

## IMMUNOGLOBULIN IDIOTOPES EXPRESSED BY T CELLS

### I. Expression of Distinct Idiotopes Detected by Monoclonal Antibodies on Antigen-specific Suppressor T Cells\*

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The structure of receptor(s) for antigen on T lymphocytes is not yet completely understood. There is substantial evidence, however, indicating that the receptor carries determinants which are similar (cross-reactive) to immunoglobulin variable region (IgV)<sup>1</sup> structures. Numerous studies using antibodies against immunoglobulin (Ig) idiotypes have demonstrated that T cells of various phenotypes can be either stimulated (activated) or inhibited by anti-idiotypic antibodies and that soluble T cell products bear the idiotypes (reviewed in 1). The presence of antibody-like idiotypes on specific, antigen-binding suppressor T cells (T<sub>s</sub>) has been shown using T<sub>s</sub>-generated by azobenzenearsonate (2), hen egg lysozyme (3), 4-hydroxy-3-nitrophenyl acetyl (NP) (4), and *Streptococcus pneumoniae* R36a (Pn) (5). Furthermore, an idiotypic expressed on antibody to L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup> (GAT) was also found on GAT-specific suppressor factors extracted from activated T cells (6). In all these models, however, the idiotypic was detected by conventional, heterologous antibody produced either in guinea pigs or rabbits.

It has become generally accepted that an "idiotypic" defined by conventional antisera may be in fact a group of distinct determinants, idiotopes (Id), which may be expressed independently of each other. This has been demonstrated in studies with monoclonal antibodies directed against individual Id of IgV (7-13) and in structural studies that showed that antibodies bearing a particular idiotypic display a high degree of heterogeneity in reaction with anti-idiotypic antisera (14) as well as a significant difference in amino acid sequences (15-17). It appears that the conventional anti-idiotypic antisera may react with different Id determinants of a given idiotypic family, fortuitously. Furthermore, such antisera may cross-react with the specific nonimmunoglobulin receptors expressed on somatic cells other than lymphocytes, as has been shown with receptors for insulin (18) and alprenolol (19). Because of the complexity of idiotypes, the reactivity of conventional antisera with T cells does

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<sup>1</sup> Abbreviations used in this paper: BA, *Brucella abortus*; C, complement; GAT, L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup>; Id, idiotypic; IgV, immunoglobulin variable region; NP, 4-hydroxy-3-nitrophenyl acetyl; PC, phosphorylcholine; Pn, *Streptococcus pneumoniae* strain R36a; PnC, cell-wall polysaccharide from Pn; RBC, erythrocyte; T15, idiotypic family expressed on TEPC-15 myeloma protein; TNP, trinitrophenyl; T<sub>4</sub>, T helper cell; T<sub>s</sub>, T suppressor cell.

not allow a definitive conclusion on the similarity between T receptors and IgV region; a comparative analysis of several individual Id would be more informative.

We have recently used a panel of monoclonal antibodies against the T15 idiotypic family to analyze the expression of individual Id on Pn-reactive B cells (20). The antibodies inhibit the induction of primary, T-independent response and the specific plaque formation by lymphocytes immunized with Pn in vitro. The inhibitory effects of monoclonal antibodies directed against Id within and without the paratopic region are comparable. The Id appear to be expressed independently, in various combination patterns ("idiograms"), which characterize B cells of a given inbred strain (8, 20).

Based on these results it has become possible to investigate the idiotypic pattern of Pn-specific  $T_s$  in BALB/c strain that have been previously shown to express a cross-reactive T15 marker detected by conventional antisera (5). Using a functional assay of inhibition of  $T_s$  activity by monoclonal antibodies in vitro, we show herein that the idiogram of Pn-educated  $T_s$  overlaps with that of Pn-reactive B cells, that is, the  $T_s$  express most, but not all, known Id of the T15 family.

