SUPPRESSION BY AUTOGENOUS COMPLEMENTARY IDIOTYPES: THE PRIORITY OF THE FIRST RESPONSE*

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Conceptually, one antibody could be directed against a combining site structure of another antibody; thus, the two antibodies would combine with each other and might be considered at least partially complementary. Since the combining regions of antigen receptors on B lymphocytes are apparently identical with the combining regions of the antibodies that the cells produce (1–8), then complementary antibodies might also be considered complementary anti-receptor antibodies (9). By convention, one antibody is described as "idiotype," and the specific anti-antibody for it as anti-idiotypic. But these distinctions may be arbitrary when considering the functional relationships between complementary antibodies or receptors. Thus, for convenience in this paper, we will refer to idiotype and anti-idiotype as complementary idiotypes or antibodies.

Our evidence for complementary responses is derived from a model developed by Cosenza and Köhler (4, 5). In their studies, A/He mice were immunized with the phosphorylcholine (PC)[1] binding IgA myeloma protein produced by the plasmacytoma TEPC-15 (T15), induced in and carried by serial transplantation in BALB/c mice. The antibody raised to T15 neutralized the specific antibody activity of mouse IgM to PC, and it specifically suppressed the response to immunization with PC antigens when it was given passively to adult BALB/c mice or was added to cultures of BALB/c spleen cells. Thus, the antibody raised against an antibody, i.e. T15, had activity against a normally induced antibody, and apparently it functioned as an anti-receptor antibody (5).

Interestingly, sera from mice immunized repeatedly with a PC antigen contained not only antibody against PC but also antibody reactive with T15 (10). Presumably, such complementary antibodies occurring in the same individual might be autoregulatory. This possibility was explored in the present experiments by preimmunizing mice with T15 before measuring their response to immunization with PC, or alternatively, by preimmunizing with PC before measuring the response to immunization with T15. We find that prior repeated immunization with T15 markedly suppressed the response to PC and vice versa; however, with other immunization schedules, complementary responses occurred simultaneously or sequentially in individual mice.

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[1] *Abbreviations used in this paper:* FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; HBSS, Hanks' balanced salt solution; KLH, keyhole limpet hemocyanin; M315, MOPC-315; MBSA, methylated bovine serum albumin; PC, phosphorylcholine; PFC, plaque-forming cells; T15, TEPC-15.

Materials and Methods

Mice. All mice, except as noted below, were A/He females 8- to 12-wk old purchased from The Jackson Laboratory, Bar Harbor, Maine. The biological function of two pools of antisera raised in A/He mice was checked in cultures of BALB/c spleen cells (5) and in neonatal BALB/c mice (11). The BALB/c mice were purchased from Cumberland View Farms, Clinton, Tenn.

Antigens and Immunizations. A formalin-killed vaccine of *Diplococcus pneumoniae*, R36A (4), is referred to as vaccine. Mice were immunized with 0.2 ml of vaccine containing 10^9 organisms/ml which produces peak 4-day responses (5). PC is the major antigenic determinant mice respond to when immunized with the vaccine; the antibody is predominantly, but not solely, IgM (4, 11, 12). The C-polysaccharide extracted from R36A vaccine induces a response to PC in doses of 1–10 μg but induces immunologic paralysis to PC when given in doses of 400 μg (13).

A comparable IgM response is induced by PC coupled to various protein carriers (14); in the present experiments *p*-azophenylphosphorylcholine conjugated to keyhole limpet hemocyanin (PC-KLH) was also used as a PC-containing antigen (14). Mice were injected intravenously with 0.2 ml containing 0.7 mg PC-KLH/ml, or were injected in each hind foot pad with 0.05 ml of a mixture of 1 part PC-KLH, 0.7 mg/ml; 1 part methylated bovine serum albumin (MBSA), 1 mg/ml; 1 part *Bordetella pertussis* vaccine (strain 37790, lot T-59 187 kindly supplied through Dr. H. Campbell, Jr., Biological Development, Eli Lilly & Co., Indianapolis, Ind.); and 3 parts Freund's incomplete adjuvant (FIA) (Difco Laboratories, Detroit, Mich.) for the first immunization. A mixture of 2 parts PC-KLH, 1 part MBSA, and 3 parts FIA was used for subsequent immunizations in hind foot pads.

2,4-Dinitrophenyl (DNP)-Ficoll ($DNP_{39}AECM_{83}$) prepared by Dr. John Inman was kindly supplied by Dr. Donald E. Mosier, NIAID, National Institutes of Health, Bethesda, Md. Mice were immunized intravenously with 0.2 ml of a solution containing 10 μg DNP-Ficoll in saline.

