was similarly circumvented in the present studies by giving αPC intraperitoneally.

TABLE I

Suppression by a Complementary Response

Ex-peri-ment	Preim-muniza-tion*	Challenge immuniza-tion	In vivo‡	In vitro§	
			αPC PFC/spleen	αPC PFC/culture Whole cells	B cells
1	None	PC-TNP	15,000 (4.17 ± 0.21)	319 (2.51 ± 0.11)	168 (2.22 ± 0.08)
	H8	PC-TNP	350 (2.54 ± 0.11)	46 (1.66 ± 0.05)	65 (1.81 ± 0.02)
	H8	PC-TNP	200 (2.30 ± 0.13)	28 (1.45 ± 0.02)	26 (1.41 ± 0.05)
2	None	PC-TNP	6,960 (3.84 ± 0.32)	168 (2.22 ± 0.07)	250 (2.40 ± 0.10)
	αH8	PC-TNP	240 (2.38 ± 0.08)	25 (1.39 ± 0.01)	20 (1.30 ± 0.04)

* In experiment 1, active immunization was completed 1 wk or 6 mo before challenge immunization. In experiment 2, αH8 (0.2 ml, 1:2) was given 1 wk before challenge immunization.

‡ Each group contained five or six mice. Differences between the control and the preimmunized groups in both experiments were significant, $P = < 0.01$. The mean numbers of αTNP PFC/spleen for the groups in experiment 1 were 56,000 and 45,000 and 65,000, respectively; and 25,100 and 32,500, respectively, for experiment. 2.

§ Cells were pooled from two or three mice and cultures were immunized with PC-TNP. Differences between the nonimmunized and preimmunized groups in both experiments were significant, $P = < 0.01$. The mean numbers of αTNP PFC/culture in experiment 1 were 303, 428, and 568 for whole cells; and 130, 101, and 298 for B cells. In experiment 2, mean numbers were 940 and 1230 for whole cells and 860 and 860 for B cells.

During the course of the present work, previous observations were confirmed. For example, A/He mice immunized with H8 were unresponsive to immunization with PC (experiment 1, Table I). In other experiments, mice were immunized simultaneously with H8 in adjuvants in one footpad and with PC in adjuvants in the other footpad as previously described (12). Simultaneous αPC and αH8 PFC responses were demonstrated in corresponding popliteal lymph nodes assayed 4 d after the third immunization, and sera had titers of αH8 of 7–10 and αPC of 5–7. Unseparated cells and B cells were prepared for culture from spleens of simultaneously immunized mice 1 wk and 1 mo after the third immunization. The PFC responses to PC and TNP of both unseparated cells and B cells were as high as for cells obtained from normal mice or from mice remotely immunized with PC (data not shown).

These observations taken together support previous findings that a prior immune response suppresses the complementary response, but reciprocal regulation is not effective when complementary responses are simultaneous. But it was indeterminant whether B cells from suppressed mice were reversibly suppressed or were made tolerant.[2]

Elimination of Cells Responsive to PC and the Apparent Absence of Suppressor Cells. Mice or spleen cells from mice recently or remotely actively immunized with H8 were immunized with PC. As shown in Table I, experiment 1, both mice and cells in culture were unresponsive to PC but responded normally to TNP. The absence of response of unseparated cells in culture might be due to suppression by T cells and/ or αH8 secreted by B lymphocytes. However, purified B cells were unresponsive, and spleen cells from mice immunized with H8 as in this experiment do not contain PFC to H8-coated SRBC (12), and do not suppress when cocultured with normal cells

[2] For convenience, we assume that cells made tolerant are eliminated, although it is possible that the cells are made irreversibly unresponsive without being eliminated.