### Materials and Methods

**Mice.** BALB/c strain mice were obtained from a colony maintained at Ciba-Geigy AG, Basel, Switzerland. For suppression of T15, mice were injected with 50  $\mu$ g of monoclonal anti-T15 antibody MaId5-4 intraperitoneally within 24 h after birth. They were used for the experiments within 2–4 mo of age, together with sex- and age-matched control mice. The serum titer of T15 Id detectable by a reverse hemagglutination assay using MaId5-4-coated RBC was  $\sim 10^{-4}$  (corresponding to 20  $\mu$ g/ml) in normal, adult BALB/c mice, whereas the level of Id in T15-suppressed mice was undetectable ( $<0.1$   $\mu$ g/ml). The injection of MaId5-4 induces a chronic suppression of T15 (21). Additional normal BALB/c and C57Bl/6 strain mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

**Antigens.** Sheep and burro erythrocytes (RBC) were purchased from the Colorado Serum Co., Denver, CO. The *S. pneumoniae* strain R36a (Pn) was grown in Todd-Hewitt Broth (BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, MD) and formaldehyde-treated Pn antigen for stimulation of lymphocyte cultures was prepared according to DuClos and Kim (22). The optimal immunogenic concentration of each batch was determined empirically. The TNP-*Brucella abortus* (TNP-BA) conjugate was prepared and provided by Dr. James J. Mond from the Uniformed Services University of Health Sciences, Bethesda, MD. Extraction of cell wall polysaccharide (PnC) from Pn was previously described (23).

**Monoclonal Anti-Idiotypic Antibodies.** Antibodies were products of cloned hybridomas generated by fusion of lymphocytes taken from mice (BALB/c, A/J, or SJL) immunized against HOPC-8 or TEPC-15 proteins with myeloma cell lines according to Kohler et al. (24). The details of the production and maintenance of the hybridoma clones and their specificity for idiotopes of the HOPC-8/TEPC-15 family have been described elsewhere (8, 11–13). Antibodies were obtained by repeated salt precipitation of either peritoneal ascites from hybridoma-bearing mice or supernatants from cultures of hybridoma cell lines. All anti-idiotypic antibodies react specifically with both HOPC-8 and TEPC-15 myeloma proteins but not with other mouse myeloma proteins (including the PC-binding proteins, M603 and M511) nor with different classes of serum Ig. The exception is antibody B36-75, which reacts with HOPC-8 but not with TEPC-15 (8). ABI-2 antibody (11, 12) was provided by Dr. John F. Kearney from the University of Alabama (Birmingham, AL). The Ig class of the monoclonal anti-Id antibodies and their binding properties in respect to PC inhibition of Id-(anti-Id) reactivity are summarized in Table I.

The ability of the monoclonal anti-Id antibodies to inhibit PC-reactive B cells from BALB/c mice in vitro has been studied in detail (20). The antibodies inhibit the response to Pn when added into the cultures together with the antigen on day 0, and they also inhibit the plaque formation by differentiated, antigen-stimulated cells in agarose (see the description of the two assays below). The inhibition is specific, in that the response of BALB/c lymphocytes

TABLE I  
*Inhibitory Effect of Five Monoclonal Anti-T15 Antibodies on Pn-specific B Cells from BALB/c Mice*

Clone	Antibody Isotype	Hapten inhibition*		Effective protein amount‡	Inhibition of primary response in vitro§		Inhibition of PFC assay	
		PC chloride	PC-KLH		Normal mice	T15-suppressed mice¶	Normal mice	T15-suppressed mice*
				<i>μg</i>				
AB1-2**	γ1	—	+	1	85%	<15%	85%	<15%
MaId5-4‡‡	γ1	—	+	0.5	92%	<15%	86%	<15%
B36-75§§	γ1	10 <sup>-3</sup>	20	50	62%	<15%	53%	<15%
B36-82§§	μ	—	30	0.1	78%	<15%	80%	<15%
B24-50§§	γ2b	—	—	15	55%	<15%	60%	<15%

\* Concentration of antigen (mol of PC-chloride, μg/ml of PC-KLH) required for 50% inhibition of binding of <sup>125</sup>I-HOPC8 to anti-idiotypes. The information on AB1-2 and MaId-4 is only qualitative (+ or -).

‡ Smallest amount of protein (μg added into the culture vessel or the PFC assay mixture) that still yields the maximum inhibition (20).

§ Inhibitory effect of antibodies added into the cultures of responder cells with Pn on day 0. The effect was calculated as percent inhibition of specific antibody forming cells detectable on day 4. The values represent means from numerous experiments (20). The control response (in absence of anti-Id) ranged from 200 to 800 PFC/well.

|| Mean inhibitory effect (percent) of anti-Id added into the plaque assay mixture.

¶ Splenic lymphocytes from BALB/c mice inoculated with MaId5-4 hybridoma protein, neonatally.

\*\* Ref. 11, 12.

‡‡ Ref. 13.

