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

By CONSTANTIN BONA* and WILLIAM E. PAUL

From the Laboratory of Immunology, National Institute of Allergy, and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

It has been proposed that idiotypic determinants expressed on precursors of antibody-secreting cells and T lymphocytes might serve as sites for the specific regulation of lymphocyte function (1–8). Several instances in which T lymphocytes regulate production of antibodies of a given idiotype or allotype have been reported (9–12). Such regulator cells have usually been demonstrated in animals which have been intentionally pretreated with anti-idiotype or anti-allotype antibody. The general validity of the concept that lymphocyte function may be regulated through immunoglobulin determinants would be strengthened by the discovery of a naturally occurring idiotype-specific suppressor system.

In the course of examining the expression on anti-trinitrophenyl (TNP)[1] antibodies of the idiotype(s) associated with the TNP-binding myeloma protein MOPC 460, we have encountered an example of such natural suppression. We found that anti-TNP antibodies produced by BALB/c mouse cells, in vivo or in vitro, in response to some thymus-independent (TI) TNP antigens include a component that express 460 idiotype(s) (Id). In a more detailed examination of the in vitro response to TNP-Nocardia water-soluble mitogen, we observed that removal of T lymphocytes from spleen cell populations increased the number of cells secreting 460Id-bearing molecules. Addition of T lymphocytes from normal BALB/c mice suppressed the 460Id component of the B-cell response to TNP-NWSM. Plate-binding experiments indicated that the suppressive action of the T-lymphocyte population depends on a cell which can bind to MOPC 460 myeloma protein.

Materials and Methods

Animals. Adult mice (older than 8 wk) were used in all experiments. BALB/cAnN, A/He, and C57BL/6 mice were obtained from the Small Animal Section, Division of Research

* Maître de Recherche CNRS and Pasteur Institute, Paris, France; visiting scientist in the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

[1] *Abbreviations used in this paper*: BL, bacterial levan; DNP, dinitrophenyl; HA, hemagglutination; HI, hemagglutination inhibition; Id, idiotype; IdX, a major cross-reactive idiotype; LPS, lipopolysaccharide; NWSM, Nocardia water-soluble mitogen; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TNP, trinitrophenyl.

Services, National Institutes of Health. BALB/c *nu/nu* and +/*nu* littermates were obtained from Sprague Dawley Co., Madison, Wis. C.B20 mice were obtained from Litton Bionetics (Bethesda, Md.).

Antigens. TNP derivatives of Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.), levan, and dextran were prepared according to Inman et al. (13) and TNP-*Brucella abortus* according to Mond et al. (14). TNP was coupled to other carriers by adding 10 mg of carrier to a solution containing 6 mg of trinitrobenzenesulfonate in 5 ml of cacodylate buffer (0.28M), pH 5.9. The pH was raised to 11.5 with NaOH. After a 3-h incubation at room temperature with stirring, the solutions were dialyzed for 48 h against 0.15 M NaCl. Bacterial levan was prepared from *Aerobacter levanicum* (15) and Nocardia water-soluble mitogen (NWSM) from *Nocardia opaca* (16) as previously described. *Serratia marcescens* lipopolysaccharide (LPS) was prepared by trichloroacetic acid extraction (17).

Myeloma Proteins. MOPC 460 (IgA, κ, specific for dinitrophenyl (DNP) and TNP), MOPC 167 (IgA, κ, specific for phosphorylcholine), EPC 109 (IgA, κ, specific for levan and inulin), and XRPC-24 (IgA, κ, specific for galactan) myeloma proteins were kindly donated by Dr. Michael Potter, National Cancer Institute, National Institutes of Health.

Preparation of Anti-Idiotypic Serum. Anti-idiotypic sera against XRPC-24 (X24) and EPC 109 (E109) were raised in A/He mice, according to a previously described technique (18). These antisera were made specific by adsorption on MOPC 167 Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). Anti-idiotypic serum against MOPC-460 (anti-460Id) was prepared in BALB/c mice by immunization with 75 μg protein in CFA, followed 5 d later by a similar injection in IFA, then by 6 weekly injections of 75 μg protein in saline. By direct HA tests, this serum lacked activity against MOPC 167- and E109-coated SRBC; consequently, it did not require absorbtion with myeloma proteins to render it specific. A preparation was specifically purified by absorbtion to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) coated with MOPC 460 and elution with glycine-HCl buffer (0.1 M, pH 2.8).