Two myeloma proteins were used as antigens; the protein produced by the T15 plasmacytoma is an IgA which binds PC (but not DNP); and the protein produced by the MOPC-315 (M315) plasmacytoma, is an IgA which binds DNP (but not PC). The tumors were carried in the ascitic form, and the proteins were recovered from ascitic fluid. The ascitic fluid was partially reduced and alkylated. T15 was absorbed on PC-Sepharose, eluted with 10^{-2} M PC, extensively dialyzed, and concentrated or lyophilized. M315 was absorbed on trinitrophenyl-Sepharose, eluted with normal acetic acid, dialyzed, and concentrated. For immunization, the purified proteins, 1 mg/ml, were homogenized in a mixture of 1 part protein, 1 part MBSA albumin, and 2 parts Freund's adjuvant. Freund's complete adjuvant (FCA) (Difco Laboratories) was used for the first immunization; FIA was used for subsequent immunizations. Each immunization was in two depots of 0.05 ml, either in abdominal skin or in hind foot pads.

Spleen and Lymph Node Cells. Individual spleens were gently teased apart in 5.0 ml of Hanks' balanced salt solution (HBSS) using forceps and a wire screen. The cells were washed twice in 5.0 ml of HBSS and resuspended in 10.0 ml HBSS. The number of cells recovered per spleen was determined on an aliquot using a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.). 1–2 × 10^8 nucleated cells were usually recovered per spleen; 25 or 50 μl (i.e., 1/200 or 1/400 of a spleen) was plated on each slide for assaying numbers of plaque-forming cells (PFC).

After two or more foot pad injections, the popliteal nodes measured 3–5 mm in diameter. The two nodes were pooled and a suspension of single cells was prepared in the same manner as for spleens, except that after the final wash the cells were resuspended in 2.0 ml HBSS. The number of cells recovered per node was determined on a portion of the sample using a Coulter Counter; 5 × 10^6–2 × 10^7 cells were usually recovered from two nodes. The numbers of cells recovered from nodes were equivalent whether foot pads had been injected with adjuvants alone or with the adjuvants containing the antigens used. Either 25 or 50 μl (i.e., the equivalent of 1/20 or 1/40 of a single lymph node) was plated on each slide for assaying numbers of PFC.

Antibody Titers and PFC. Titration of serum antibody to PC and enumeration of cells producing such antibody were done using sheep erythrocytes (SRBC) coated with the C-polysaccharide of R36A vaccine (PC-SRBC) (4). Titration of serum antibody to T15 determinants and measurement of cells producing such antibody were done using SRBC coated with T15 (T15-SRBC). Chromium chloride was used for coating with both antigens; reproducible coating was achieved using chromium chloride in Tris buffer, pH 5.4, and carefully filtered and washed SRBC (13).

Assays for antibody were done in microtiter plates. Sera were diluted in phosphate-buffered

saline containing 5% heat-inactivated fetal calf serum. Wells contained 50 μl of diluted serum and 50 μl of a 0.15% suspension of coated or noncoated erythrocytes. Trays were shaken, incubated at 37°C for 1 h, and placed overnight in the refrigerator. The titer of antibody was taken as the reciprocal, $\log_2$, of the highest serum dilution which caused agglutination as indicated by a dispersed pattern of sedimentation of erythrocytes.[2]

For assaying numbers of PFC, 25 or 50 μl of cell suspension was mixed with 50 μl of a 5% suspension of target erythrocytes in 0.3 ml of 0.5% agarose in HBSS. The mixture was spread on a glass slide. Slides were prepared in duplicate for direct plaques against PC-SRBC and SRBC, and for indirect plaques against T15-SRBC and SRBC. Most PFC against PC are direct (14), and most PFC against T15 are indirect (13). Except when noted, only direct PFC to PC-SRBC and indirect PFC to T15-SRBC and SRBC were assayed. When direct PFC to TNP were assayed, the procedure was the same as previously reported (14). For direct PFC, slides were incubated first in a moist chamber and then with complement. For indirect PFC, slides were incubated with developing serum after the initial incubation; the slides were drained and washed once in HBSS and then incubated with complement. Each incubation period was 1 h. Complement was frozen guinea pig serum (Grand Island Biological Co., Grand Island, N. Y.) diluted 1:30 in HBSS. Developing serum was a goat anti-mouse Ig exhaustively absorbed with T15 and diluted 1:1,000 in HBSS. In some preliminary experiments, PFC against T15-SRBC were enumerated using Cunningham chambers and equal parts of fresh whole normal mouse and guinea pig serum as complement (13, 15); in repeated experiments, equivalent results were obtained using either this procedure or the developing serum and agarose slides.