TABLE II

Normal Cells Are Not Suppressed by Cells from Mice Actively Immunized with H8

| Normal cells | NWP cells from | | αPC PFC/culture‡ | |
	Normal mice	Mice immunized with H8*		
10^7			340	(2.53 ± 0.09)
5×10^6			250	(2.40 ± 0.04)
5×10^6	5×10^6		240	(2.38 ± 0.06)
5×10^6		5×10^6	310	(2.49 ± 0.08)
	10^7		<50	—
		10^7	<50	—
10^7	Normal serum		450	
10^7		Serum from mice immunized with H8	<50	—

* Mice were actively immunized with H8 1 mo earlier. Serum pooled from normal or immunized mice was added to cultures in a final dilution of 1:200.
‡ Cultures were immunized with PC-TNP. Responses of cultures of normal cells with added NWP cells were not significantly different. All cultures of normal cells, with or without added NWP cells or serum, contained 900–1,300 PFC to TNP.

(data not shown). Furthermore, as shown in Table I, experiment 2, purified B cells from mice passively immunized 1 wk earlier with αH8 are also unresponsive.

In additional experiments of similar design, the responses of spleen cells obtained 1 or 4 d after mice received αH8 were reduced by ~40 and 80%, respectively. Thus, the effect of αH8 in vivo on cells responsive to PC in vitro must be quite rapid, but cumulative for several days.[3] Together the observations indicate that B lymphocytes responsive to PC are made tolerant by active immunization with H8 or passive immunization with αH8, a conclusion consistent with earlier studies showing that a xenogeneic anti-idiotypic antiserum eliminated idiotypic cell precursors responsive to group A streptococci (4).

In attempts to determine whether spleens of suppressed mice contained suppressor cells, whole cells or NWP cells were prepared from mice, recently or remotely actively immunized with H8 or passively immunized with αH8. In different experiments, the cells were added in various numbers to cultures of either whole spleen cells or purified splenic B cells obtained from normal mice. The results recorded for one experiment (Table II) were typical for this type of experiment in that NWP cells from unresponsive mice either did not suppress or caused no more suppression than NWP cells obtained from normal mice. In contrast to the ineffectiveness of cells, serum from the mice actively immunized with H8 caused marked suppression of responses to PC and no suppression of responses to TNP.

Immature or regenerating B cells are inactivated or are "made tolerant" more readily by antigen or anti-idiotypic antibody (43–52) than mature B cells. Therefore, we tested whether cells from suppressed mice inhibited regeneration of αPC responsivity in sublethally irradiated mice. Unseparated or NWP cells obtained from mice

[3] Spleen cells obtained from young adult BALB/c mice 1–4 d after receiving αH8 (αT15) responded normally in culture, although cells obtained from young adults neonatally injected with αH8 were tolerant (8). Thus, αH8 appears to have comparable effects on adult A/He mice and neonatal BALB/c mice. Presumably, the reversible suppression observed in adult BALB/c mice is due to the high levels of circulating idiotype and the relatively large numbers of cells responsive to PC, which must reduce both the concentration and duration of action of passively given αH8.

actively immunized with H8 or obtained from normal mice were transferred to normal adult recipients given 300 rad. This dose of irradiation eliminates recognizable lymphocytes in bone marrow and lymphoid tissue and abolishes responsivity to immunization with PC for about 2 wk; recovery associated with extensive regeneration of lymphoid tissue is ~50% at 3 wk and is complete by 4–5 wk. Therefore, mice were irradiated and given putative suppressor cells or pooled serum from suppressed mice; the mice were immunized with PC-TNP 3–5 wk later. In repeated experiments, suppression by cells was marginal and only found when large numbers of cells were given; variability between mice was often great. Furthermore, NWP cells from normal mice usually caused equal or almost as great suppression of recovery as cells from mice immunized with H8. However, small amounts of serum from suppressed mice containing αH8 invariably suppressed recovery of the response to PC but not to TNP. The results combined from two experiments using the same protocol and presented in Table III reflect the overall findings for this series of experiments. Although the experiments did not exclude a role for suppressor cells, suppression could clearly be initiated by Ig alone; therefore, the direction of the work was turned to test the role complementary Ig might play in blocking or neutralizing regulation by αH8.

