

CELLULAR BASIS OF REGULATION OF EXPRESSION OF IDIOTYPE

II. Immunity to Anti-MOPC-460 Idiotypic Antibodies Increases the Level of Anti-Trinitrophenyl Antibodies Bearing 460 Idiotypes

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It has recently been shown that an idiotypic determinant(s) (460-Id)¹ found on the 2,4-dinitrophenyl (DNP) and 2,4,6-trinitrophenyl (TNP)-binding myeloma protein, MOPC-460, is expressed on some anti-TNP antibodies produced by BALB/c mice after immunization with certain thymus-independent (TI) (1) and thymus-dependent (2) TNP antigens. In vitro studies indicate that the 460-Id-bearing component of the anti-TNP response to the TI antigen, TNP-Nocardia water-soluble mitogen (NWSM), is regulated by a 460-Id-specific suppressor T cell found in the spleen of normal BALB/c mice (1).

In this communication, we report the results of experiments in which we studied the effect of anti-460-Id antibodies and of anti-[anti-460-Id] antibodies on the response to two TI TNP antigens. Mice actively immunized with MOPC-460 or acutely pretreated with anti-460-Id antibodies made an anti-TNP response to TNP-NWSM and to TNP-levan which lacked a 460-Id⁺ component. By contrast, mice actively immunized with purified anti-460-Id antibodies or acutely pretreated with anti-[anti-460-Id] antibodies showed an increase in the 460-Id⁺ component of their anti-TNP response. Furthermore, T cells from mice immunized to anti-460-Id antibodies failed to suppress, in vitro, the 460-Id⁺ component of the response of normal B cells to TNP-NWSM. These results suggest (a) that 460-Id-specific suppressor T cells normally regulate in vivo responses to TI TNP-antigens; (b) that anti-[anti-460-Id] antibodies can eliminate 460-Id-specific suppressor T cells; and (c) that 460-Id-specific suppressor T cells and anti-460-Id antibodies share idiotypic determinants.

Materials and Methods

Mice. BALB/cAnN mice, 8–12 wk old, were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md.

Antigens. 2,4,6-trinitrophenyl (TNP)-Nocardia water soluble mitogen (NWSM) was pre-

¹ *Abbreviations used in this paper:* AECM, aminoethylcarbonylmethyl; C, complement; HA, hemagglutinin titer; Id, idiotype; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; NWSM, Nocardia water soluble mitogen; PFC, plaque-forming cell; RIA, radioimmunoassay; SRBC, sheep erythrocytes; TI, thymus-independent; TNP, 2,4,6-trinitrophenyl.

pared as previously described (1). An aminoethylcarbonylmethyl (AECM) derivative of levan, derived from *Aerobacter levanicum*, was the kind gift of Dr. C. Glaudemans, National Institute of Arthritis, Metabolic and Digestive Diseases, National Institutes of Health, Bethesda, Md. It contained 1 AECM group per 21 sugars. TNP-levan, containing 1 TNP per 24 sugars, was prepared by the reaction of trinitrobenzenesulfonate with AECM-levan.

Myeloma Proteins. MOPC-460, an IgA, κ -myeloma protein with DNP- and TNP-binding activity, and EPC-109 and UPC-61, IgA, κ -myeloma proteins with $\beta(2-6)$, $\beta(2-1)$ fructosan-binding activity were kindly donated by Dr. Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Preparation of Anti-Idiotype Antibodies. Anti-460-Id antibodies were prepared by immunization of BALB/c mice with 75 μ g of MOPC-460 myeloma protein emulsified in complete Freund's adjuvant. This was followed 5 d later by a similar dose of myeloma protein in incomplete Freund's adjuvant and then by six weekly injections of 75 μ g of protein in saline. Anti-460-Id antibodies were also obtained from a hybridoma [No. 77-14-11-F6(51)] derived by Buttin et al. (3). These antibodies were used in the radioimmunoassay for 460-Id. Antibodies specific for the idiotypic determinants of anti-460-Id antibodies (anti-[anti-460-Id] antibodies) were produced by immunization of BALB/c mice with purified anti-460-Id antibodies coupled to keyhole limpet hemocyanin (KLH). Anti-460-Id antibodies were purified by absorption of an ammonium sulfate fraction of BALB/c anti-460-Id serum to a Sepharose 4B-MOPC-460 column (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) followed by elution with a glycine-HCl buffer (0.1 M; pH 2.8). The purified antibodies were conjugated to KLH by mixing purified antibody with KLH, at a final concentration of 0.5 mg/ml, in the presence of 0.05% glutaraldehyde. The reaction was allowed to proceed until the solution was opalescent; it was then stopped by the addition of lysine to a final concentration of 0.05 M. This was followed by extensive dialysis. The KLH-anti-460-Id conjugate was then used to immunize BALB/c mice according to the same schedule as that described for immunization with MOPC-460. Anti-E109-Id antibodies were produced in A/He mice as previously described (1).