§§ Ref. 8.

to other antigens such as TNP-BA, TNP-Dextran, or sheep RBC is not affected (12, 20). The relative inhibitory activity of different anti-Id hybridoma proteins (percent suppression) for normal BALB/c shown in Table I is a mean value from numerous assays on lymphocytes pooled from several mice as well as cells from individual donors (20). The proteins had no effect on Pc-reactive cells from T15-suppressed BALB/c mice (suppression <15%, in either test) (see also Results).

*Separation of B and T Cells.* B cells were prepared by cytotoxic depletion of T cells from spleen cell suspension using repeated treatment (twice) with monoclonal anti-Thy-1.2 antibody (25; a generous gift of Dr. Ann Marshak-Rothstein, Massachusetts Institute of Technology, Boston, MA) and a low-toxicity rabbit serum as a source of complement (C). The details of this procedure were described elsewhere (26). The response of the remaining B cell-rich population to concanavalin A was depressed by 90% compared with control cells treated with C alone, whereas the mitogenic response to a bacterial lipopolysaccharide remained undiminished.

T cells were prepared by panning of spleen cell suspension on plastic petri dishes coated with a goat antiserum against mouse Ig (27). The nonadherent fraction usually contained <2% of cells with surface Ig detectable by immunofluorescence.

*Induction and Testing of Specific Suppressor Cells.* Spleen cells or purified T cells were resuspended in a culture medium consisting of enriched Eagle's medium (28) and 10% fetal calf serum (Grand Island Biological Co., Grand Island, NY). Cells were incubated in Marbrook culture chambers (Bellco Glass, Inc., Vineland, NJ) (28) together with a concentration of Pn supraoptimal on antibody response, for 3 d (5). Parallel cultures were also set up without Pn. At the end of incubation, cells were washed, resuspended in appropriate culture medium, and added into the fresh responder cultures (1–3 × 10<sup>5</sup> Pn-educated cells in 50 μl of medium per well). Sheep RBC-specific T<sub>s</sub> cells were induced by a 3-d incubation with the antigen in vitro and tested as previously described (29).

*Responder Cultures and Plaque-forming Cell (PFC) Assays.* The primary antibody response to Pn

or TNP-BA was initiated in flat-bottomed wells of 96-well plates (3042, Falcon Microtest II; Falcon Labware, Oxnard, CA) in quadruplicate. Each well contained  $10^6$  splenocytes or B cells and optimal dose of antigen ( $10^4$ – $10^6$  Pn or  $10^5$  TNP-BA) in a total volume of 150  $\mu$ l of a culture medium containing  $2 \times 10^{-5}$  M 2-mercaptoethanol and 10% fetal calf serum (HY-Clone, Sterile Systems, Logan, UT) as described elsewhere (20). The culture wells were fed daily with 10  $\mu$ l of a cocktail (20).

The induction of primary response of splenocytes to sheep RBC in the multiwell culture system was previously described (29). Antibody PFC generated in the responder cultures after 4 d of incubation were enumerated by plaque assays with sheep RBC, TNP-coupled burro RBC (30), or PnC-coupled burro RBC (23) using either microscopic slide-assay with agarose (31) or a liquid-phase assay in chambers constructed from microscopic slides (modified Cunningham's system) (32).

*Inhibition of Lymphocytes by Monoclonal Anti-Id Antibodies.* The minimal concentrations of hybridoma proteins required for inhibition of both Pn-reactive B cells and specific PFC were determined in an earlier study (20). The amounts of proteins used in the present experiments were 5- to 10-fold higher than those given in Table I. The controls for the assays described below included the addition of equal volume (20–50  $\mu$ l) of diluent (culture medium or a balanced salt solution).

Attempts to inhibit the generation of  $T_s$  were carried out by adding anti-Id proteins into the Marbrook cultures (50  $\mu$ l/vessel) at the beginning of a 3-d incubation of purified T cells with Pn. To test the effect of anti-Id(s) on effector  $T_s$ , the hybridoma proteins were added into the assay cultures of responder cells with educated  $T_s$ .

The effect of anti-Id on induction of antibody response of spleen cells or B cells (20) was assessed by adding the hybridoma proteins into the responder culture wells on day 0 and enumerating the PFC response on day 4. The ability of anti-Id to inhibit the mature antibody-forming cells was determined by addition of the proteins into the plaque assay reaction mixture (20). In either assay the percent inhibition was calculated:  $100 - ((\text{PFC with anti-id})/(\text{PFC with diluent})) \times 100$ .