Protection from Suppression. Both antibody (IgM or IgG) to an antigen and the complementary antibody are potent, specific immunosuppressants when given several days before or 24 h after active immunization of animals that have not been previously immunized with the antigen (53). To test the separate and combined effects that the complementary immunoglobulins αPC and αH8 might play in regulation, doses of each that caused comparable suppression were arbitrarily selected. Doses of αPC (0.2 ml, 1:2 or 1:4) and αH8 (0.2 ml, 1:4 or 1:8) each caused >50 but <100% suppression when given alone 1 d before or at the time of immunization with PC. In different experiments, mice given αH8 were injected separately with αPC either on the same day or 1 d later; the mice were actively immunized with PC either 1 or 7 d after receiving αPC.

The results (Table IV) demonstrate that αPC prevented suppression produced by αH8 and vice versa. Presumably the complementary antibodies neutralized each other so that neither caused suppression. The effect of αPC in preventing suppression was specific to the extent that neither normal BALB/c serum absorbed on PC-Sepharose (0.2 ml of undiluted serum/mouse) nor IgA (non-PC-reactive) produced by the plasmacytoma MOPC315 (M315; 0.2 ml) containing 0.2 mg purified protein/

TABLE III

Suppression by Cells or Complementary Idiotype Given Passively to X-irradiated Recipients

NWP cells or antibody*	αPC PFC/spleen‡
None	11,800 (4.07 ± 0.34)
Normal cells	5,200 (3.71 ± 0.35)
H8-immunized cells	2,200 (3.34 ± 0.80)
αH8 antibody	900 (2.95 ± 0.45)

* The results for two comparable experiments have been combined; each combined group contained seven or eight mice. Recipients received 300 rad 2 h before receiving 5×10^7 cells or 0.2 ml of serum pooled from immunized donors. All mice were immunized with PC 3 wk later.

‡ The difference between groups receiving no cells or αH8 antibody was significant, $P = < 0.01$. However, the differences between groups receiving no cells or normal cells, or normal cells and H8 immunized cells were not significant. The mean number of PFC/spleen for four unirradiated normal mice was 21,000 (4.32 ± 0.18).

TABLE IV
αPC *Prevents Suppression Caused by Complementary Idiotype and Vice Versa*

Day 0*		Day 7	Day 11‡
αH8	αPC	Challenge immuniza-tion	αPC PFC/spleen
0	0	PC-TNP	21,000 (4.32 ± 0.21)
+	0	PC-TNP	3,400 (3.53 ± 0.35)
0	+	PC-TNP	7,200 (3.86 ± 0.31)
+	+	PC-TNP	23,400 (4.37 ± 0.26)

* Each group contained four or five mice; αH8 (0.2 ml, 1:4) and/or αPC (0.2 ml, 1:2) were given 7 d before challenge immunization.
‡ Differences in responses between groups receiving either αH8 or αPC alone and untreated mice or mice receiving both αH8 and αPC were significant, $P = < 0.05$. The mean numbers of αTNP PFC/spleen for the groups were 62,000, 66,000, 82,000, and 68,000, respectively.

mouse) prevented suppression caused by αH8 (data not shown). Thus, the results for these controls were consistent with previous findings that showed that reciprocal regulation of the αPC response by αH8 was specific and not caused by immunization with M315 (12). Also, the experiment was repeated, except that 0.3 ml whole A/He anti-PC serum was substituted for αPC; the antiserum was as effective as αPC in preventing suppression by αH8.

In other experiments, it was shown that treatment with αPC permitted recovery of the H8 (T15) idiotype rather than permitting or promoting expression of a different idiotype. For example, 10 mice were suppressed by giving αH8 (0.2 ml, 1:4 given twice 1 wk apart). Five of the suppressed mice and five normal mice were treated with αPC (0.2 ml, 1:2 given once a week for 3 wk, beginning 1 wk after the second injection of αH8). All 15 mice were challenged with PC and TNP 4 wk after the treatment was completed. The long interval between suppression and challenge permitted comparable recovery of total responses to PC with means of: 16,800 (4.23 ± 0.025), 13,200 (4.12 ± 0.21), and 18,000 (4.26 ± 0.18) PFC/spleen for the three groups; however, all five suppressed but untreated mice had <10% PFC of T15 idiotype, whereas all mice in the other two groups had >50% PFC of T15 idiotype with means of 73 and 65% PFC of T15 idiotype for these groups. The three groups had comparable responses to TNP.