Antibody Assays. TNP-coated sheep erythrocytes (SRBC) were prepared according to the technique of Rittenberg and Pratt (4). Levan-SRBC were prepared using O-steroyl levan as described by Hammerling and Westphal (5). MOPC-460-, EPC-109-, and UPC-61-coated SRBC were prepared using the chromic chloride method (6). Anti-TNP, anti-levan, and anti-460-Id serum antibody titers were determined by agglutination of coated SRBC. The hemagglutinin (HA) titer reported is the \log_2 of the reciprocal of the highest dilution giving agglutination. Serum 460-Id titers were determined by the capacity of serum and serum dilutions to inhibit the agglutination of MOPC-460-coated SRBC by anti-460-Id antibodies. The hemagglutination inhibition titer reported is \log_2 of the reciprocal of the highest dilution of serum which caused inhibition of hemagglutination.

Serum levels of 460-Id were also measured by a radioimmunoassay in which the capacity of serum and serum dilutions to inhibit the binding of 125 I-labeled F(ab) fragments of MOPC-460 by hybridoma anti-460-Id antibodies was determined (3). F(ab) fragments of MOPC-460 myeloma protein were prepared according to the techniques of Kuetter et al. (7) and labeled with 125 I through the use of chloramine T (8). Rabbit anti-mouse Fc antibodies were used to precipitate complexes of MOPC-460 F(ab) fragments and anti-460-Id antibodies. These anti-Fc antibodies were prepared by immunization of rabbits with two injections of 1 mg each of purified H chains followed by absorption of the serum on an AH-Sepharose column (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) to which F(ab) fragments of A/J IgG had been linked (9). The H chains were purified from pooled A/J IgG by reduction and alkylation, followed by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) equilibrated with 5 M guanidine (10). F(ab) fragments of A/J IgG were prepared as described by Porter et al. (11).

Separation of T and B Lymphocytes. Thymus-dependent (T) lymphocytes were purified by passage over nylon wool columns. Of the cells obtained, 95–98% could be killed by anti-Thy 1.2 and complement (C) (12). These cells failed to proliferate in vitro to lipopolysaccharide (LPS) or NWSM. Cell preparations enriched in thymus-independent (B) lymphocytes were obtained by treating spleen cells with anti-Thy 1.2 serum (Litton-Bionetics, Bethesda, Md.) and C as previously described (1). These cells gave in vitro thymidine-incorporation responses

to concanavalin A of less than twice background in experiments in which unseparated cells gave responses which were 50–120 times background. The B-cell preparations responded well to LPS and NWSM. Such responses could be abolished by treating B cells with rabbit anti-mouse Ig and C (13).

In Vitro Responses to TNP-NWSM. B lymphocytes (5×10^5) were cultured alone or with T lymphocytes (5×10^5) in microtiter plates (tissue culture, cluster 36, Costar, Data Packaging, Cambridge, Mass.) in a modified Mishell-Dutton medium containing 10% fetal calf serum and 10^{-5} M 2-mercaptoethanol in the presence or absence of TNP-NWSM ($3 \mu\text{g}/\text{ml}$) for 3 d.

Plaque-Forming Cell (PFC) Assay. The number of cells secreting antibodies specific for TNP and levan was determined by a hemolytic plaque assay, as previously described (1, 14). The percentage of anti-TNP PFC-secreting anti-TNP antibodies carrying the 460-Id and of anti-levan PFC-secreting anti-levan antibodies carrying E109-Id was determined by adding BALB/c anti-460-Id or A/He anti-E109-Id antisera to the agarose, as previously described (14). The difference between the number of PFC obtained in the absence and presence of anti-Id represents the number of PFC-secreting antibody bearing the Id under study. The standard error of these numbers of PFC-secreting Id-bearing antibodies is $SE = \sqrt{SE_1^2 + SE_2^2}$, in which SE_1 is the SE of the number of PFC in absence of anti-Id sera, and SE_2 is the SE of the number of PFC in the presence of anti-Id sera.