## Results

*Induction of Pn-specific Suppressor Activity in Cultures of Purified T Cells.* The suppressor activity of Pn-educated splenocytes resides in Thy-1.2-positive cell population (5). For the sake of this study, however, it was important to determine whether suppressor cells can be induced in cultures of purified, naïve T cells and to confirm that the effector  $T_s$  inhibit the Pn-reactive B cells directly. The results in Table II show that purified splenic T cells incubated with Pn for 3 d and then added into fresh assay cultures were about as effective in suppressing the anti-Pn response as unseparated, antigen-educated splenocytes, and that both spleen and B responder cells were

TABLE II  
*Direct Suppressor Effect of Pn-educated T cells on Pn-reactive B Cells*

Responder cells ( $10^6$ )	Pn-specific PFC/well (mean $\pm$ SEM) with cells added				
	None (control)	T cells* ( $3 \times 10^5$ )		Spleen cells ( $3 \times 10^5$ )	
		media educated‡	Pn edu- cated§	media educated‡	Pn edu- cated‡
Spleen cells	338 $\pm$ 82	346 $\pm$ 68	60 $\pm$ 12	290 $\pm$ 24	58 $\pm$ 20
B cells	190 $\pm$ 42	386 $\pm$ 128	42 $\pm$ 14	254 $\pm$ 30	48 $\pm$ 26

\* T cell-rich fraction obtained by panning of splenocyte suspension on anti-Ig plates.

‡ Cells incubated in culture media for 3 d.

§ Cells incubated with a high dose of Pn.

|| Spleen cells treated twice with anti-Thy-1.2 + C.

TABLE III  
*Specificity of Suppressor Cells Generated by Pn in Cultures of Purified T Cells\**

Responder spleen cells plus	Specific PFC/well (mean $\pm$ SEM) against:	
	PnC-RBC	TNP-RBC‡
Media (control)	254 $\pm$ 96	816 $\pm$ 72
T cells, media educated	278 $\pm$ 78	888 $\pm$ 174
T cells, Pn educated	98 $\pm$ 32	900 $\pm$ 148

\* T cells were purified and incubated with or without Pn, as in Table II.

‡ Hapten-specific response in cultures stimulated with TNP-BA.

TABLE IV  
*Failure of anti-Id Proteins to Inhibit the Generation of Suppressor Cells by Pn in Cultures of Normal Purified T Cells\**

Suppressor cells		Pn-specific PFC/well (mean $\pm$ SEM)	
Cells added ( $3 \times 10^6$ /well)	Anti-Id present during education	Experiment 1	Experiment 2
T cells, media educated (control)	None	520 $\pm$ 48	276 $\pm$ 51
T cells, Pn educated	None	37 $\pm$ 16	40 $\pm$ 24
	AB1-2	65 $\pm$ 20	80 $\pm$ 12
	MaId5-4	76 $\pm$ 32	66 $\pm$ 30
	B36-75	23 $\pm$ 15	91 $\pm$ 9
	B36-82	44 $\pm$ 28	56 $\pm$ 24
	B24-50	50 $\pm$ 20	Not tested

\* T cells were prepared and cultured with or without Pn, with addition of 1–250  $\mu$ g of indicated monoclonal antibody or with the diluent (control). After 3 d, T cells were washed and added ( $3 \times 10^5$ ) into culture wells containing  $10^6$  of normal splenic B cells (experiment 1) or spleen cells (experiment 2) plus Pn antigen. The PFC response was determined after 4 d.

suppressed. Normal, media-incubated T cells did not suppress; in fact, there was an enhancement of anti-Pn response in B cell cultures that received the control, media-incubated T cells. The enhancement that has been consistently seen in our experiment seems to indicate that the response to Pn is partially T dependent (J. Cerny and C. Heusser, unpublished observations). The suppression generated in T cell cultures with Pn is antigen-specific in that the  $T_s$  do not inhibit the anti-TNP response in cultures stimulated with TNP-BA (Table III).