The effect of treatment with αPC was tested next on mice suppressed by giving αH8 at the time of sublethal irradiation, a procedure that can cause prolonged and profound suppression (Table III). Mice were irradiated with 300 rad and injected with αH8 on the same day. Mice were treated with αPC given either 1, 3, and 5 or 7, 9, and 11 d after irradiation. In different experiments, mice were actively immunized with PC 3-5 wk after irradiation. Although the magnitude of responses in different experiments varied depending on dosages of αH8 and αPC and the interval between treatment and challenge, the essential finding demonstrated for one experiment (Table V) was consistent; i.e., treatment with αPC promoted recovery of responsivity to PC. Because sublethal irradiation with 300 rad reduces the total number of lymphocytes in the bone marrow and spleen to <5% and the number of pre-B cells to <1% of normal (54; and Ms. Deborah Dessner, personal communication), αH8 given after irradiation presumably tolerizes regenerating immature cells responsive to PC, and treatment with αPC prevents this.

TABLE V

Treatment with αPC Promotes Recovery from Suppression Caused by Irradiation and Complementary Idiotype

Day 0*		Days 7-11	Day 35	Day 39‡
Irradiation (300 rad)	αH8	αPC	Challenge immunization	αPC PFC/spleen
+	0	0	PC-TNP	27,800 (4.44 ± 0.41)
+	+	0	PC-TNP	8,600 (3.93 ± 0.24)
+	0	+	PC-TNP	6,000 (3.78 ± 0.18)
+	+	+	PC-TNP	28,200 (4.45 ± 0.38)

* Each group contained five or six mice. αH8 (0.2 ml, 1:4) was given on the day of irradiation, and αPC (0.2 ml whole) was given 7, 9, and 11 d after irradiation. Mice were challenged with PC-TNP 35 d after irradiation.

‡ Differences in responses between groups receiving either αH8 or αPC alone, and untreated mice or mice receiving both αH8 and αPC were significant, $P = < 0.05$. The mean numbers of αTNP PFC/spleen for the groups were 62,000, 74,000, 83,000, and 156,000, respectively.

Rescue from Suppression. In the above experiments, treatment with αPC alone prevented suppression or promoted recovery of mice given αH8 passively. We next attempted rescue in mice made unresponsive by active immunization with H8.

Using mice immunized with H8 1-3 mo previously, the variables tested included: (a) sublethal irradiation alone, (b) treatment with αPC alone, (c) combined treatment with irradiation and αPC, and (d) varying amounts of αPC used for treatment and intervals between treatment and challenge immunization. X-irradiation alone was not effective, which was not surprising because circulating αH8 actively produced before x-irradiation should have had the same effect of tolerizing regenerating lymphocytes as αH8 given passively to normal sublethally irradiated mice (see above experiments). Also, treatment with αPC alone was ineffective, although this might not have been the case if larger doses of αPC (>1.5 ml total) had been used and/or the interval between beginning treatment and challenge immunization was longer (>5 wk).

Rescue was observed only in mice that were irradiated and treated with amounts of αPC that produced serum levels approximating those obtained with active immunization. Rescue was not demonstrable at 2 wk and was only partial at 4-5 wk. For example, in one experiment, 18 mice to be tested were immunized with αH8 1 mo earlier; six were irradiated and injected with 0.5 ml of αPC 1, 3, and 5 d after irradiation, six were irradiated only, and six received αPC only. Three groups of six normal control mice were treated similarly. 5 wk after irradiation, all mice were challenged with PC-TNP. The six test mice irradiated and treated with αPC had a mean of 2,300 (3.36 ± 0.82) PFC/spleen to PC; all 12 test mice in the other two groups had <200 PFC/spleen. Irradiation and/or treatment with αPC had no affect on responses to PC of the three groups of normal control mice. All groups had comparable responses to TNP. These findings were confirmed in two additional experiments of similar design.

