INDUCTION OF IDIOTYPE-SPECIFIC SUPPRESSOR T CELLS WITH ANTIGEN/ANTIBODY COMPLEXES*

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A central question in immunology is how the antibody response is regulated in a specific manner. Early observations indicated that the response is controlled through feedback inhibition since the passive administration of antibody could markedly suppress the response to the corresponding antigen (1–3). This suppression may be mediated through the formation of antigen/antibody complexes since several investigators have found immune complexes to be immunosuppressive (4–6).

Jerne (7) has proposed that an autoimmune response to self-idiotopes (Id)[1] might form the basis of an immunoregulatory network. Thus, the injection of antigen elicits the production of antibodies whose variable regions carry unique antigenic determinants (Id). Once these Id (expressed either on cells or as antibodies) reach a critical threshhold, they are thought to become immunogenic and to induce an auto-anti-Id response. Indeed, the production of anti-Id antibody has been demonstrated during a normal immune response (8, 9). These results are particularly striking since the induction of iso-anti-Id antibodies usually requires prolonged immunization using potent adjuvants (10). However, the explanation may be that during the normal immune response, antigen becomes complexed with antibody rendering it highly immunogenic. Support for this was provided by Klaus (11) who showed that preformed complexes readily induced high titers of anti-Id antibody.

The immune response is also regulated by cells that help or suppress antibody production; for example, specific suppressor T cells (Ts) can be induced by cultivation of lymphocytes with antigen (12, 13). Compared with the antigen-specific suppressor cells, the evidence for the immunoregulatory role of anti-Id suppressor cells is less compelling. Such cells have been generated by the administration of anti-Id antibody (14, 15), immunization with purified myeloma proteins in adjuvant (16), or administration of idiotype coupled to syngeneic lymphocytes (17–18). It has not been well established whether anti-Id suppressor cells for the antibody response become activated during an antigen-triggered immune response, although Bona and Paul (19)

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[1] *Abbreviations used in this paper:* BA, *Brucella abortus*; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; Id, idiotope; PBS, phosphate-buffered saline; PC, phosphorylcholine; PFC, plaque-forming cell; Pn, *Streptococcus pneumoniae* R36 vaccine; PnC, cell wall polysaccharide extracted from Pn; RAMIg, rabbit anti-mouse Ig; RBC, erythrocyte; T15, idiotype family expressed on TEPC-15 myeloma protein; TNP, trinitrophenyl; Ts, suppressor T cell.

have shown that M-460 idiotype-binding Ts pre-exist for the response to trinitrophenyl (TNP)-Ficoll in BALB/c mice.

In previous experiments, we attempted to induce idiotype-specific suppressor cells by culturing BALB/c spleen cells with a high dose of bacterial vaccine prepared from *Streptococcus pneumoniae* R36a (Pn). However, the suppressor cells induced with Pn were idiotype-positive T cells rather than antiidiotypic (20), and more recently, it was shown that these Ts express some, but not all, T15 idiotopes also expressed on B cells (21). The failure to induce anti-Id suppressor cells with antigen alone, together with the findings of Klaus (11) that antigen/antibody complexes readily induced anti-Id antibody led us to look for the induction of Ts with immune complexes.

As a model, we have used the in vitro antibody response of BALB/c mice to a Pn vaccine (22). The cell wall polysaccharide (PnC) extracted from Pn was used to form complexes with TEPC-15, an IgA myeloma protein that binds to phosphorylcholine (PC) determinants on the PnC. We report here that these antigen/antibody complexes can induce specific suppressor T cells, and that these cells appear to be idiotype specific.

Materials and Methods

Mice. BALB/c mice were purchased from the Charles River Breeding Laboratories, Wilmington, MA, or in later experiments, from The Jackson Laboratory, Bar Harbor, ME. T15 idiotype-suppressed mice and their age-matched controls were generously provided by Dr. Christoph Heusser, Ciba-Geigy Ltd., Basel, Switzerland. The T15-suppressed mice had been prepared by injecting BALB/c mice with 50 μg of monoclonal anti-T15 antibody MaId5-4 intraperitoneally within 24 h of birth. Preimmune adult sera from the suppressed mice did not have detectable antibodies (<0.1 μg/ml) with the MaId5-4 idiotope as measured by a reverse hemagglutination assay using MaId5-4-coated erythrocytes (RBC) (23). Serum from the normal BALB/c mice had about 20 μg/ml T15 Id.

Antigens. Sheep and burro RBC were purchased from the Cleveland Scientific Co., Cleveland, OH. TNP-conjugated *Brucella abortus* (TNP-BA) was prepared and provided by Dr. James J. Mond, Uniformed Services University of Health Sciences, Bethesda, MD. A formaldehyde-treated vaccine was prepared from Pn as described by DuClos and Kim (24), and the optimal immunogenic concentration of each batch was determined empirically. PnC was extracted from Pn as described by Anderson and McCarty (25).