Coating of Sheep Erythrocytes (SRBC). SRBC were coated with TNP as described by Rittenberg et al. (19). SRBC were coated with MOPC 460 myeloma proteins by the chromium chloride method (18); the proteins were coupled at a final concentration of 1.0 mg/ml. SRBC were coated with D-steroyl derivatives of bacterial levan, inulin, and with galactan, prepared as described by Hammerling and Westphal (20).

Determination of Anti-TNP Titers by Hemagglutination. Hemagglutination (HA) of TNP-coated SRBC was expressed as $\log_2$ of the highest dilution giving complete agglutination.

Determination of Serum Titer for MOPC-460 Idiotype. A hemagglutination inhibition (HI) method described earlier (15) was used to quantify the level of 460Id in sera. In these experiments, antibodies to MOPC 460 and SRBC coated with MOPC 460 were used. The titer is $\log_2$ of the highest dilution of serum which causes inhibition of hemagglutination.

Separation of T and B Lymphocytes. T cells were purified by passing spleen cells on a nylon wool column (21). Of the cells obtained, 95–98% could be killed by anti-Thy 1.2 and complement. These cells did not give proliferative response to LPS or NWSM. B-cell preparations were obtained by treating spleen cells with anti-Thy 1.2 serum (Litton Bionetics) and complement as previously described (21). These cells gave responses to Con A of less than twice background in experiments in which unseparated spleen cells gave responses which were 50 to 120 times background. Conversely, the B-cell preparations responded well to LPS and NWSM.

Separation of MOPC 460-Specific Lymphocytes. Petri dishes were coated with MOPC 460, EPC 109, MOPC 167, or purified anti-MOPC 460 antibodies according to the technique described by Mage et al. (22). Nylon wool-purified T-lymphocyte populations (10^7/ml per dish) in RPMI-1640 containing 5% heat-inactivated fetal calf serum were incubated on the plate for 1 h. The nonadherent cells were recovered by gentle shaking; the adherent cells were then obtained by scraping with a rubber policeman.

Plaque-Forming Cell Assay. Cells were cultivated in micro-titer plates (tissue culture, cluster 36, Costar, Cambridge, Mass.) in Mishell-Dutton medium as previously described (14). The number of plaque-forming cells (PFC) specific for TNP, bacterial levan (BL), or galactan was determined as described (4, 19). The percentage of PFC-secreting antibody carrying a given idiotype was determined by adding anti-idiotype serum at a final concentration of 1:100 to the agarose. The sera had been previously absorbed with SRBC coated with the same antigen as

that used in the plaque assay. The difference between the numbers obtained in the absence and in the presence of anti-idiotype serum represents the number of PFC-secreting antibody bearing the corresponding idiotype.

Results

Proportion of Anti-TNP Antibodies Carrying MOPC 460Id in Normal BALB/c Mice. Anti-TNP hemagglutinins and PFC responses were studied in BALB/c mice immunized with various T-independent TNP antigens including TNP-Ficoll, TNP-LPS, TNP-NWSM, TNP-BA, TNP-levan, and TNP-dextran. A significant increase in anti-TNP-PFC and anti-TNP-agglutinins was obtained after immunization with each of these antigens. Inhibition of anti-TNP-PFC by BALB/c anti-460Id antibodies indicates that after immunization with TNP-Ficoll, TNP-LPS, and TNP-BA few, if any, of the PFC secrete 460Id-bearing molecules. Similarly, serum HI results show few, if any, 460Id-bearing molecules. By contrast, immunization with TNP-NWSM, TNP-levan, and TNP-dextran leads to the appearance of a significant proportion of anti-TNP PFC that secrete anti-TNP antibodies which can be bound by BALB/c anti-460Id. 460Id HI titers in the serum of such mice are low (2–3) but statistically significant. The results of one experiment of this type are presented in Table I. Similar results were obtained in a second experiment.

Specificity of Inhibition of Plaques by Anti-Idiotype Antibodies. To verify the specificity of the inhibition of the anti-TNP plaques by anti-460Id, we tested the capacity of this serum to inhibit formation of plaques on inulin-SRBC, BL-SRBC, galactan-SRBC, and unconjugated SRBC by spleen cells from BALB/c mice immunized with TNP-NWSM, BL, galactan, in the form of gum gatti, and sheep erythrocytes (Table II). In addition, we examined the effect of anti-X24Id and anti-E109Id on each of these plaque-forming systems.