The number of direct and indirect PFC-secreting antibody specific for MOPC-460 was determined using MOPC-460-coated SRBC.

Results

Production of Syngeneic Anti-460-Id Antibodies in BALB/c Mice. To determine whether the production of anti-TNP antibodies expressing 460-Id could be influenced by pretreatment with anti-460-Id antibodies, or by a state of active immunity to 460-Id determinants, we immunized BALB/c mice with MOPC-460, as described in Materials and Methods. Such mice developed antibodies which agglutinated MOPC-460-coated SRBC, but not MOPC-167-coated SRBC (Table I A). Because both MOPC-460 and MOPC-167 are IgA, κ -myeloma proteins, this result indicates that a syngeneic anti-460-Id antibody response has occurred and confirms a previous report by Sakato and Eisen (15). Furthermore, these immune animals have splenic PFC specific for MOPC-460, but not for U-61 as shown by selective lysis of MOPC-460-coated SRBC (Table I B) and selective inhibition of this lysis by MOPC-460 (Table I C).

Influence of Anti-460-Id Antibodies on Production of 460-Id⁺ Anti-TNP Antibodies. We have previously shown that a small but significant fraction of anti-TNP antibodies produced by BALB/c mice in response to immunization with TNP-NWSM carry 460-Id determinants. In the experiment illustrated in Table II, 23% of anti-TNP-PFC produced in response to TNP-NWSM immunization could be inhibited by incorporation of anti-460-Id in the agarose, indicating that the anti-TNP antibodies produced by these cells bore 460-Id. BALB/c mice which had been immunized with MOPC-460 (Id-2 mice) failed to develop any PFC which could be inhibited by anti-460-Id, although they made a substantial anti-TNP antibody response. Similarly, normal BALB/c mice pretreated with three doses of $0.1 \mu\text{g}$ of anti-460-Id or more failed to develop anti-TNP PFC which secreted 460-Id⁺ antibodies. Pretreatment with three doses of $0.01 \mu\text{g}$ of anti-TNP-Id had no effect. These indicate that modest to high doses of anti-460-Id inhibit the activation of 460-Id⁺ anti-TNP B cells, whereas low doses show neither inhibitory nor stimulatory activity.

Production of Syngeneic Antibodies Specific for Anti-460-Id (Anti-[Anti-460-Id] Antibodies). BALB/c mice immunized with KLH conjugates of anti-460-Id antibodies produced antibodies which were capable of agglutinating SRBC coated with

TABLE I
Anti-Idiotypic Antibody Response to MOPC-460 by BALB/c Mice

A. Hemagglutinin response (n = 12)		B. PFC response (n = 5)			C. Specificity of anti-460-Id PFC		
SRBC coated with	HA titer (log ₂)	SRBC coated with	PFC/10 ⁷ spleen cells		Inhibitor	Amt	Direct anti-MOPC-460 PFC/10 ⁷ spleen cells
			Direct	Indirect			
MOPC-460	5.83 ± 0.68	MOPC-460	61.3 ± 15.5	15.8 ± 3.6	None	—	147 ± 9
MOPC-167	0	U-61	3.3 ± 3.3	6.0 ± 2.8	MOPC-460	1	89 ± 7
						10	30 ± 3
						100	15 ± 4
					U-61	1	148 ± 13
						10	141 ± 11
						100	81 ± 13

TABLE II
Number of PFC-Secreting Anti-TNP Antibodies Carrying 460-Id in Normal BALB/c Mice Injected with Anti-460-Id Antibodies and in Id-2 BALB/c Mice

BALB/c mice*	Dosage	No. of mice tested	Anti-TNP PFC		Anti-TNP (HA)	Anti-MOPC 460-Id (HA)
			Total	460-Id*		
	μg			%		
Normal	—	3	305 ± 15	23.0 ± 2.0	8.0 ± 1.0	0.5 ± 0.5
Id-2	—	6	245 ± 46	1.5 ± 0.9	6.5 ± 1.0	4.2 ± 0.9
Normal pretreated	0.01	3	343 ± 17	22.0 ± 2.0	6.0 ± 0	0
With purified	0.1	3	156 ± 25	1.5 ± 1.5	7.0 ± 1.0	0
Anti-MOPC-460-Id	1	3	118 ± 6	0	8.5 ± 0.5	0
Antibodies	10	3	165 ± 24	14.0 ± 12.0	8.0 ± 0	3.0 ± 1.0
	100	3	244 ± 2	13.0 ± 1.0	5.0 ± 1.0	5.0 ± 2.0