Monoclonal Antibodies and Myeloma Proteins. The TEPC-15 and McPC-603 myeloma cell lines (26) were grown as ascites in BALB/c mice. The AB1-2 (anti-T15) hybridoma cell line (27) was generously provided by Dr. John Kearney, University of Alabama, Birmingham, AL. The myeloma proteins were prepared by ammonium sulfate precipitation of ascites fluid as described previously (28). Monoclonal anti-Thy-1.2 antibody H0 13-4 (29) was conjugated with fluorescein isothiocyanate (FITC) (Sigma Chemical Co., St. Louis, MO) as described elsewhere (30).

Preparation of Immune Complexes. TEPC-15 or McPC-603 myeloma proteins were mixed with PnC at a 10:1 or 50:1 (wt/wt) ratio, respectively, and incubated at 37°C for 30 min and then at 4°C overnight. The complexes were then washed twice at 800 *g* and resuspended in saline. An aliquot was diluted in 0.1 N NaOH, and the protein concentration was estimated by measuring the optical density at 280 nm ($E^{1cm}_{0.1\%} = 1.4$). The amount of complexes added to lymphocyte cultures was based on the protein content of the immune complexes.

"Panning" Techniques. The methods for lymphocyte separations were based on the methods described by Mage et al. (31) and Wysocki and Sato (32). Rabbit anti-mouse immunoglobulin (RAMIg) was prepared by hyperimmunizing rabbits with normal mouse IgG (prepared by affinity chromatography on protein A-Sepharose) in complete Freund's adjuvant. Mouse IgG Sepharose columns were prepared by conjugating protein A-purified mouse IgG onto cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). The rabbit serum diluted 1:2 in carbonate buffer, pH 8.3, was added to the column and the nonspecific proteins were washed through with buffer. The RAMIg was eluted with 0.2 N acetic acid in 0.5

M NaCl, pH 2.5, dialyzed against phosphate-buffered saline (PBS), filter sterilized, and stored at 4°C. Bacteriological grade petri dishes (1005; Falcon Labware, Oxnard, CA) were incubated with 10 ml RAMIg diluted to 10 μg/ml in Tris-buffered saline, pH 9.5, for 1 h at room temperature. The plates were washed five times with saline and then stored at 4°C in PBS/5% fetal calf serum (FCS) until used.

For lymphocyte separations, spleen cells were treated with Tris-ammonium chloride buffer to remove RBC (33) and adjusted to 10^7 cells/ml in PBS/5% FCS. 5-ml aliquots of the cell suspension were added to the RAMIg plates and incubated at 4°C for 1 h. The plates were then washed to remove the nonadherent cells, which were routinely <5% surface Ig-positive and >85% Thy-1.2-positive as assessed by membrane fluorescence with FITC-RAMIg and FITC-conjugated anti-Thy-1.2, respectively.

Two methods for positive selection of T cells were used. In the first method, the cells nonadherent to RAMIg plates were treated with a murine anti-Thy-1.2 monoclonal antibody (1:100) for 30 min on ice. The cells were washed in the cold and then added back onto RAMIg plates and incubated for 1 h at 4°C. The plates were washed five times with cold PBS and then the adherent cells were removed by directly pipetting PBS/5% FCS onto the surface of the plates. Removal of the adherent cells was monitored using an inverted microscope.

The second method for positive selection of T cells was a modification of the technique recently described by Fong et al. (34). In these experiments, cells were treated with FITC-conjugated anti-Thy-1.2 antibody for 30 min on ice, washed, and added to plates coated with affinity-purified goat anti-FITC antibody, a gift from Dr. James Monthony, Bio-Rad Laboratories, Richmond, CA. After 1 h at 4°C., the plates were washed and the adherent cells collected as described above. These cells were routinely 98–100% Thy-1.2-positive.

Idiotype-binding Assay. The TEPC-15 myeloma protein was radiolabeled with ^{125}I, and the idiotype-binding assay was performed as described elsewhere (28). For the binding assay, aliquots of 10^6 purified T cells were pelleted and resuspended in a total volume of 0.1 ml of Hanks' balanced salt solution (HBSS) (Microbiological Associates, Walkersville, MD) with 5% FCS in the presence of increasing amounts of unlabeled TEPC-15 or MOPC-315 myeloma proteins together with 5 μg of ^{125}I-TEPC-15 (≅10,000 cpm/μg protein). The cells were incubated overnight at 4°C and then washed four times with cold HBSS/5% FCS. The resulting cell pellets were then counted in a Beckman Gamma 4000 spectrometer. The maximum idiotype binding was ~150 ng/10^6 T cells.

Antibody Response in Lymphocyte Cultures. The primary antibody plaque-forming cell (PFC) response was initiated in Marbrook culture chambers (Bellco Glass, Inc., Vineland, NJ) as described previously (35). Direct PFC were enumerated on day 5 by a modification of the Jerne plaque assay using RBC, TNP-coupled RBC (36), or RBC coupled with PnC (28) as the target cells. In some experiments, AB1-2 antibody was incorporated into the plaque mixture to determine the number of T15 idiotype-positive plaques (27, 37).