Anti-460Id inhibited 21% of anti-TNP plaques elicited by TNP-NWSM immunization whereas it did not cause statistically significant inhibition of control plaques. Anti-E109Id inhibited <1% of anti-TNP plaques and anti-X-24 inhibited only 5% of these plaques.

In Vitro Responses to TNP-NWSM. To study the regulation of expression of 460Id on anti-TNP antibodies, we established a primary in vitro anti-TNP antibody response to TNP-NWSM. We found that culturing 5×10^5 spleen cells in microtiter plates for 3 d with 3 μg of TNP-NWSM gave a maximal response which, in three experiments was 133 ± 47 PFC per culture.

This response did not appear to require the presence of detectable numbers of T lymphocytes. Thus, spleen cells were first positively selected for Ig-bearing cells by adherence to anti-Ig-coated plates and then treated with anti-Thy 1.2 and C to eliminate any residual T cells. Such cell populations have excellent anti-TNP responses to TNP-NWSM which were only modestly depressed from that of unseparated cells. More importantly, addition of nylon wool purified T cells to anti-Thy 1.2 and C-treated spleen cells caused only a modest enhancement of their response (Table III).

Furthermore, in vivo immunization of *nu/nu* and littermate BALB/c mice with 30 μg of TNP-NWSM led to comparable responses in both groups at 5 d after immunization. *Nu/Nu* mice had 155 ± 12 direct anti-TNP PFC per 10^6 cells and littermates 185 ± 16 per 10^6 cells.

Effect of T Lymphocytes on the Expression of 460Id on Anti-TNP Antibodies. In the

TABLE I

Properties of Anti-TNP Antibodies Produced in BALB/c Mice

Immunogen	Anti-TNP PFC/10^6 cells		Serum titer (log_2)	
	Total	% MOPC 460Id$^+$*	Anti-TNP (HA)	MOPC 460Id (HI)*
TNP-Ficoll, 20 μg	613 ± 16‡	6 ± 2	12 ± 1	1 ± 0.3
TNP-NWSM, 30 μg	116 ± 22	36 ± 3	8 ± 1	2 ± 1
TNP-LPS, 10 μg	138 ± 46	0	7 ± 1	0
TNP-levan, 50 μg	517 ± 202	35 ± 6	11 ± 1	3 ± 0
TNP-BA, 0.1 ml of 1% suspension	120 ± 21	10 ± 10	9 ± 1	1 ± 0
TNP-dextran, 100 μg	265 ± 64	11 ± 1	12 ± 1	2 ± 1

* BALB/c anti-MOPC 460Id was used for inhibition of PFC and determination of HI titer.

‡ Mean ± SE of three mice studied for each immunogen.

experiment shown in Table IV, BALB/c spleen cells cultured with TNP-NWSM gave a response in which only 10% of the anti-TNP PFC observed at 3 d could be inhibited by anti-460Id. When T lymphocytes were depleted from this spleen cell population by treatment with anti-Thy 1.2 and C, the proportion of PFC secreting 460Id-bearing anti-TNP antibody in the subsequent response rose to 41%. Addition of nylon wool-purified T lymphocytes to this B-lymphocyte population completely abolished the 460Id-positive component of the response, although little change was seen in the total number of anti-TNP PFC. The capacity of the nylon wool-purified lymphocytes to diminish the proportion of anti-TNP antibodies bearing 460Id was ablated by treatment with anti-Thy 1.2 and C, further verifying the role of T lymphocytes in this suppression. In contrast, T lymphocytes obtained from allotype congenic C.B20 mice, which possess IgC_H and IgV_H genes of C57BL/Ka on a BALB/c background, failed to suppress the 460Id component of the anti-TNP response of BALB/c B cells.

Results demonstrating specific inhibition by T lymphocytes of the 460Id component of the anti-TNP-NWSM response have been obtained in a total of seven experiments.

Specificity of T Lymphocytes for 460Id Determinants. To determine whether the suppression of expression of 460Id depended on T lymphocytes specific for 460Id or anti-460Id determinants, we adsorbed T-lymphocyte populations on plates coated with MOPC 460, EPC 109, MOPC 167, or purified A/He anti-460Id antibody and then tested their ability to suppress 460Id$^+$ PFC responses.