The magnitude of a response was calculated from counts of two slides minus the background response to SRBC, which rarely exceeded 1,000 PFC of any kind for the spleen and 100 PFC of any kind for a lymph node. The experiments reported in detail included more than 100 normal A/He mice assayed for "background" spleen PFC against PC-SRBC, T15-SRBC, and SRBC. The numbers of PFC against PC-SRBC and T15-SRBC were rarely greater than PFC against SRBC; therefore, the background response of normal mice was not taken into account in reporting responses.

Results

The conditions for the experiments to be reported in detail were selected on the basis of previous findings (4, 5, 13, 14) and on a series of preliminary experiments which established appropriate doses of antigen, tissue sites, and times for assaying responses. In the present studies three PC antigens were used: R36A vaccine, PC-KLH, and the C-polysaccharide of R36A vaccine. Each of these in saline injected intravenously induced similar spleen PFC responses to PC-SRBC but little or no response detectable in lymph nodes. Repeated injections of PC-KLH in adjuvants (using either FCA or *B. pertussis* vaccine in FIA for the first injection) in hind foot pads induced a high PFC response in popliteal lymph nodes but little or no detectable response in other lymph nodes or the spleen.

Whereas the response to PC could be measured in either the spleen or popliteal lymph nodes depending on the immunization procedure, this was not the case for T15. Mice repeatedly injected intravenously with a range of doses of T15 in saline had no detectable spleen PFC or serum antibody response to T15-SRBC. However, T15 mixed with adjuvants and injected in abdominal skin or hind foot pads two or more times at weekly intervals induced serum antibody to T15-SRBC. Responses were highest when FCA rather than FIA was used for the

[2] Sera pools from normal A/He mice may have very low titers against SRBC; titers of normal serum against PC-SRBC or T15-SRBC were never higher than titers against SRBC alone. Immunization with PC or T15 did not cause any detectable rise in titers to SRBC. No attempt was made to adjust titers because of low "background" titers to SRBC.

first immunization. (*B. pertussis* vaccine was not tested.) By using injections in hind foot pads, the predominant response was initially limited to the popliteal lymph nodes, though a considerable splenic response did occur after four or more injections (unpublished observations). The following experiments demonstrate the kind of responses obtained using the immunization procedures selected for subsequent experiments.

The Response to Immunization with PC or T15. Mice were injected every 7 days either intravenously with vaccine or in hind foot pads with PC-KLH or T15 in adjuvants. Groups of three mice were sacrificed 2, 4, and 7 days after each immunization. Peak spleen PFC responses were always 4 days after the last immunization with vaccine. No demonstrable response occurred after a single immunization in hind foot pad with either PC-KLH or T15. The results obtained 4 days after one to three immunizations are shown in Table I. Though not reported in Table I, immunization with PC antigens did not induce a detectable response to T15, and immunization with T15 did not induce a detectable response to PC.

Preimmunization with T15 Suppresses the Response to PC. In preliminary experiments mice were challenged intravenously with vaccine 1 wk after the last of weekly immunizations in foot pads with T15; the response to PC was assayed 2, 4, 5, and 10 days later. Mice preimmunized once with T15 invariably had normal responses to PC. An occasional mouse (2 of 16 in four experiments) immunized twice with T15 had reduced responses to PC. In contrast, mice preimmunized three times with T15 always had reduced responses to PC (exp. 1, Table II), a finding confirmed in two additional experiments also reported in Table II. Mice challenged for as long as 5 wk after the last immunization with T15 were markedly suppressed. Preimmunization with T15 did not suppress responses to SRBC or TNP; furthermore, preimmunization with a non-PC-binding IgA mouse myeloma protein failed to suppress the response to PC. Thus, insofar as was tested, suppression by preimmunization with T15 was specific.