To augment recovery, mice actively immunized with H8 were irradiated, treated with αPC, injected with PC at 1 mo, and with PC-TNP for the final challenge immunization at 2 mo. The results presented in Table VI show almost complete recovery of response to PC in mice treated with αPC; furthermore, 40% of PFC of

TABLE VI

Rescue of a Clone Suppressed by Autogenously Produced Complementary Idiotype

Active immuniza-tion*	X-irradiation (300 rad)‡	Treat-ment§	Challenge im-mmunization‖	αPC response¶		
				PFC/spleen	T15 idiotype	PFC T15 idiotype
					%	
None	+	None	PC-TNP	12,600 (4.10 ± 0.12)	28	3,529
H8	+	None	PC-TNP	1,200 (3.08 ± 0.38)	0	—
H8	+	αPC	PC-TNP	8,000 (3.90 ± 0.29)	40	3,200

* Each group contained four mice.
‡ Irradiation was given 2 wk after active immunization.
§ Each treated mouse received five intraperitoneal injections of 0.5 ml of αPC given every other day beginning 2 d after irradiation. αPC was diluted 1:4 for the first injection, 1:2 for the second and third injections, and was used whole for the fourth and fifth injections.
‖ All mice were immunized with PC 1 mo after irradiation and with PC-TNP 2 mo after irradiation.
¶ The difference in response between the actively immunized group not treated with PC and either other group was significant, $P = < 0.05$. The mean numbers of αTNP PFC/spleen for the groups were 93,000, 85,000, and 105,000, respectively.

suppressed mice treated with αPC were of T15 idiotype, again indicating that treatment with αPC (T15 idiotype) permitted recovery of the T15 idiotype.

Discussion

Precise and specific regulation of immunity is a common objective in experimental and clinical immunology. Evidence for regulation usually depends on demonstrating specific suppression caused by antigen, lymphocytes, or the products of the immune response. In different models, the mediators of regulation may act directly on effectors or indirectly through suppression of helper activity. We failed to find consistent or impressive differences between suppressor function of cells obtained from suppressed or normal mice. Certainly our findings do not exclude a role for T suppressor (or absence of T helper) and/or accessory cells in causing or sustaining suppression, but apparently Ig can initiate whatever mechanism is ultimately responsible for suppression, including direct inactivation of B cells without involvement of T cells (28, 55). Because αH8 continues to circulate at appreciable titer for many months after immunization with H8, Ig alone may sustain suppression. There is the additional possibility that the αH8 that effectively eliminates complementary B cells may also inactivate or eliminate complementary T helper or suppressor cells. In support of this possibility, αH8 injected into BALB/c mice eliminated or inactivated PC-specific helper cells (39).

Termination of suppression presumably occurs because the suppressor system is exhausted or is eliminated. X-irradiation may reduce, at least temporarily, lymphocytes involved in suppression; x-irradiation may also stimulate proliferation of the suppressed, as well as nonsuppressed, clones during the massive regeneration of lymphoid tissue that occurs after sublethal irradiation (56). Antibody given passively during the phase of rapid regeneration may have the additional effect of neutralizing complementary antibody and tolerizing or eliminating regenerating lymphocytes that bear complementary receptors.

The condition of immunologic unresponsiveness must be a function of not only the state of the suppressor system but also of the state of the suppressed clones. If the rate

of regeneration of a clone is slow, taking many weeks or months, the current state of the suppressor system may not be relevant to understanding critical events that depleted mature responsive cells earlier. For example, it requires several months for either neonatal liver cells or spleen cells from mice made neonatally tolerant to PC to restore the response to PC of either heavily irradiated syngeneic recipients or unirradiated genetically unresponsive NBF_1 males, although both kinds of recipients support an αPC response of normal spleen cells on adoptive transfer (57, 58). Similarly, several months were required for Ig^- stem cells prepared from BALB/c spleens to establish responsivity to PC in NBF_1 mice (59). As far as we know, the limiting factor in restoring or establishing these responses in x-irradiated or NBF_1 recipients is the inherent slow rate of maturation of B cells from stem cells rather than the absence of idiotype-specific helper cells (20, 60) or other reactants that may be required for B cells to mature and respond.