*Monoclonal Anti-Id Antibodies Fail to Inhibit the Generation of  $T_s$  In Vitro.* Our first approach to assessment of Id expression on T cells was an attempt to inhibit the generation of  $T_s$  by addition of monoclonal anti-Id antibodies into the cultures of naive T cells with Pn at time zero. The anti-Id used for the experiment were previously shown to inhibit the activation of B cells by Pn in vitro (20). The protein concentration used herein is  $\sim$ 10-fold higher than the effective end-point concentration shown in Table I. T cells were incubated for 3 d, washed, and tested for their ability to suppress

the response to Pn. We did not, however, see any diminution of the suppressive activity of  $T_s$  educated with Pn in the presence of either of the anti-Id as compared with the activity of control  $T_s$  (Table IV). In other trials not shown here, we used a mixture of the anti-Id antibodies, and readed the proteins to the T cell cultures on the second day of incubation with Pn, but still no diminution of  $T_s$  activity was observed. The anti-Id antibodies also failed to inhibit the generation of  $T_s$  in unfractionated spleen cell suspension.

*Inhibition of Educated, Effector  $T_s$  by Monoclonal Anti-Id Proteins.* Because it appeared that anti-Id proteins were unable to interfere with the generation of  $T_s$ , we set out to determine whether those antibodies might block the effect of mature  $T_s$  on responder B cells. For this, it was necessary to use T15-negative responder cells that would not themselves be inhibited by addition of the anti-Id into the assay cultures. Such cells were obtained from the spleen of BALB/c mice injected neonatally with monoclonal anti-T15 antibody MaId5-4. Preliminary experiments were carried out to ascertain that the splenic lymphocytes from T15-suppressed mice did respond to Pn in vitro and that the response was suppressed by Pn-educated T cells from normal BALB/c but not by any of the anti-Id antibodies.

Fig. 1 shows an experiment in which a mixture of two anti-Id antibodies, AB1-2 plus MaId5-4, was added to cultures containing Pn-educated  $T_s$  and spleen cells from T15-suppressed mice. The splenocytes from T15-suppressed mice responded well to Pn (group I) and the response was not inhibited by the anti-T15 Id antibodies (group II). The normal T cells incubated in culture medium did not inhibit the response (group III). There was a significant suppressive effect on Pn-educated T cells ( $T_s$ ); however, the suppression was overcome by addition of anti-Id antibody mixture to the assay culture. Thus, the response in the cultures containing  $T_s$  without anti-Id (group IV, 270 PFC/well) was suppressed by 64% and 60%, respectively, compared with control groups I and III. The response in cultures containing both  $T_s$  and anti-Id (684 PFC/well, group V) was significantly higher ( $P < 0.01$ ), and it was suppressed by only 9%, -6%, and 26%, respectively, compared with control groups I, III, and II. The comparison with group II is the most rigorous as it takes into account an eventual effect of anti-Id antibody on the responder cells themselves. In subsequent experi-

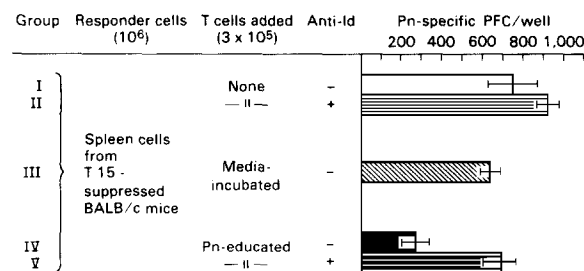


FIG. 1. Abrogation of the effect of  $T_s$  by monoclonal anti-idiotypic antibodies. Spleen cells from T15-suppressed BALB/c mice (responder cells,  $10^6$ /well) were stimulated with Pn in vitro, and the specific antibody PFC were enumerated 4 d later. Addition of anti-Id (mixture of Ab1-2 and MaId5-4 proteins) into the culture wells, on day 0 did not affect the response (group II). Addition of syngeneic, splenic T cells ( $3 \times 10^5$ /well), which were educated for 2 d with Pn (group IV) suppressed the response by 60% compared with control cultures with media-educated T cells (group III) and by 64% compared with group I. In contrast, the response in cultures that received both Pn-educated T cells and anti-Id (group V) was comparable to control responses (suppression by 9% vs. group I, 26% vs. group II, and -6% vs. group III).