Preimmunization with T15 also suppressed the response to PC-KLH injected intravenously (unpublished observations) or injected with adjuvants in hind foot pads. For the latter observation, mice received three weekly injections of T15 given in depots in abdominal skin using FCA for the first immunization. Beginning 1 wk after the last preimmunization, mice were injected twice at weekly intervals in hind foot pads with PC-KLH in FIA using pertussis vaccine for the first immunization. Popliteal lymph nodes were assayed 4 days after the second injection. Four of four preimmunized mice had fewer than 100 PFC to PC-SRBC per popliteal lymph node while mice immunized only with PC-KLH had a mean of 2,192 (3.34 ± 0.05) PFC to PC-SRBC per popliteal lymph node.

Preimmunization with PC Suppresses the Response to T15. In a series of experiments mice were preimmunized either once, twice, or three times with PC-KLH in adjuvants or intravenously with vaccine. The mice were challenged in hind foot pads with T15 beginning 1 wk after the last immunization with PC. Popliteal lymph nodes were assayed 4 days after the second injection of T15. Mice preimmunized once or twice with PC had normal or only slightly lower responses to T15; in five experiments mice preimmunized three times with a PC antigen had responses to T15 suppressed by three- to fivefold, as is shown for one such experiment in Table III. Controls in this series of experiments included

TABLE I

Response to Immunization with PC or T15

Immunization*		Response‡ No. of immunizations		
		1	2	3
R36A vaccine i.v.	Log_{10} PFC per spleen to PC	4.41 ± 0.21	4.22 ± 0.16	3.88 ± 0.12
	Log_2 serum antibody titer to PC	6	5	6
Pc-KLH + adjuvants in foot pads	Log_{10} PFC per lymph node to PC	<2.0	3.82 ± 0.09	3.68 ± 0.07
	Log_2 serum antibody titer to PC	0	4	4
T15 + adjuvants in foot pads	Log_{10} PFC per lymph node to T15	<2.0	3.44 ± 0.15	3.85 ± 0.05
	Log_2 serum antibody titer to T15	0	4	8

* Mice were immunized every 7 days.

‡ Responses were measured 4 days after the last immunization. Each mean ± SEM calculated on log transformed data is for a group of three mice. Each antibody titer is for serum pooled from three mice.

TABLE II

Preimmunization with T15 Suppresses the Response to PC

Immunization*		Response‡	
Preimmunization	Challenge immunization	Log_{10} PFC per spleen to Pc	Log_{10} PFC per spleen to TNP
Exp. 1			
T15	Pc	2.45 ± 0.67 } $P < 0.015$	-
None	Pc	4.48 ± 0.10 }	-
Exp. 2			
T15	Pc	2.12 ± 0.18 } $P < 0.001$	-
None	Pc	4.58 ± 0.07 }	-
Exp. 3			
T15	Pc	2.76 ± 0.59 } $P < 0.050$	3.05 ± 0.01
None	Pc	4.24 ± 0.11 }	3.02 ± 0.14
M315	Pc	4.72 ± 0.10	3.15 ± 0.14
T15	DNP	<2.0	4.80 ± 0.10
None	DNP	<2.0	4.81 ± 0.12
M315	DNP	<2.0	5.03 ± 0.07

* Mice were preimmunized in hind foot pads once a week for 3 wk and challenged intravenously 1 wk after the third preimmunization in experiments 1 and 3 and after 5 wk in exp. 2.

‡ Responses were assayed 4 days after the challenge immunization. Each mean ± SEM calculated on log transformed data is for a group of four or five mice. Additional controls in exps. 1 and 2 included groups preimmunized with T15 and untreated mice injected intravenously with 0.2 ml 5% SRBC; preimmunization with T15 enhanced mean responses to SRBC by two- and threefold.

preimmunized mice challenged in foot pads with 20% SRBC substituted for T15, or preimmunized mice challenged intravenously once with TNP-Ficoll. Preimmunization with PC had no effect or slightly enhanced responses to these antigens.

Equivalent suppression of the responses to T15 was obtained when relatively large amounts of serum from mice immunized three or more times with PC was given passively to normal mice. For example, in one experiment mice were each

TABLE III
Preimmunization with Pc Suppresses the Response to T15

Immunization*		Response‡	
Preimmunization	Challenge immunization	Log_{10} PFC per lymph node to T15	Log_{10} PFC per lymph node to SRBC
Exp. 1			
Pc	T15	2.95 ± 0.19 } $P < 0.005$	<2.0
None	T15	3.75 ± 0.30 }	<2.0
Exp. 2			
Pc	T15	2.51 ± 0.22 } $P < 0.050$	<2.0
None	T15	3.22 ± 0.18 }	<2.0
Pc	SRBC	<2.0	3.20 ± 0.06 } $P > 0.200$
None	SRBC	<2.0	3.20 ± 0.15 }

* In exp. 1 mice were preimmunized in abdominal skin once a week for 3 wk and challenge immunized in hind foot pads beginning 1 wk after the third preimmunization. In exp. 2 mice were passively immunized with syngeneic antiserum having an anti-PC titer of 7; 0.8 ml was given 4 h before the first and 0.6 ml was given 4 h before the second challenge immunization.