Results

Specific Suppression Induced by Antigen/Antibody Complexes. To determine the effects of specific immune complexes on the T15 idiotype-dominated response of BALB/c mice to Pn vaccine, the soluble PnC extracted from Pn bacteriae was used to form complexes with TEPC-15, an IgA myeloma protein that binds to PC determinants on the PnC. A 10:1 (wt/wt) ratio of TEPC-15 to PnC gave insoluble complexes that were in the equivalence zone. The immune precipitates were washed and added to BALB/c spleen cells in Marbrook vessels together with Pn vaccine and TNP-BA. After 5 d in culture, the number of PFC specific for each antigen was determined. The results in Table I show that as little as 2 μg of the TEPC-15/PnC complex had a marked suppressive effect on the PFC response to Pn. The suppression was specific since the response to TNP-BA was not affected. In other experiments (not shown), complexes formed in either antigen or antibody excess were equally suppressive.

Induction of Specific Suppressor Cells with Antigen/Antibody Complexes. In the next

TABLE I

*Specific Suppression Induced by Antigen/Antibody Complexes In Vitro**

TEPC-15/PnC complex added (day 0)	Specific PFC per culture vs:		
	PnC	Percent of control	TNP
None	760 ± 56	Control	2,812 ± 204
20 μg	63 ± 17	8	3,206 ± 386
2 μg	353 ± 140	46	2,906 ± 643
0.2 μg	570 ± 149	75	2,834 ± 642

* BALB/c spleen cells (10^7) were incubated in Marbrook vessels and stimulated with 10^6 Pn vaccine and TNP-BA. The indicated amounts of immune complexes were added at time 0. Direct PFC were assayed on day 5 using burro RBC coated with PnC or TNP as the indicator cells. The results are expressed as the mean of triplicate cultures assayed in duplicate ± SE.

TABLE II

*Induction of Specific "Suppressor Cells" with Antigen/Antibody Complexes**

Cells added (2×10^6/culture) after induction with:	PFC per culture specific for:			
	PnC	Percent of control	TNP	Percent of control
Medium	293 ± 22	Control	4,548 ± 920	Control
TEPC-15 (60 μg)	384 ± 71	131	6,340 ± 560	139
PnC (6 μg)	253 ± 44	86	5,200 ± 312	114
TEPC-15/PnC (20 μg)	50 ± 23	17	3,872 ± 1,040	85

* BALB/c spleen cells were incubated in Marbrook vessels in medium alone or with the indicated reagent. After 2 d, the cells were harvested, washed twice, and added to Marbrook vessels together with fresh BALB/c spleen cells (10^7), and stimulated with 10^6 bacteriae from a Pn vaccine and with TNP-BA. After 5 d, the number of direct PFC per culture was determined as in Table I.

experiments, we looked at whether the suppression induced by TEPC-15/PnC complexes was due to the induction of suppressor cells. Spleen cells from BALB/c mice were incubated in Marbrook vessels with either the antigen (PnC) or antibody (TEPC-15), alone or with preformed immune complexes. After 2 d, the cells were harvested, washed, and added to fresh responder spleen cell cultures together with Pn vaccine and TNP-BA. 5 d later, direct PFC were determined for each antigen. The results (Table II) indicate that preincubation with immune complexes apparently caused the induction of specific suppressor cells whereas incubation with either antigen or antibody alone had no effect.

Although it appeared that we had induced specific suppressor cells, we were concerned that residual antigen/antibody precipitates were being carried over into the test cultures causing direct suppression as in Table I. Thus, we prepared complexes with PnC- and FITC-conjugated TEPC-15 in an attempt to monitor carry-over of the complexes. After the cells had been incubated for 2 d with the FITC-labeled complexes, they were harvested, washed, and examined for fluorescent particles. This type of examination showed that many "bits" of fluorescent material were in fact carried over with the cells; however, little, if any, of the material appeared to be membrane associated. Thus, a means of separating cells from the free immune precipitates was required.

Positive Selection of Suppressor Cells. To characterize the phenotype of the suppressor

cell while at the same time removing free complexes, we chose a positive selection procedure to purify Thy-1.2-positive cells to assay for suppressor cell activity. A T cell-enriched population was obtained by absorbing BALB/c spleen cells on RAMIg plates to remove B cells. The nonadherent cells were cultured with FITC-labeled complexes for 2 d and then a positive selection procedure was performed by treating the cells with a murine monoclonal anti-Thy-1.2 antibody and then adding these cells to RAMIg plates. After 1 h at 4°C, the nonadherent cells were removed, and the plates were washed five times. The adherent cells were then harvested and examined microscopically for the presence of fluorescent material and for suppressor cell activity. None of the positively selected cells had membrane fluorescence, and very few bits of free fluorescent material could be seen when the cells were examined under a UV microscope. In an attempt to quantify the amount of complexes associated with the unfractionated cells, the cells were solubilized with 0.1 N NaOH and the fluorescence measured with a fluorimeter. Using this procedure, the positively selected cells had <100 ng of complex per 10^6 cells whereas the unfractionated cells had ~2 μg of complex per 10^6 cells. However, as shown in Table III, the positively selected cells incubated with the TEPC-15/PnC complexes were as suppressive as the unselected population.