TABLE V  
*Inhibition of Effector, Pn-specific Suppressor T Cells by Monoclonal Anti-Idiotopic (Anti-T15) Antibodies*

Experiment	Responder cells ( $10^6$ ) plus $3 \times 10^5$ cells added	Pn-specific PFC/well (mean $\pm$ SEM) with anti-Id added:					
		None (diluent)	AB1-2	MaId5-4	B36-75	B36-82	B24-50
A	None (control 1)*	681 $\pm$ 79	771 $\pm$ 109	777 $\pm$ 97	663 $\pm$ 62	660 $\pm$ 49	747 $\pm$ 102
	T media educated (control 2)‡	420 $\pm$ 37	NT	NT	NT	NT	NT
	T Pn educated§	72 $\pm$ 16 (89%)¶ (83%)**	486 $\pm$ 22 (37%)¶ (-15%)**	354 $\pm$ 37 (55%)¶ (16%)**	360 $\pm$ 49 (45%)¶ (14%)**	105 $\pm$ 18 (84%)¶ (75%)**	474 $\pm$ 60 (37%)¶ (-13%)**
B	None (control 1)*	564 $\pm$ 106	688 $\pm$ 40	788 $\pm$ 40	600 $\pm$ 47	544 $\pm$ 101	636 $\pm$ 73
	T media-educated (control 2)‡	458 $\pm$ 35	NT	NT	NT	NT	NT
	T Pn-educated§	184 $\pm$ 25 (67%)¶ (60%)**	488 $\pm$ 71 (21%)¶ (-7%)**	400 $\pm$ 42 (49%)¶ (13%)**	396 $\pm$ 79 (34%)¶ (14%)**	152 $\pm$ 25 (72%)¶ (67%)**	384 $\pm$ 44 (40%)¶ (16%)**

\* Fresh responder spleen cells from T15-suppressed BALB/c mice cultured with Pn or with Pn plus anti-Id antibody indicated.

‡ Responder cultures as above, with addition of  $3 \times 10^5$  T cells preincubated in culture medium for 3 d.

§ As above, with T cells preincubated with Pn. Figures in frames indicate the cultures with strong suppressive effect of  $T_s$ .

|| Not tested.

¶ Inhibited of response (%) with respect to control 1 (responder cells alone or with the respective anti-Id).

\*\* Inhibition of response (%) in respect to control 2 (responder cells plus media-educated T cells).

TABLE VI  
*Monoclonal Anti-T15 Antibodies Do Not Inhibit Sheep RBC-Specific Suppressor T Cells\**

Strain	Responder cells ( $10^6$ ) plus $1 \times 10^5$ cells added	Sheep RBC-specific PFC/well with anti-Id added:					
		None	AB1-2	MaId5-4	B36-75	B36-82	B24-50
BALB/c	None (control 1)	5,707	6,080	7,733	NT	NT	4,587
	T media educated (control 2)	10,720					
	T SRBC educated	1,440 (75%)‡ (87%)§	1,120 (82%) (90%)	1,227 (84%) (88%)	2,027 (NT) (81%)	2,027 (NT) (81%)	1,635 (64%) (85%)
C57BL/6	None (control 1)	2,837	2,432	2,731	2,880	2,645	2,581
	T media-educated (control 2)	2,122					
	T SRBC educated	277 (90%)‡ (87%)§	341 (86%) (84%)	256 (91%) (88%)	171 (94%) (92%)	235 (91%) (89%)	427 (83%) (80%)

\* The experimental set-up was the same as in Table V except that  $T_s$  was generated with sheep RBC and tested on sheep RBC-stimulated responder spleen cells. Both  $T_s$  and responders were from the same strain, either BALB/c or C57BL/6. Percent inhibition (see footnotes to Table V).

ments, therefore, the abolition of  $T_s$  activity by anti-Id was always measured against two controls: (a) responder cells plus media-incubated T cells, and (b) responder cells plus anti-Id.

The effect of several individually tested anti-Id antibodies on Pn-specific  $P_s$  is shown in Table V. None of the antibodies had a significant effect on the responder cells from T15-suppressed mice, and all but one of them have either diminished or abolished the suppression mediated by  $T_s$ . The exception was antibody B36-82, which consistently failed to affect the suppressor activity. The number of PFC in cultures with  $T_s$  plus B36-82 was not significantly different from that in cultures with  $T_s$  only ( $P > 0.1$  in both experiments 1 and 2, Table V) and the percent of suppression did

not change relative to either control group.