‡ Responses were assayed 4 days after the second challenge immunization. Each mean ± SEM calculated on log transformed data is for a group of five mice in exp. 1 and for a group of three mice in exp. 2. In other experiments, preimmunization with PC as done in exp. 1 enhanced responses to SRBC or TNP-Ficoll by two- to threefold.

injected intraperitoneally with a total of 1.4 ml of serum having a titer of 7 for PC; 0.8 ml was injected 4 h before the first immunization in hind foot pads; 0.6 ml was injected 1 wk later, 4 h before the second immunization in hind foot pads. Whereas the passively given antiserum suppressed immunization with T15, it had no detectable effect on immunization with SRBC (exp. 2; Table III).

The Failure of T15 Alone or the C-Polysaccharide of R36A Vaccine Alone to Prevent Induction of Complementary Responses. Preimmunization with T15 suppressed immunization with PC, presumably by inducing antibody to T15; however, it might be argued that T15 was released from the depots of T15 in adjuvants and suppressed by combining with the PC antigens. This seems unlikely for several reasons. First, sera from mice bled after one or more immunizations with T15 in adjuvants had no detectable agglutinins for PC-SRBC; second, mice immunized with T15 using only FIA were not suppressed (unpublished observations). Furthermore, injections of T15 at the time of immunization with PC had relatively little effect on the response to PC. This was demonstrated by injecting mice with either 75 μg or 375 μg of T15 in saline 1 day before intravenous immunization with vaccine. The smaller amount, equal to the total amount used to immunize three times with T15 in adjuvants, did not suppress while the larger dose suppressed only slightly, though the mice had high agglutinin titers for PC from the T15 given passively (Table IV). The failure of T15 to suppress is consistent with the observation that the Fc portion of Ig plays an important role in antibody-mediated suppression of the antibody response (16, 17).

The effect of a circulating PC antigen on immunization with T15 was also tested. Intravenous injection of 4 μg of the C-polysaccharide extracted from

TABLE IV
The Failure of T15 in Saline to Inhibit Immunization with PC

Immunization*		Response‡	
Preimmunization	Challenge immunization	Log_{10} PFC per spleen to PC	Log_2 serum antibody to PC
375 μg T15	PC	4.07 ± 0.05	8
75 μg T15	PC	4.71 ± 0.11	5
None	PC	4.65 ± 0.10	3

* Mice were injected intravenously with T15 in saline 1 day before intravenous challenge immunization with R36A vaccine.

‡ Responses were assayed 4 days after challenge immunization. Each mean ± SEM calculated on log transformed data is for a group of five mice. Each antibody titer is for serum pooled from five mice.

R36A vaccine induces a good anti-PC response in mice, whereas injection of 400 μg induces immunologic paralysis to PC (13). Injection of the larger dose results in levels of polysaccharide of about 50 μg/ml 1 day later and 5 μ/ml of serum 1 wk later (13). Mice were injected intravenously with 400 μg of the C-polysaccharide either 1 wk or 1 day before beginning immunizations with T15 in adjuvants; popliteal lymph nodes were assayed 4 days after the second weekly injection of T15. As shown in Table V, a "paralyzing dose" of C-polysaccharide had little effect on the response to T15. Thus, the suppression of the response to T15 produced by repeated immunization with PC was probably due to induction of antibody to PC rather than to release of PC antigen which combined with T15.

Taken together, our findings indicate that antibody passively given or actively produced can suppress induction of a complementary response. In the following experiments, we tested the effect of an active response on a previously induced complementary response.

The Failure of Immunization with T15 to Suppress either a Secondary or a Sustained Response to PC. Mice were immunized intravenously with vaccine, and then beginning 1 wk later were immunized at weekly intervals for 3 wk with T15 in adjuvants. 1 wk after the third immunization with T15, the mice were again injected intravenously with vaccine. 4 days later spleens and lymph nodes were assayed. The results (Table VI) demonstrate that intervening immunization with T15 suppressed only very slightly the "secondary" PFC response to PC, though it did lower the serum antibody titer by greater than 10-fold. As noted in previous experiments, the lymph nodes of mice immunized intravenously once with PC and then in foots pads with T15 had almost as many PFC to T15 as mice immunized with T15 alone; agglutinin titers for T15-SRBC were also equivalent. Thus, the initial single immunization with PC had not suppressed the final response to immunizations with T15, just as intervening immunization with T15 did not suppress the secondary PFC to PC.