Immature B cells in general are made tolerant more readily than mature B cells (43–47) with the provision that differences also exist between subpopulations (48, 49, 51, 52). Under usual circumstances, immature B cells specific for self antigens are presumably eliminated, whereas the remaining clones mature. Under appropriate conditions of antigen stimulation, mature B cells proliferate, differentiate, synthesize, and secrete Ig. The Ig secreted may then serve to protect the clone in two ways: (a) by tolerizing complementary clones, and (b) by protecting immature cells of the clone from tolerization by persisting antigen or complementary reactants if they are present. It is possible that analogous events occur when mature B cells in the adult are eliminated and clones are regenerated from stem cells.

Clearly, mature and immature B cells can be made tolerant by sufficient amounts of complementary antibody actively produced or passively given. Once mature cells are eliminated, Ig of the specificity produced by the clone will be absent or disappear. We assumed, therefore, that recovery of a suppressed clone required replenishment from stem or precursor cells and, in the absence of protective Ig, the maturing cells may be made tolerant by persisting antigen and/or complementary reactants so that tolerance is perpetuated. In any event, these considerations were consistent with our previous and present observations, which show the role of complementary antibodies in causing or preventing suppression, depending on the sequence of their action. These considerations also provided the rationale for giving Ig passively to rescue responses already suppressed.

There is presumably a selective advantage for having immune responses to infectious or noxious agents that are not suppressed prematurely or inappropriately. The early production of IgM may help serve this function. Although either antibody or the complementary antibody is a potent and specific immunosuppressant when given at the time of primary immunization in vivo or in vitro (53, 61–63), the role of actively produced antibodies in the homeostasis of ongoing immune responses remains, in our opinion, poorly understood. It is possible that only in the absence of an early and/or vigorous antibody response does a first immunization stimulate a complementary or anti-idiotypic response that is effective in regulating the first response. For example, contact hypersensitivity produced in mice by skin painting with dinitrofluorobenzene (DNFB) is apparently limited in severity and duration by autogenously produced anti-idiotypic antibody (36); interestingly, the procedure used to induce delayed-type hypersensitivity in these experiments does not result in detectable circulating antibody

to DNFB (Dr. Henry Claman, personal communication). Specific T blast cells stimulated by DNFB may initiate the anti-idiotypic response, a suggestion that is compatible with the demonstration that T blast cells selected for in culture can be used as antigen to stimulate an anti-idiotypic response in a normal host (18, 64). In another experimental model, autogenously produced anti-idiotypic antibody apparently causes feedback suppression of the antibody response of mice to TNP-Ficoll (32, 34, 35), but mice have only about 10% as high levels of antibody to the antigen as mice immunized with TNP coupled to more conventional carriers (Dr. Edmond Goidl, personal communication). In this instance, autogenous B or T blast cells may be effective immunogens for stimulating complementary B lymphocytes in the absence of early appreciable circulating αTNP antibody.

Absent or suppressed immunity can be rectified by adoptive transfer of appropriate syngeneic cells in some experimental models, but for outbred individuals, it may be more reasonable to attempt rescue from autogenous stem cells rather than create a chimera. The present studies suggest that passively given antibody may be used as a specific reagent for aiding in the rescue of clones having the same specificity as the passively given antibody. It seems likely from experimental observations and theoretical considerations discussed that sublethal x-irradiation or other procedures that destroy mature lymphocytes will be a useful or necessary adjunct.

Summary

A/He mice actively producing complementary or anti-idiotypic antibody directed against a combining site structure for phosphorylcholine (PC) have profound and long-lasting suppression of their response to PC. B cells from unresponsive mice are unresponsive in vitro, and attempts to demonstrate suppressor cells in unresponsive mice were unsuccessful. Although the process ultimately responsible for suppression has not been defined, suppression can be initiated by anti-idiotypic antibody alone and prevented by complementary Ig; i.e., by anti-PC antibody. Furthermore, a suppressed anti-PC response can be rescued by sublethal irradiation and anti-PC antibody given passively. The recovery of the suppressed response is slow and presumably results from maturation from "stem" cells, which are protected from tolerization by the passively given antibody. Thus, by extrapolation, one of the functions of secreted Ig may be to protect the clone that produces it.

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