Idiotype Specificity of Immune Complex-induced Ts. In the next set of experiments, we tested the effects of Ts induced with TEPC-15/PnC complexes on normal ($T15^+$) and T15-suppressed BALB/c mice. The T15-suppressed mice, which had been prepared by neonatal injection of BALB/c mice with a monoclonal anti-T15 antibody (MaId 5-4) (21), were generously provided by Dr. Christoph Heusser, Ciba-Geigy Ltd. After induction of Ts by incubation of cells with TEPC-15/PnC complexes for 2 d, free complexes were removed by positive selection for T cells. To further rule out contamination of these cells by B cells or small amounts of antigen/antibody complexes that might attach to the RAMIg-coated dishes, we used a different selection procedure (see Fig. 1) in which the cells were treated with FITC-conjugated monoclonal anti-Thy-1.2 antibody and then added to plates coated with affinity-purified

TABLE III

Specific Suppressor T Cells Induced By TEPC-15/PnC Complexes

Day −2.* Preincubation of T cells with:	Day 0.‡ Positive selection for T cells	Day +5. Specific PFC per culture vs:	
		PnC	Sheep RBC
Medium	−	817 ± 126	ND§
	+	590 ± 55	3,680 ± 378
TEPC-15/PnC complexes	−	185 ± 23	ND
	+	158 ± 36	3,730 ± 316

* T cells (BALB/c spleen cells nonadherent to RAMIg plates) were cultured in Marbrook vessels (10^7/culture) in medium alone or with 50 μg/culture of TEPC-15/PnC complexes.

‡ On day 0, the cells were harvested and treated with a murine monoclonal antibody (anti-Thy-1.2) and then added to RAMIg plates. After 1 h at 4°C, the nonadherent cells were removed. After the plates were washed five times, the adherent cells were collected and compared with unselected cells for suppressor cell activity by adding 10^6 cells from each group to Marbrook vessels containing 10^7 fresh BALB/c spleen cells together with either sheep RBC or Pn vaccine. Direct PFC were assayed on day 5 using sheep RBC or PnC-coated sheep RBC as indicator cells in the PFC assay.

§ Not determined.

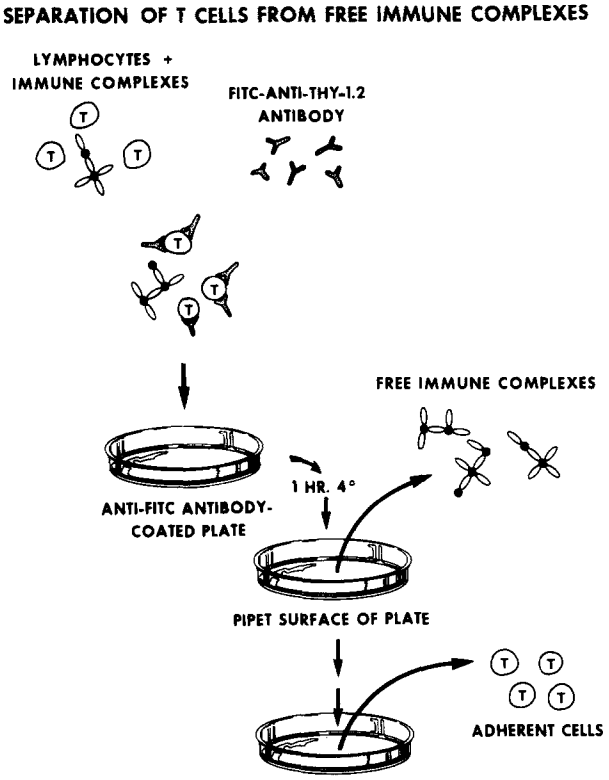

FIG. 1. Positive selection procedure for removal of free immune complexes.

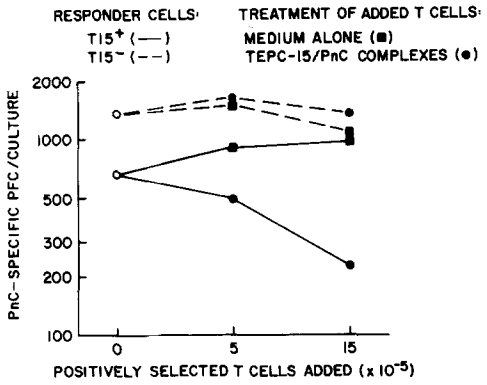

FIG. 2. Assay of immune complex-induced Ts on normal ($T15^+$) or T15-suppressed ($T15^-$) BALB/c mice. BALB/c T cells (10^7/culture) were cultured with TEPC-15/PnC complexes (50 μg/culture) or medium alone in Marbrook vessels. After 2 d, T cells were positively selected as described in Fig. 1 and added to fresh responder spleen cells from normal or T15-suppressed BALB/c mice. Direct PFC were assayed on day 5. PFC from the normal BALB/c control cultures could be inhibited by 73% by incorporating AB1-2 monoclonal anti-T15 antibody into the plaquing mixture (27, 37), whereas PFC from the T15-suppressed mice were not inhibited by AB1-2.

goat anti-FITC antibody. The adherent T cells selected in this manner were then tested for the ability to inhibit the anti-Pn response of normal and T15-suppressed BALB/c mice. The results (Fig. 2) show that the Ts did not affect the response of the

T15-suppressed mice whereas the response of the normal BALB/c mice was suppressed. The degree of suppression was dose dependent, and in one experiment, the response of spleen cells from T15-suppressed mice was inhibited by as much as 50%; however, within the same experiment, the response of normal BALB/c spleen cells was inhibited by 80% or more.