* All groups of mice were immunized with 30 μg TNP-NWSM 7 d after completion of pretreatment.

purified anti-460-Id (HA titer 3.2 ± 0.3) but not SRBC coated with BALB/c IgG_{2a} or with UPC-10 myeloma protein. To determine whether these serum hemagglutinins were anti-(anti-460-Id) antibodies or anti-TNP antibodies bearing 460-Id determinants, we performed two types of experiments. First, we absorbed the putative anti-[anti-460-Id] antiserum on TNP-KLH-Sepharose 4B to remove any anti-TNP antibodies which might have been present. Such treatment did not diminish the HA titer on anti-460-Id-coated SRBC. Secondly, we demonstrated that the binding of ¹²⁵I anti-460-Id hybridoma by anti-[anti-460-Id] immunoglobulin adsorbed on a plastic surface was not inhibited by TNP-lysine or TNP-glycine. Buttin et al. (3) have shown that both TNP-lysine and TNP-glycine inhibit the binding of MOPC-460 by the anti-460-Id hybridoma. These results indicate that the immunoglobulins which can agglutinate anti-460-Id-coated SRBC lack anti-TNP activity. This strongly suggests that these hemagglutinins are anti-[anti-460-Id] antibodies, although we cannot entirely exclude the possibility that they are 460-Id⁺ immunoglobulins which lack anti-TNP activity.

Enhancement of the 460-Id⁺ Component of the Anti-TNP Response in Id-3 Mice and in Normal Mice Pretreated with Anti-[Anti-460-Id] Antibody. Mice which had been immunized with KLH-conjugates of anti-460-Id antibodies and which developed an HA response

TABLE III
Proportion of Anti-TNP-Antibodies Carrying 460-Id in Normal BALB/c and BALB/c Mice Producing Anti-[Anti-460-Id] Antibodies (Id-3 Mice)

	TNP-NWSM		TNP-levan	
	Normal	Id-3	Normal	Id-3
Number of mice	5	3	5	4
Anti-TNP PFC/10 ⁶ cells	100 ± 14	84 ± 8	276 ± 15	239 ± 64
Percent 460 ⁺	27 ± 12	54 ± 5	12 ± 1	34 ± 5
Anti-levan PFC/10 ⁷ cells	ND*	ND	203 ± 130	284 ± 41
Percent E109 ⁺	ND	ND	42 ± 5	45 ± 20
Anti-TNP HA titer	6 ± 0.5	8 ± 2	12 ± 0.3	12 ± 0
460-Id HI titer	0.5 ± 0.5	2 ± 1	1 ± 0.5	3.5 ± 0.5
µg 460-Id/ml (RIA)‡	22 ± 6	57 ± 21	14 ± 6	60 ± 25

Normal BALB/c or Id-3 BALB/c mice were immunized with 30 µg of TNP-NWSM or 50 µg of TNP-levan. These were sacrificed 5 d later and humoral and spleen PFC responses tested.

* Not done.

‡ Radioimmunoassay.

specific for anti-460-Id-coated SRBC (Id-3 mice) were immunized with either TNP-NWSM or TNP-levan. These mice produced an anti-TNP antibody response in which the 460-Id⁺ component was substantially increased as compared to normal BALB/c mice immunized with the same TNP antigens (Table III). Thus, of the anti-TNP PFC produced by Id-3 mice immunized with TNP-levan, 34% could be inhibited with anti-460-Id, whereas only 12% of the anti-TNP PFC produced by normal BALB/c mice immunized with TNP-levan were inhibitable. The *P* value for the difference of these values was < 0.005. A similar difference was observed in the anti-TNP response to TNP-NWSM, although it did not reach levels of statistical significance. In addition, serum concentration of 460-Id molecules were also higher in Id-3 mice than in normal mice, as judged both by the 460-Id HI titer and by radioimmunoassay. On the other hand, the total anti-TNP response in normal and Id-3 mice was essentially the same and Id-3 mice immunized with TNP-levan displayed a proportion of E109⁺ anti-BL PFC which was similar to that of normal mice.