Five experiments of this type are presented in Table V. The results are reported as the percent of TNP-PFC which express the 460Id. Addition of 5×10^5 nylon wool purified T cells to 5×10^5 B cells significantly diminished the expression of 460Id. Adsorbtion of T-lymphocyte populations on MOPC 460-coated plates removed their inhibitory activity. T lymphocytes which were recovered from MOPC 460-coated plates were strongly inhibitory of the 460Id component of the anti-TNP response. By contrast, T cells adsorbed on EPC 109, MOPC 167, or anti-460Id plates retained suppressive activity whereas T cells recovered from EPC109 plates had little or no inhibitory activity. These results indicate that a cell critical to the suppression of 460Id response is specific for the 460Id itself.

Finally, we examined the capacity of smaller numbers of T lymphocytes recovered from MOPC 460 plates to inhibit the 460Id component of the anti-TNP response.

TABLE II

*Specificity of Inhibition of Anti-TNP, Anti-Inulin, Anti-Galactan, and Anti-SRBC PFC by Various Anti-Idiotype Sera**

BALB/c immunized with:	Indicator in PFC assay	No serum	BALB/c anti-460 1:100		A/He anti-E109 1:100		A/He anti-X24 1:300	
		PFC/10^6	PFC/10^6	Id^+ PFC/10^6	PFC/10^6	Id^+ PFC/10^6	PFC/10^6	Id^+ PFC/10^6
TNP-NWSM, 30 μg	TNP-SRBC	570 ± 15‡	453 ± 28	*117 ± 31*§	567 ± 29	3 ± 32	541 ± 14	29 ± 20
Inulin-BA, 0.1 ml (0.1%) in CFA	In-SRBC	60 ± 4	56 ± 3	4 ± 5	18 ± 5	*42 ± 6*	66 ± 9	−6 ± 9
Galactan, 50 μg	Gal-SRBC	244 ± 23	206 ± 13	38 ± 26	233 ± 68	11 ± 71	25 ± 4	*219 ± 23*
SRBC, 0.1 ml 20%	SRBC	130 ± 11	116 ± 9	14 ± 14	104 ± 5	26 ± 12	128 ± 5	2 ± 12

* Spleens from three mice for each group were pooled.

‡ Number of PFC per 10^6 spleen cells ± SEM.

§ Difference between number of PFC/10^6 in the absence of serum and in the presence of anti-idiotype serum. SE of the difference was calculated as follows: SE of difference = $\sqrt{SE_1^2 + SE_2^2}$, where SE_1 is SE of mean number of PFC in absence of serum and SE_2 is SE of mean number of PFC in presence of anti-idiotype serum. Values in italics indicate that the difference between number of PFC in absence and presence of anti-idiotype serum is significant ($P < 0.05$).

TABLE III

T-Independence of Anti-TNP Response of BALB/c Mice to TNP-NWSM

Cell type	PFC/culture
5×10^5 Spleen cells (unseparated)	147 ± 7*
5×10^5 B cells obtained by anti-Thy 1.2 + C treatment	92 ± 13
5×10^5 B cells recovered from anti-Ig plates‡ and then treated with anti-Thy 1.2 + C	82 ± 11
5×10^5 B cells obtained by anti-Thy 1.2 + C + 5×10^5 nylon wool T cells	110 ± 6

* Mean ± SE of four replicate wells. Spleen cells from normal BALB/c mice were treated as indicated in the table and then cultured in microtiter wells with TNP-NWSM (3 μg/ml). The number of anti-TNP PFC were measured on day 3.

‡ Plates were coated with purified rabbit anti-mouse IgG, κ antibodies.

Significant inhibition, when compared to the same number of T cells recovered from EPC 109 plates, was obtained when 1.0×10^5 or more T cells were added to 5×10^5 B cells. Table VI presents results of one of three replicate experiments, each of which yielded similar findings.