In the next set of experiments, we prepared complexes of the PnC antigen with two different PC-binding myeloma proteins, TEPC-15 and McPC-603. Both proteins are IgA, κ antibodies; however, the anti-PnC response of BALB/c mice is dominated by antibodies that share Id with the TEPC-15 myeloma protein ($T15^+$) whereas the McPC-603 protein is $T15^-$. Both types of complexes were then compared for their ability to activate cells that would suppress the $T15^+$ anti-PnC response of BALB/c spleen cells. Splenic T cells were cultured in Marbrook vessels with either TEPC-15/PnC or McPC-603/PnC complexes (50 μg/culture). Both complexes were formed at antigen/antibody equivalence. After 2 d, the T cells were positively selected and tested for the ability to suppress the response of normal and T15-suppressed BALB/c mice. The results (Table IV) show that Ts induced with McPC-603 complexes induced the same amount of suppression as those induced by TEPC-15 complexes. Again, the response of normal BALB/c spleen cells was markedly suppressed and, as mentioned above, the response of T15-suppressed mice was inhibited to a lesser degree.

The results thus far suggested that the Ts was specific for certain T15 Id that are expressed by normal BALB/c mice but are not produced (or which may be expressed to a lesser degree) during the anti-Pn response of T15-suppressed BALB/c mice. In the final set of experiments, we wished to determine whether the Ts had receptors for TEPC-15-related Id. Thus, cells were cultured with TEPC-15/PnC complexes for 2 d and then the T cells were positively selected as described in Fig. 1. These cells were then incubated with ^{125}I-TEPC-15 at 4°C overnight. The cells were washed four times and the cell-bound radioactivity was determined. The results (Fig. 3) demonstrate that the cells that had been precultured with TEPC-15/PnC complexes bound twice as much radiolabeled TEPC-15 as did the control cultures and that this binding could be specifically inhibited with unlabeled TEPC-15 but not with MOPC-315, a different

TABLE IV

Induction of Ts with McPC-603/PnC and TEPC-15/PnC Complexes

Responder spleen cells	T cells added after incubation for 2 d with:	PnC-specific antibody response	
		PFC per culture	Percent of control
Normal BALB/c	Medium	1,495 ± 345	Control
	TEPC-15/PnC	170 ± 62	11
	McPC-603/PnC	280 ± 53	19
T15-suppressed BALB/c	Medium	1,390 ± 148	Control
	TEPC-15/PnC	707 ± 195	51
	McPC-603/PnC	640 ± 100	46

BALB/c splenic T cells (cells nonadherent to RAMIg plates) were cultured in medium alone or with 50 μg/culture of TEPC-15/PnC or McPC-603/PnC complexes. After 2 d, the T cells were positively selected as shown in Fig. 1, and 2×10^6 cells from each group were added to Marbrook vessels containing 10^7 spleen cells from either normal or T15-suppressed BALB/c mice together with the Pn antigen. Direct PnC-specific PFC were assayed on day 5.

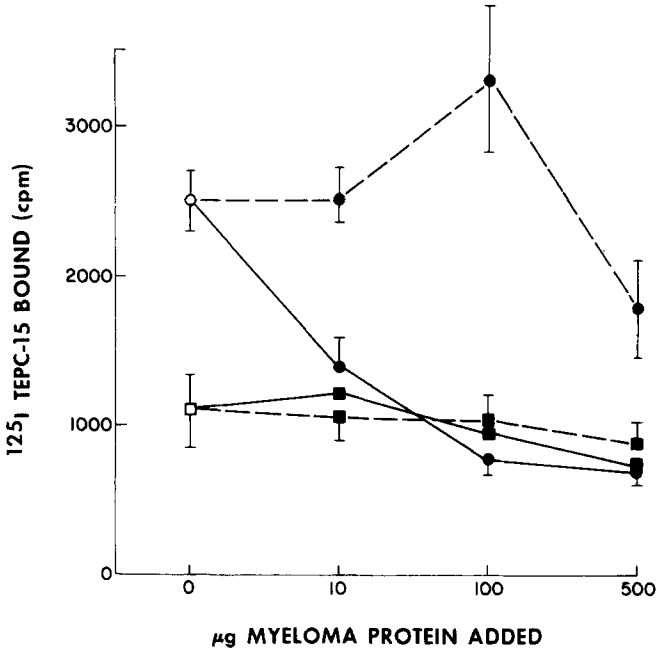

FIG. 3. Binding of radiolabeled TEPC-15 in the presence of unlabeled TEPC-15 or MOPC-315 myeloma proteins. BALB/c T cells were incubated with 50 μg/culture of TEPC-15/PnC complexes (○) or with medium alone (□) for 2 d. The T cells were then positively selected as described in Fig. 1 and incubated overnight at 4°C with 5 μg ^{125}I-TEPC-15 in the presence of the indicated amount of unlabeled TEPC-15 (——) or MOPC-315 (-----). The cells were washed four times and then the radioactivity associated with the cell pellet was determined. Each point represents the mean of four cultures ± SD.