Furthermore, when normal BALB/c mice were pretreated with three doses of 100 µg of an ammonium sulfate fraction of anti-[anti-460-Id] antiserum, previously absorbed on a TNP-lysine-Sepharose-4B column, both the proportion of the 460-Id⁺ anti-TNP PFC and the serum 460-Id concentration produced in response to TNP-NWSM were significantly increased (Table IV). This result and that of the previous experiment indicate that anti-[anti-460-Id] antibodies enhance the number of anti-TNP PFC which secrete 460-Id⁺ antibodies.

Absence of 460-Id-Specific Suppressor T Cells in Id-3 Mice. In a previous study, we have shown that naturally occurring 460-Id-specific suppressor T cells regulate the activation of those cells potentially capable of producing 460-Id⁺ anti-TNP antibodies. In the following experiment, we compared the suppressive effect of nylon wool-enriched T lymphocytes from normal and Id-3 mice. Data presented in Table V show that T lymphocytes from normal BALB/c mice inhibited the 460-Id⁺ component of the in vitro anti-TNP response of normal and of Id-3 B lymphocytes to TNP-NWSM.

TABLE IV
Proportion of Anti-TNP Antibodies Carrying 460-Id in BALB/c Mice Pretreated with Anti-[Anti-460-Id] Antibodies*

Pretreatment of mice	TNP-NWSM	N	Anti-TNP-PFC		Anti-TNP HA titer	μg 460-Id/ml (RIA)
			PFC/ 10^6 cells	Percent 460-Id ⁺		
-	-	3	35 \pm 3	0	4 \pm 0.5	<0.1
-	+	8	519 \pm 49	32 \pm 3	6.3 \pm 0.7	12.5 \pm 7.3
+	-	3	27 \pm 4	43 \pm 8	2 \pm 0.7	<0.1
+	+	5	597 \pm 24	52 \pm 4	7.8 \pm 1.1	48.5 \pm 7.8

* Mice were pretreated three times, at 3-d intervals, with 100 μg of an ammonium sulfate fraction of BALB/c anti-[anti-460-Id] antibodies and immunized 1 d after completion with 30 μg TNP-NWSM. Mice were sacrificed 5 d later and humoral and spleen PFC responses tested. The anti-[anti-460-Id] antibody used in the experimental group had been passed over a TNP-KLH-Sepharose-4B column.

TABLE V
Proportion of PFC-Secreting Anti-TNP Antibodies Carrying 460-Id in Cultures of T and B Lymphocytes from Normal and Id-3 BALB/c Mice

Donor of lymphocytes		Anti-TNP-PFC		
B	T	PFC/culture	460-Id ⁺ PFC/culture	Percent 460-Id ⁺ PFC
Normal	—	191 \pm 26	73 \pm 27	38
Normal	Normal	105 \pm 6	12 \pm 7	11
Normal	Id-3	134 \pm 13	55 \pm 13	41
Id-3	—	432 \pm 15	224 \pm 18	52
Id-3	Normal	440 \pm 18	42 \pm 26	10
Id-3	Id-3	367 \pm 41	206 \pm 43	56

B lymphocytes (5×10^5) were cultured alone or with 5×10^5 T lymphocytes in microtiter wells with 5 $\mu\text{g}/\text{ml}$ of TNP-NWSM for 4 d. Anti-TNP PFC in the absence or presence of BALB/c anti-460 antiserum (1/100 dilution) were measured using TNP-SRBC.

By contrast, T cells obtained from Id-3 mice failed to show any detectable inhibition of the 460-Id⁺ anti-TNP response of either normal or Id-3 B cells. This result indicates that Id-3 mice lack, or are deficient in, 460-Id-specific suppressor T cells. The absence of such cells may be a major factor in the heightened 460-Id⁺ anti-TNP response of Id-3 mice.