In another experiment mice were immunized intravenously every week with PC, and beginning 1 wk after the first immunization they were also immunized in hind foot pads with T15. Mice were killed after they had received four immunizations with PC and three immunizations with T15. Immunization with T15 significantly enhanced rather than suppressed the PFC response to PC, though serum antibody titers to PC were lowered by more than 10-fold (Table

TABLE V

The Failure of a Paralyzing Dose of C-Polysaccharide of R36A Vaccine to Inhibit Immunization with T15

Immunization*		Response‡	
Preimmunization	Challenge immunization	Log_{10} PFC per lymph node to T15	Log_{10} PFC per spleen to PC
C-polysaccharide – 7 days	T15	3.65 ± 0.21	–
C-polysaccharide – 1 day	T15	3.49 ± 0.09	–
None	T15	3.84 ± 0.13	–
C-poplysaccharide – 7 days	PC	–	3.15 ± 0.06 } $P < 0.001$
None	PC	–	4.74 ± 0.22 }

* Mice were injected intravenously with 400 μg of the C-polysaccharide of R36A vaccine before two challenge immunizations in hind foot pads with T15 in adjuvants or a single intravenous injection of R36A vaccine.

‡ Responses were assayed 4 days after the second challenge immunization with T15 or 4 days after the single challenge immunization with vaccine. Each mean ± SEM calculated on log transformed data is for a group of four mice.

VII). Neither the numbers of lymph node PFC nor the levels of serum antibody to T15 were affected appreciably by immunization with PC. The experiment was repeated except that mice were killed after five immunizations with PC and after four immunizations with T15. Again, immunization with T15 enhanced the number of PFC to PC but reduced serum antibody to PC significantly, and as in the experiment reported in Table VI immunization with PC did not measurably affect the response to T15.

The Biological Activity of Serum from Mice Immunized with T15 or PC. Sera were pooled from mice bled 4 days after three immunizations with T15; one pool was from appropriate mice in experiments done early in the course of this work, and the other pool was from appropriate mice in experiments done near the conclusion of the work reported here. The first pool had a titer of 11 and the second pool a titer of 9 for T15-SRBC. Neither pool had detectable agglutinins for PC-SRBC. Both pools, final dilution of 1:200 in cultures of BALB/c spleen cells, completely suppressed the response to immunization with vaccine while control cultures without added sera had >1,000 PFC to PC per culture. These sera did not suppress the response in vitro to immunization with SRBC or TNP. 12 neonatal BALB/c mice from three litters were given 0.1 ml of one or the other pool; the mice were completely unresponsive to PC when immunized with vaccine 6–18 wk later; all of 9 littermates which did not receive sera and were similarly immunized with vaccine had >25,000 PFC per spleen. Four adult A/He mice given 0.3 ml of the first pool 1 day before immunization with vaccine had PFC responses to PC of <500 per spleen while three controls immunized similarly with vaccine had >10,000 PFC per spleen; the second pool was not tested in adult mice. Thus, the A/He mice as immunized with T15 in these experiments produced antisera which had activity similar to sera obtained from mice immunized more intensely as reported in previous experiments (6, 7).

In addition, serum pooled from five mice immunized three times with T15 in adjuvants and a pool from five mice immunized three times with R36A were tested for inhibition of plaque formation using cells producing complementary

TABLE VI

A Secondary PFC Response to PC Was Not Suppressed by Immunization with T15

Immunization*			Response‡,§	
Preimmunization	Intervening immunization	Challenge immunization	Log_{10} PFC per spleen to Pc	Log_2 serum antibody titer to Pc
PC	T15	Pc	4.39 ± 0.06 } $P < 0.050$	3
PC	None	Pc	4.76 ± 0.14	7
None	T15	Pc	<2.0 } $P < 0.001$	1
None	None	Pc	4.34 ± 0.09	2
PC	T15	None	3.22 ± 0.15 } $P > 0.400$	2
PC	None	None	3.29 ± 0.05	1

* Mice preimmunized once with R36A vaccine given intravenously were injected in foot pads with T15 in adjuvants beginning 1 wk later. 1 wk after the third injection of T15, the mice were challenged intravenously with vaccine. Controls were injected as indicated.