IgA myeloma protein. Thus the results indicate that cultivation of T cells with TEPC-15/PnC complexes induced the expression of receptors on these cells for T15-related Id or an expansion of cells bearing idiotype receptors.

Discussion

The results of this study demonstrate the importance of immune complexes in regulation of the immune system. We have shown that antigen/antibody complexes can induce specific Ts that are not induced by either free antigen or antibody under similar conditions. To demonstrate that the suppression was due to the Ts and not to carry-over of free immune complexes, it was necessary to employ a positive selection procedure in which T cells were immobilized on plates that could then be washed to remove free complexes. This positive selection procedure did not result in significant enrichment of the Ts population since the population used for induction of Ts had already been enriched for T cells by absorption of spleen cells on RAMIg-coated plates before culture with immune complexes.

Since the immune complexes contain both antigen and antibody, it was possible that the Ts could be specific for either the antigen or the idiotype, or the Ts preparation could have been a mixture of both idiotype- and antigen-specific Ts. To examine this question, we compared the effect of the Ts on the response to Pn of normal BALB/c mice (which is dominated by the T15 idiotype family) and BALB/c

mice in which the T15 Id had been chronically suppressed by the neonatal injection of a monoclonal anti-T15 antibody, MaId5-4. By 8 wk of age, the overall response to Pn in the latter group is normal although the response is $T15^-$ (23). The T15-suppressed mice prepared with a monoclonal antibody differ from those prepared using conventional antisera in that the overall ($T15^-$) response to Pn recovers by 8 wk in the monoclonal antibody-suppressed mice whereas in the latter group the overall response recovers to a variable degree (38, 39). These results are discussed in detail elsewhere (23).

The immune complex-induced Ts appears to be idiotype-specific since the Ts exhibits a relatively weak effect on the anti-Pn response of T15-suppressed mice whereas the response of normal BALB/c mice was markedly suppressed. In two of four experiments, the PFC response of T15-suppressed mice was completely unaffected by the complex-induced Ts whereas in the remaining experiments the response was inhibited by 30 and 50%, respectively. This suggests that our Ts preparation may contain some antigen-specific suppressor cells in addition to the predominant idiotype-specific Ts population.

In an attempt to more precisely determine the fine-specificity of the Ts, we compared complexes formed with two different PC-binding myeloma proteins for the ability to suppress the antibody response to Pn. The results showed that complexes formed with McPC-603, which expresses a different set of Id than TEPC-15, were as effective as TEPC-15 complexes at inducing Ts that could inhibit the anti-Pn response of spleen cells from normal BALB/c mice but which were much less suppressive when assayed for the ability to inhibit the response of spleen cells from T15-suppressed mice. Thus, the Ts may recognize a cross-reactive Id common to TEPC-15 and McPC-603 proteins. Such idiotypic cross-reactions between TEPC-15 and McPC-603 have been demonstrated serologically (40). Alternatively, the complex of antigen and antibody may express new or "altered" Id that may be shared between TEPC-15 and McPC-603 complexes. In support of this view, Nemazee and Sato (41) have shown that in the formation of immune complexes, new Id are expressed that can be detected by monoclonal antibodies (41).

Further evidence for the idiotype specificity of the Ts is our finding that cells cultured with TEPC-15/PnC complexes expressed receptors for T15-related Id. However, whether these receptors are on the Ts remains to be determined since the Ts activity could not be depleted on idiotype-coated dishes (data not shown). This may simply indicate that the adsorption procedure is inefficient. Alternatively, it may suggest that the Ts readily shed their idiotype receptors during culture with the antigen/antibody complexes only to reexpress them when added to the test cultures.

Previously, we had shown that antigen-specific Ts could be induced by culturing spleen cells for 2–3 d with formalin-treated Pn, a vaccine prepared from whole bacteria (20, 21). In the present experiments, the soluble PnC extracted from Pn did not induce suppressor cells. The amount of PnC used herein may simply be suboptimal for the induction of antigen-specific suppressor cells; however, it is also conceivable that Ts are more readily induced with a particulate form of antigen. The Pn antigen-specific Ts appears to act directly on B cells (20); however, we do not know whether the idiotype-specific Ts acts directly on B cells or whether the Ts acts on a $T15^+$ helper cell. The latter possibility must be considered since careful examination has revealed that the response to Pn in BALB/c mice is partially T dependent (21).

Anti-Id Ts have been induced by a variety of experimental manipulations including the administration of anti-Id antibody (14, 15), immunization with purified myeloma proteins in adjuvant (16), or by injection of idiotype-conjugated cells (17, 18). In addition, idiotype-positive first order suppressor cells (Ts-1) or soluble factors derived from such cells have been shown to induce second order (Ts-2) suppressor cells that are antiidiotypic (42). Which of these mechanisms for the induction of anti-Id Ts represents the major pathway for idiotypic regulation of a normal immune response remains to be determined although the induction of Ts with immune complexes must be considered a prime candidate since potent Ts could be induced within 2 d with antigen/antibody complexes whereas neither free (soluble) antigen nor free antibody alone could induce Ts under similar conditions.