Discussion

In the studies presented here, we have demonstrated that BALB/c mice with active or passive immunity to the idio-type(s) of MOPC-460 fail to express 460-Id⁺ antibodies in their humoral response to TNP-levan and TNP-NWSM. Conversely, mice with active or passive immunity to idiotypic determinants of anti-460-Id antibodies express a heightened 460-Id⁺ component in their anti-TNP response to TNP-NWSM and to TNP-levan. The mechanism through which immunity to MOPC-460-Id eliminates a 460-Id⁺ response has not been established. However, based on studies in the phosphoryl choline-T15 system and in the bacterial levan-E109 system (16, 17) elimination or inactivation of precursors of cells capable of secreting 460-Id⁺ anti-TNP antibodies by anti-Id antibody is a major possibility. In addition, in preliminary experiments we

have shown that such mice possess suppressor T lymphocytes capable of inhibiting a 460-Id⁺ response to TNP-NWSM but we have not yet established whether suppressor activity in mice actively immune to MOPC-460 exceeds that in normal mice. The heightened 460-Id⁺ response of mice with active or passive immunity to anti-460-Id antibodies correlates with the absence of 460-Id-specific suppressor T lymphocytes in these mice, and is very likely a result of the absence of such cells. Furthermore, it seems likely that anti-[anti-460-Id] antibodies have reacted with and eliminated these 460-Id-specific suppressor T cells, suggesting that these suppressor T cells and anti-460-Id antibody share common idiotypic determinants. This would be consistent with a similarity in the structure of the 460-Id-specific receptor on the suppressor T cell and of the binding site of the anti-460-Id antibody.

Furthermore, these results suggest that the *in vitro* regulation by suppressor T cells of the 460-Id⁺ component of the anti-TNP response also explains the relatively small component of 460-Id⁺ molecules in the *in vivo* anti-TNP response. This would suggest that a regulatory network consisting of Id-bearing B cells and of T cells specific for idio type is an important feature of the normal immune response. Whether spontaneous development of anti-[anti-460-Id] antibodies and T cells also occurs and acts to regulate the natural level of suppression is a provocative but unresolved issue.

Other instances in which an idio type-anti-idio type network has been employed to explore the role of such a mechanism in regulation should be mentioned. In one striking example, rabbit anti-Id antibody to anti-ribonuclease obtained from a single donor was prepared. Rabbits immunized to these anti-Id antibodies and then immunized with ribonuclease developed anti-ribonuclease antibodies with an idio type similar to that expressed by the antibodies used to prepare the initial anti-Id antibody. Because sharing of idiotypes in anti-ribonuclease antibodies of normal rabbits is rarely observed (18), these results suggest that immunization with anti-Id antibody perturbed the existing balance, either by directly activating Id-bearing B or T cells or by eliminating suppressor T cells which normally limited the expression of that idio type. A similar observation has been made in response to certain polysaccharide antigens (19).

These examples and our results give clear illustrations of the ways in which regulatory interactions among a network (20) of Id-bearing and Id-specific antibodies and cells might occur, as well as emphasizing that second degree idio type-anti-idio type (Id-2-anti-Id-2) interactions could profoundly effect the expression of Id-1 (e.g., 460-Id⁺ anti-TNP antibodies) determinants on the antibodies produced as a result of conventional immunization. The extent to which anti-[anti-460-Id] antibodies occur and to which anti-460-Id T cells are eliminated in normal strains immunized with TNP-NWSM or TNP-levan would shed considerable light on the physiologic relevance of these systems.

Summary

The antibody response of BALB/c mice to trinitrophenyl (TNP)-levan or TNP-*Nocardia* water-soluble mitogen (NWSM) includes a small but significant fraction of antibodies which share idiotypes (Id) with the dinitrophenyl (DNP)- and TNP-binding myeloma protein MOPC-460. Active immunization of BALB/c mice with MOPC-460 or passive administration of anti-460-Id antibodies suppresses the 460-Id⁺ component of the anti-TNP response. By contrast, active immunization of BALB/

c with anti-460-Id antibodies or passive administration of BALB/c anti-[anti-460-Id] antibodies leads to an enhanced 460-Id⁺ component in the anti-TNP antibodies produced in response to TNP-levan or TNP-NWSM. This enhanced 460-Id⁺ response appears to be a result of the elimination of suppressor T lymphocytes specific for the 460-Id as T lymphocytes from such mice are unable to suppress the in vitro 460-Id⁺ response to TNP-NWSM whereas normal T cells are suppressive. These results indicate that suppressor cells specific for 460-Id normally regulate the activation of precursors of cells capable of secreting 460-Id⁺ anti-TNP antibodies.

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