*Lack of Inhibition of Sheep RBC-specific T<sub>s</sub> by Anti-T15 Id Antibodies.* The specificity of the apparent abolition of the Pn-specific suppressor T cell activity by monoclonal anti-T15 Id antibodies was tested using T<sub>s</sub> specific for another antigen, sheep RBC. The purpose of the control study was to exclude the possibility that the monoclonal anti-T15 may detect an Ig determinant(s) cross-reactive with a T cell-specific surface antigen. A monoclonal antibody with such a fortuitous cross-reactivity has been recently mentioned by Pillemer and Weissman (33). The experimental design of the control experiment with sheep RBC-generated T<sub>s</sub> was the same as that with Pn. The study was carried out with syngeneic cells (T<sub>s</sub> and responders) from both BALB/c and C57BL/6 strain. However, none of the anti-Id antibodies had any detectable effect on the activity of sheep RBC-specific T<sub>s</sub> in either strain (Table VI).

### Discussion

Id associated with T15/48 idiotype family were defined operationally by monoclonal anti-idiotypic antibodies (anti-Id). Previous studies (8, 20) strongly suggested that the five anti-Id selected for our experiments react with distinct idiotypic determinants on a T15<sup>+</sup> immunoglobulin molecule. Screening of anti-PC serum antibody from different mouse strains by radioimmunoassay with anti-Id have shown that the Id are independently expressed, that is, all anti-Id reacted with BALB/c anti-PC antibody, but only some of them bound to antibodies from other inbred strains, in a random pattern (8). Further analysis of PC-reactive B cells and the mature, PC-specific PFC from individual mice of BALB/c and especially C57BL/6 strains confirmed the independence of Id expression. Even though some Id were more frequently detected than others, there was no linkage pattern discernable. The study also suggested that the T15<sup>+</sup> response of BALB/c mice to PC (presented on *S. pneumoniae*) is idiotypically heterogeneous and that the idiotypic repertoire expressed by the B cells may change in the course of antigen-driven differentiation, presumably by a process of somatic mutation (20). The hapten-inhibition analysis of the interaction between monoclonal anti-Id and PC-reactive myelomas (HOPC-8 or TEPC-15) indicate that the Id we detect occupy different sites of the Ig in respect to the paratope (8, and Table I).

The expression of five distinct Id on T cells was monitored by a functional assay (i.e., inhibition). First, we find that none of the anti-Id inhibited the generation of suppressor T cells by Pn in vitro; not even a mixture of several anti-Id(s) was effective (data not shown). Because all of the anti-Id did inhibit the activation of Pn-reactive B cells (i.e., precursors of antibody-forming cells) (20), the apparent failure to inhibit the T<sub>s</sub> activation does not seem to reflect an innate deficiency of the monoclonal proteins. One may still argue that the mode of antigen receptor expression on resting precursors T cells is much that it is not properly accessible to the anti-Id. However, in our earlier experiments, we observed an inhibition of T<sub>s</sub> education by a conventional mouse (A strain) antiserum against TEPC-15 (5). Thus it is possible that the receptor on T<sub>s</sub> precursors excludes the Id detectable by the selected monoclonal antibodies but includes other idiotypic determinants recognized by the polyvalent conventional antibody. A similar conclusion was made by Benca et al., (34) who found that PC-specific helper T cells (T<sub>H</sub>) were inhibitable by conventional (A strain) antiserum against HOPC-8 but not by a monoclonal antibody AB1-2 generated against the



same myeloma protein (34). On the other hand, one of the other anti-Id used in our study, MaId 5-4, was shown to inhibit PC-specific  $T_H$  activity (35).

However, we found that the monoclonal anti-Id did reverse the suppressive effect of activated  $T_s$  on response to Pn. To show this, we used responder cells from syngeneic (BALB/c) mice injected neonatally with monoclonal antibody MaId 5-4 against TEPC-15. The treatment leads to chronic suppression (deletion) of T15/H8 idiotype-bearing clones (21), which are replaced by PC-reactive clones bearing a different, heretofore unidentified idiotype (36, 37). The MaId 5-4 appears to be directed against a very common Id of the T15 family that is present on most anti-PC antibodies (13) and on virtually all PC-reactive B cells from BALB/c as well as C57BL/6 strain (20). Thus it was expected that Pn-reactive cells from the suppressed T-15-mice would not express any other Id of T15 family, as was indeed shown by the failure of any of the anti-Id to inhibit the response (Table V). The ostensibly  $T15^-$  B cells were nonetheless readily suppressed by Pn-educated  $T_s$  from normal, T15-positive mice, which substantiates the earlier notion that the effectorial specificity of the  $T_s$  is towards the antigen rather than the idiotype of B cells (5).