In terms of the immune network, our results suggest that immune complexes induce a different regulatory pathway than does antigen: antigen-induced Ts are antigen specific (20, 43), whereas we show here that complex-induced Ts are predominantly idiotype specific. Whether both types of Ts coexist during a normal immune response or whether one cell type (e.g., the antigen-specific Ts) precedes the other (e.g., idiotype specific) cell remains to be determined. In this regard, it is interesting that Kim and Greenberg (44) have shown that mixing antigen-specific Ts with idiotype-specific Ts results in the abrogation of suppression, suggesting that the two Ts would not function when present at the same time.

Our results indicating that antigen/antibody complexes readily induce idiotype-specific Ts suggest that this may be an important mechanism for regulating a normal immune response. Thus, our findings lend further support to the network theory of the immune system (7).

Summary

The effects of immune complexes on the antibody response of BALB/c mice to *Streptococcus pneumoniae* R36a (Pn) were investigated. The cell wall polysaccharide (PnC) extracted from Pn was used to form complexes with TEPC-15, a myeloma protein that binds to phosphorylcholine determinants on the PnC. Complexes formed at equivalence were cultured with splenic T cells from BALB/c mice for 2 d, and then the cells were added to fresh BALB/c spleen cell cultures to test their effect on the antibody response to Pn, a response dominated by the T15 idiotype family. The results indicate that TEPC-15/PnC complexes induced potent suppressor T cells (Ts) whereas cells cultured with free antigen or free antibody alone had no effect on the plaque-forming cell response to Pn. The suppression was specific since the response to control antigens such as sheep erythrocytes was unaffected. The suppression appears to be idiotype-specific since the Ts had a relatively weak (and in some cases no) effect on the anti-Pn response of BALB/c mice that had been suppressed for T15 idiotopes by neonatal injection of a monoclonal anti-T15 antibody, MaId 5-4. Furthermore, cells cultured with TEPC-15/PnC complexes were shown to express specific receptors for TEPC-15 idiotopes. The results indicate that antigen/antibody complexes may have important immunoregulatory effects because they are potent inducers of idiotype-specific Ts.

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References

1. Uhr, J. W., and G. Möller. 1968. Regulatory effect of antibody on the immune response. *Adv. Immunol.* **8:**81.
2. Möller, G., and H. Wigzell. 1965. Antibody synthesis at the cellular level. Antibody-induced suppression of 19S and 7S antibody response. *J. Exp. Med.* **121:**969.
3. Henry, C., and N. K. Jerne. 1967. Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. *J. Exp. Med.* **128:**133.
4. Uhr, J. W., and J. B. Baumann. 1961. Antibody Formation. I. The suppression of antibody formation by passively administered antibody. *J. Exp. Med.* **113:**935.
5. Henney, C. S., and K. Ishizaka. 1970. Studies on the immunogenicity of antigen-antibody precipitates. II. The suppressive effect of anti-carrier and anti-hapten antibodies on the immunogenicity of dinitrophenylated human γG globulin. *J. Immunol.* **104:**1540.
6. Feldmann, M., and E. Diener. 1970. Antibody-mediated suppression of the immune response in vitro *J. Exp. Med.* **131:**247.
7. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris).* **125C:**373.
8. Kluskens, L., and H. Köhler. 1974. Regulation of immune response by autogenous antibody against receptor. *Proc. Natl. Acad. Sci. USA.* **71:**5083.
9. Cosenza, H. 1976. Detection of anti-idiotype reactive cells in the response to phosphoryl-choline. *Eur. J. Immunol.* **6:**114.
10. Sakato, N. and H. N. Eisen. 1975. Antibodies to idiotypes of isologous immunoglobulins. *J. Exp. Med.* **141:**1411.
11. Klaus, G. G. B. 1978. Antigen-antibody complexes elicit anti-idiotypic antibodies to self-idiotopes. *Nature (Lond.).* **272:**265.
12. Kontiainen S., and M. Feldmann. 1976. Suppressor cell induction *in vitro.* I. Kinetics of induction of antigen-specific suppressor cells. *Eur. J. Immunol.* **6:**296.
13. Eardley, D. D., and R. K. Gershon. 1976. Induction of specific suppressor T cells *in vitro. J. Immunol.* **117:**313.
14. Eichman, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* **5:**511.
15. Owen, F. L., S. T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **145:**1559.
16. Milburn, G. L., and R. G. Lynch. 1982. Immunoregulation of murine myeloma in vitro. II. Suppression of MOPC-315 immunoglobulin secretion and synthesis by idiotype-specific suppressor T cells. *J. Exp. Med.* **155:**852.
17. Dohi, Y. and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. *J. Exp. Med.* **150:**909.
18. Sy, M. S., B. A. Bach, A. Brown, A. Nisonoff, B. Benacerraf, and M. I. Geene. Antigen- and receptor-driven regulatory mechanisms. II. Induction of suppressor T cells with idiotype-coupled syngeneic spleen cells. *J. Exp. Med.* **150:**1229.
19. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T suppressor cells specific for MOPC-460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149:**592.
20. Cerny, J., M. J. Caulfield, and M. Siat. 1980. Regulation of the T15 idiotype-positive, T-independent immune responses to *Streptococcus pneumoniae* R36 (Pn). I. Specific suppressor T cells induced by Pn *in vitro* are antigen-specific and idiotype-positive. *Eur. J. Immunol.* **10:**661.
21. Cerny, J., C. Heusser, R. Wallich, G. Hammerling, and D. D. Eardley. 1982. Immunoglobulin idiotopes expressed by T cells. I. Expression of distinct idiotopes detected by monoclonal antibodies and antigen-specific suppressor T cells. *J. Exp. Med.* **156:**719.
22. Cosenza, G., and H. Köhler. 1972. Specific suppression of the antibody response by antibodies to receptors. *Proc. Natl. Acad. Sci. USA.* **69:**2701.