‡ Responses were measured 4 days after the challenge immunization. Each mean ± SEM calculated on log transformed data is for a group of four mice. Each antibody titer is for serum pooled from four mice.

§ Popliteal lymph nodes were pooled from mice in each group immunized with T15. The pools contained equivalent numbers (log_{10} = 3.10–3.12) of PFC/node to T15-SRBC. Also, all three pooled sera from these groups had a log_2 antibody titer to T15-SRBC of 11.

antibody. Spleen cells obtained from mice injected once intravenously with R36A vaccine were mixed with PC-SRBC in agarose; 50 μl of a dilution of the anti-T15 serum was added, and the mixture was spread on a slide; the slides were processed as usual for demonstrating PFC to PC. Similarly, slides were prepared using popliteal lymph node cells obtained from mice immunized twice with T15 in adjuvants, T15-SRBC, and dilutions of the anti-PC serum. Anti-PC plaque formation was completely inhibited by a dilution of the anti-T15 serum which would give an estimated agglutinin titer of three to four in the cell-agarose mixture. Similarly, the anti-PC serum completely inhibited plaque formation by cells releasing anti-T15 with a dilution of the serum which would give an estimated titer of four in the cell-agarose mixture. Neither antiserum inhibited plaque formation by cells immunized against SRBC.

Discussion

It seems reasonable to assume that antibody, whether given passively or induced actively, suppressed induction of a complementary response by interacting with complementary receptors on immunocompetent cells. Presumably, such suppression is a function of the class, quantity, and specificity of the "regulating" antibody; and possibly the resulting effect is modified by a shift in the "idiotype" of the regulated response (18). To date, we have not examined these variables in a systematic way. We know that immunization with T15 in adjuvants induces in popliteal lymph nodes cells which release IgG_1 and IgG_2 against T15 (unpublished observations). Also, we detected indirect PFC to PC in mice immunized multiple times with PC but not in mice immunized once with PC. But we have not compared classes of purified antibodies for their capacity to

TABLE VII

A Sustained Response to PC Was Not Suppressed by Superimposed Immunization with T15

Immunization*		Response‡			
		To PC		To T15	
PC	T15	Log_{10} PFC per spleen	Log_2 serum antibody titer	Log_{10} PFC for lymph nodes	Log_2 serum antibody titer
4 ×	3 ×	4.38 ± 0.29 } $P < 0.10$	2	4.15 ± 0.07	10
4 ×	None	3.87 ± 0.07 }	6	–	0
None	3 ×	<2.0	0	4.43 ± 0.21	9

* Mice immunized with both antigens were injected intravenously every 7 days with R36A vaccine and beginning with the second immunization they were also injected in hind foot pads with T15 in adjuvants. Controls were injected with one or the other antigen only.

‡ Responses were measured 4 days after the last immunization. Each mean ± SEM calculated on log transformed data is for a group of five mice. Each antibody titer is for serum pooled from five mice.

cause suppression or possibly to stimulate responses when given passively. Eichmann, using heterologous anti-idiotypic antibody, found that IgG_1 stimulated while IgG_2 suppressed responses (19). If Eichmann's findings can be extrapolated in a general way to the function of autogenous antibody, then the resultant effect of suppression or stimulation will depend on the ratio of classes of antibody produced.

Presumably, only antibodies direct against combining site structures of complementary antibody or receptors are involved in regulation of that particular response, but *a priori*, there is no reason why antibodies produced by different clones could not be directed against different portions of the receptor. If this is the case, then antibodies of different specificities may vary in effectiveness in blocking or altering receptor function. However, this variability may be minimal in our model because the response to PC is predominantly of a single idiotype (14, 20–24), though antiserum to T15 may contain antibody to determinants outside the combining region and a determinant limited to the heavy chain (20, 21, 24). We find that antiserum to PC in low titer will inhibit plaque formation by cells from mice immunized with T15 whether or not the mice have a simultaneous response to PC; similarly, antiserum to T15 in low titer will inhibit plaque formation by cells from mice immunized with PC whether or not the mice have a simultaneous response to T15 (unpublished observations). Thus, to the limited extent we have examined the problem, we have not detected a change in combining site determinants of antibodies from mice having simultaneous complementary responses.