The monoclonal anti-Id had no detectable effect on the responder cells (derived from the T15-suppressed mice) but did abolish the suppressive effect of  $T_s$  (obtained from normal donors). The reversal of suppression was specific, as there was no effect of the antibodies on sheep RBC-specific  $T_s$ . The possible role of Fc region of anti-Id antibodies in the inhibition of  $T_s$  has not yet been investigated. However, the interaction of anti-Id with  $T_s$  does not alter the effector cell specificity in that Pn-educated  $T_s$  failed to inhibit the responses to unrelated antigens, sheep RBC and TNP-BA whether or not the anti-Id(s) were added (data not shown).

We interpret the data as evidence for expression of individual Ig idiotopes on mature, effectorial  $T_s$ . Four out of five Id were detectable in that manner in our assay. One of those determinants, B 36-75, is hapten (PC)-inhibitible and, therefore, located within the paratope. Id recognized by AB1-2 and MaId 5-4 are inhibitible only with PC keyhole limpet hemocyanin, suggesting that these may be somewhere near the antigen-binding site, whereas B 24-50 seems to be entirely nonparatopic (Table I). Thus, the results indicate that the  $T_s$  may express both paratopic and nonparatopic structures of the variable region of Ig. Interestingly, a determinant not detectable on  $T_s$  was that defined by B 36-82. This Id, which is uniformly expressed by virtually all PC-reactive B cells and PFC in BALB/c strain (20), appears to reflect a structural difference between  $T_s$  receptor and Ig.

There is evidence for an alteration of idiotype during antigen-driven differentiation of B cells. The switch from IgM to IgG anti-PC antibody production is accompanied by changes in primary amino acid sequences in  $V_H$  (38) and a loss of T15 expression as measured by a conventional anti-T15 antiserum (39). Recently, a transient change in Id-460 idiotype expression has been observed within the IgM-producing, TNP-reactive B cells (40). It is tempting to speculate that a similar change in idiotopic repertoire may occur during the T cells differentiation and that this may explain why the selected monoclonal anti-Id failed to inhibit the education of precursor  $T_s$  population while they did inhibit the mature, effector  $T_s$ . Changes in Id expression on both classes of lymphocytes during their immune differentiation may play a role in the regulatory balance between idiotopic and anti-idiotopic clones.

The existence of idiotopic overlap between T and B cells has been suggested

previously. Binz and Wigzell (41) used lymphocyte absorption of anti-Id sera produced against alloantibody to show that alloreactive T cells expressed some but not all idiotypic members of B cells. On the other hand, Karwink et al. (42), working with the NP<sup>b</sup> idiotype detectable by a rabbit antiserum on NIP-specific antibody observed a decreased NP<sup>b</sup> expression in hyperimmune mice while the NIP-binding receptor from T cells retained the NP<sup>b</sup> idiotype. Our study indicates that discrepancies like that could be resolved by mapping of individual idiotopes with monoclonal reagents at various stages of antigen-driven lymphocyte differentiation.

### Summary

The idiotopic repertoire expressed by antigen-specific suppressor T cells (T<sub>s</sub>) generated by *Streptococcus pneumoniae* strain R36a (Pn) in BALB/c strain mice was investigated using a panel of five monoclonal anti-idiotopic antibodies against TEPC-15/HOPC-8 myeloma proteins. Previous studies suggested that the anti-idiotopic antibodies recognize distinct idiotopic determinants within the T15 idiotype, and that Pn-reactive B cells express all of those idiotopes as shown by a specific inhibitory effect of the anti-idiotopic antibodies on induction of anti-Pn response *in vitro* as well as on the mature antibody plaque-forming cells.

In this study we asked the question of whether anti-idiotopic (Id) can block the inductive and/or effector phases of generation of T<sub>s</sub> which act on the Pn-reactive B cells. The presence of anti-Id during the activation of T cells with Pn did not prevent the generation of T<sub>s</sub>. However, suppression mediated by T<sub>s</sub> on responder lymphocytes (cultures of spleen cells or B cells) was inhibited (reversed) by four out of five anti-Id. Some of the antibodies recognize hapten (phosphorylcholine)-inhibitable Id in the paratope of Ig whereas others are directed against nonparatopic Id. These data indicate that the antigen receptor on T<sub>s</sub> includes V<sub>H</sub> sequences both within and without the immunoglobulin in paratope, and that the Id repertoire of T<sub>s</sub> overlaps with that of B cells.

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