23. Cerny, J., R. Cronkhite, and C. H. Heusser. 1983. Antibody response of mice following neonatal treatment with a monoclonal anti-receptor antibody. Evidence for B cell tolerance and T suppressor cells specific for different idiotypic determinants. *Eur. J. Immunol.* In press.
24. DuClos, T. W., and B. S. Kim. 1977. Suppressor T cells: presence in mice rendered tolerant by neonatal treatment with anti-receptor antibody or antigen. *J. Immunol.* **119:**1769.
25. Anderson, H. C., and M. McCarty. 1951. The occurrence in the rabbit of an acute phase protein analogous to human C-reactive protein. *J. Exp. Med.* **93:**25.
26. Potter, M., and R. Lieberman. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphoryl choline. *J. Exp. Med.* **132:**737.
27. Kearney, J. F., R. Barletta, Z. S. Quan, and J. Quintans. 1981. Monoclonal versus heterogeneous anti-H-8 antibodies in the analysis of the anti-phosphorylcholine response in BALB/c mice. *Eur. J. Immunol.* **11:**877.
28. Kelsoe, G., D. Isaak, and J. Cerny. 1980. Thymic requirement for cyclical idiotypic and reciprocal anti-idiotypic immune responses to a T-independent antigen. *J. Exp. Med.* **151:**289.
29. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Rawlet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. *J. Immunol.* **122:**2491.
30. The, T. H., and T. E. W. Feltkamp. 1970. Conjugation of fluorescein isothiocyanate to antibodies. I. Experiments on the conditions of conjugation. *Immunology.* **18:**865.
31. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified antiimmunoglobulin. *J. Immunol. Methods.* **15:**47.
32. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA.* **75:**2844.
33. Boyle, W. 1968. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. *Transplantation.* **6:**761.
34. Fong, S., R. I. Fox., J. E. Rose, J. Liu, C. D. Tsoukas, D. A. Carson, and J. H. Vaughan. 1981. Solid-phase selection of human T lymphocyte subpopulations using monoclonal antibodies. *J. Immunol. Methods.* **46:**153.
35. Grinwich, K. D., T. S. Alexander, and J. Cerny. 1979. Properties of murine leukemia-associated suppressor cells. I. Preferential suppression of thymus-dependent antibody responses and the requirement for syngeneity in the K region of the H-2 complex. *J. Immunol.* **122:**1108.
36. Rittenberg, M. B., and K. L. Pratt. 1969. Antitrinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* **132:**575.
37. Cerny, J., R. Wallich, and G. J. Hammerling. 1982. Analysis of T15 idiotopes by monoclonal antibodies: variability of idiotopic expression on phosphorycholine-specific lymphocytes from individual inbred mice. *J. Immunol.* **128:**1885.
38. Strayer, D. S., H. Cosenza, W. M. F. Lee, D. A. Rowley, and H. Kohler. 1974. Neonatal tolerance induced by antibody against antigen-specific receptor. *Science (Wash. DC).* **186:**640.
39. Augustin, A., and H. Cosenza. 1976. Expression of new idiotypes following neonatal suppression of a dominant clone. *Eur. J. Immunol.* **6:**497.
40. Claflin, J. L., and J. M. Davie. 1975. Clonal nature of the immune response to phosphorylcholine (PC). V. Cross-idiotype specificity among heavy chains of murine anti-PC antibodies and PC-binding myeloma proteins. *J. Exp. Med.* **141:**1073.
41. Nemazee, D. A., and V. L. Sato. 1982. Enhancing antibody: a novel component of the immune response. *Proc. Natl. Acad. Sci. USA.* **79:**3828.
42. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T lymphocyte interactions in antigen-specific immune suppression. *Scand. J. Immunol.* **13:**1.

43. Lewis, G. K., and J. W. Goodman. 1978. Purification of functional, determinant-specific, idiotype-bearing murine T cells. *J. Exp. Med.* **148:**915.
44. Kim, B. S., and J. A. Greenberg. 1981. Mechanisms of idiotype suppression IV. Functional neutralization in mixtures of idiotype-specific suppressor and hapten-specific suppressor T cells. *J. Exp. Med.* **154:**809.