The present experiments provide no evidence that complementary antibodies cause "feedback" suppression once immunization has occurred. Apparently, once a clone has been stimulated and expanded by immunization, complementary antibody does not reduce the number of cells in the clone which can produce antibody. Thus, immunization with T15 did not suppress a secondary PFC response to PC; however, simultaneous immunization did lower serum antibody

titers to PC very substantially (Tables VI and VII). Lower levels of serum antibody may have occurred because of formation of complexes. When serum containing antibody to PC is mixed with serum containing antibody to T15, the specific antibody activity of each serum is partially quenched, though mixtures prepared in different ratios may still have activity to one, the other, or both antigens (25). Thus, serum antibody titers from mice having simultaneous complementary responses may reflect the fact that two responses have occurred, but such titers may indicate inaccurately the magnitude of each of the responses. Though it seems most likely that the lower level of serum antibody to PC resulted from formation of complexes with antibody to T15, it is conceivable that high levels of circulating anti-T15 may suppress in vivo the syntheis and/or release of anti-PC antibody from cells. If this were the case, then removal of the cells from the milieu of antibody to T15 and washing them may reverse suppression so that the cells were identified and enumerated as PFC. At present we cannot confirm or rule out this possibility.

The response of A/He mice to immunization with PC is about 5- to 10-fold lower than for BALB/c mice, but A/He mice immunized with T15 have about 5- to 10-fold higher responses than BALB/c mice (unpublished observations). Presumably these differences are a function of the relative sizes of the clones which in turn are genetically determined. However, a response may be suppressed when a complementary response has been induced first. Possibly the relative unresponsiveness of BALB/c mice to immunization with T15 is partly due to their high background anti-PC response resulting from exposure to environmental PC-containing antigens (26). Certainly we find much higher levels of background serum antibody and spleen PFC to PC in BALB/c than in A/He mice, but we have not attempted to correlate the magnitude of induced anti-T15 responses with background levels of antibody to PC in individual mice.

Specific suppression can be mediated by cells, and it is possible that suppression observed in actively immunized mice was caused by cells in addition to or rather than by antibody. But regardless of the mechanism, the present experiments and those reported earlier (27) demonstrate quite dramatically that specific suppression can be induced by immunization with antigens which can not be considered cross-reacting in any conventional sense. In both studies the myeloma proteins used as antigens were injected multiple times with adjuvants. Also, polymerization of BALB/c myeloma protein enhanced immunogenicity of the protein for BALB/c mice (28); similarly, autoantibody was purified and polymerized to induce autoanti-idiotypic antiserum (29). The relevance of these kinds of studies to autoregulation of responses may be questioned legitimately because of the vigorous manipulations necessary to make autoantibody antigenic (30). On the other hand, anti-idiotypic antibody has been used to stimulate an anti-PC response in vitro (31), and antigen-antibody complexes in antibody excess may induce complementary or autoanti-idiotypic antibodies in vivo (9, 10, 32). Conceivably, antibody of any specificity, complexed in excess with its antigen, may function as an antigen and induce a complementary response. Possibly most or all antibodies might be thought of as complementary to some other antibody in the antibody repertoire of an individual and be considered part of an extensive network (33).

Summary

Complementary idiotypes or antibodies are considered to have combining site structures which are at least partly directed against each other. Complementary antibodies were induced in A/He mice by immunization with phosphorylcholine (PC)-containing antigens and by immunization with the PC-binding IgA myeloma protein TEPC-15 (T15). Both responses were monitored by enumerating plaque-forming cells (PFC) and assaying serum antibody levels against the corresponding antigens. Mice immunized at least three times with T15 in adjuvants had markedly suppressed responses to subsequent immunization with PC; similarly, mice preimmunized multiple times with PC had suppressed responses to immunizations with T15. In contrast, mice immunized with T15 in the interval between "primary" and "secondary" immunizations with PC had undiminished PFC responses to both antigens but significantly decreased antibody titers to PC. Simultaneous responses were also induced by immunizations with T15 superimposed on weekly immunizations with PC; with this regime, immunization with T15 actually enhanced the PFC response to PC, but serum antibody to PC was significantly lower than for mice immunized with PC only. Levels of serum antibody to PC were probably lower, either because anti-PC antibody was complexed with the complementary antibody directed against T15, or because the antibody directed against T15 prevented synthesis and/or release of anti-PC antibody by cells in vivo. Thus, an established prior autogenous immune response can dramatically suppress a subsequent primary complementary response, but the effects of complementary responses on each other are more complex with different sequences of immunization. Also, the effects of variables such as the amounts and ratios of the classes of antibodies on regulation of complementary responses remain to be defined.

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