Discussion

Idiotypes of the DNP-binding myeloma proteins MOPC 460 and MOPC 315 are expressed on only a small fraction of anti-TNP antibodies in BALB/c mice or BALB/c congenic mice immunized with T-dependent TNP antigens (23, 24). We have found that after in vivo immunization of BALB/c mice with three T-independent TNP antigens (TNP-NWSM, TNP-levan, and TNP-dextran), a significant percentage of anti-TNP plaques could be inhibited by BALB/c anti-460Id antibodies. The increased proportion of $460Id^+$ TNP plaques was paralleled by an increase in the serum HI titer of 460Id. A similar proportion of cells secreting anti-TNP antibodies bearing 460Id was obtained in the in vitro response of BALB/c spleen cells to TNP-NWSM.

Surprisingly, the proportion of $460Id^+$ anti-TNP PFC generated by purified B-cell populations in response to TNP-NWSM was higher than that developed by unseparated spleen cells. Furthermore, T cells purified by nylon wool columns clearly suppressed the $460Id^+$ component of the anti-TNP PFC response of B cells. Therefore, these results indicate that the T-cell population contains a naturally occurring

TABLE IV

*Inhibitory Effect of T Cells on the Development of PFC-Secreting Anti-TNP Antibodies Bearing 460Id**

Origin of cells	Total anti-TNP PFC/culture	460-Id$^+$ PFC/culture	460-Id$^+$ PFC
			%
BALB/c spleen cells (unseparated)	147 ± 7‡	14 ± 8	10
BALB/c B cells obtained by anti-Thy 1.2 + C treatment	92 ± 13	37 ± 17	41
BALB/c B cells + nylon wool-purified T cells	110 ± 6	−12 ± 9	−10
BALB/c B cells + T cells treated with anti-Thy 1.2 + C	103 ± 9	27 ± 10	27
BALB/c B cells + *C.B20* T cells	159 ± 12	49 ± 22	31

* Unseparated spleen cells or B cells were cultured in quadruplicate at 5 × 10^5 cells/microwell, with or without 5 × 10^5 T cells, with TNP-NWSM (3 μg/ml). The number of anti-TNP PFC were determined at 3 d in the absence or presence of BALB/c anti-460Id (1:100). Results reported are total anti-TNP-PFC ± SE, anti-TNP-PFC which are inhibitable by anti-460Id (460-Id$^+$ PFC) ± SE (calculated as in Table II), and percent of anti-TNP-PFC which secrete 460Id-bearing molecules.

‡ Mean ± SE of four cultures (5 × 10^5 cells/culture).

TABLE V

*Idiotypic Specificity of Suppressor T Cells**

5 × 10^5 B cells cocultured with:	% 460Id$^+$ PFC				
	Exp. No.				
	1	2	3	4	5
Nothing	46‡	52	38	55	50
5 × 10^5 T cells	3	—§	11	32	19
5 × 10^5 T cells adsorbed on MOPC 460-coated plates	60	61	43	67	50
5 × 10^5 T cells recovered from MOPC 460-coated plates	4	10	0	32	0
5 × 10^5 T cells adsorbed on control plates‖	16	16	11	44	16
5 × 10^5 T cells recovered from E109-coated plates	48	55	55	—	—

* Anti-Thy 1.2 and C-treated BALB/c spleen cells (B cells; 5 × 10^5/well) were cultured with nothing or with 5 × 10^5 nylon column-passed spleen cells (T cells) which had been subjected to various treatments. Total number of anti-TNP PFC- and of PFC-secreting 460Id-bearing molecules were measured 3 d after stimulation with TNP-NWSM (3 μg/ml).

‡ Mean of percent of anti-TNP-PFC inhibitable by anti-460Id.

§ Not done.

‖ Control plates were as follows: exp. 1-3, E109-coated plates; exp. 4, MOPC 167-coated plates; exp. 5, purified A/He anti-460Id-coated plates.

suppressor cell which controls the expression of clone(s) capable of secreting 460Id-bearing anti-TNP antibodies.

Plate-depletion experiments demonstrated that a cell important in this suppressor activity was specific for 460Id; that is, the suppressor activity was removed when T cells were plated on MOPC 460-coated dishes but not when they were plated on E109-coated dishes. In addition, T cells, which had adhered to MOPC 460-coated dishes and were then recovered, exhibited strong inhibitory activity. On the other hand, T cells which had adhered to E109 plates did not suppress the response. T cells that recovered from MOPC 460 coated plates represent 0.1-0.5% of the applied T-

TABLE VI

Suppression of 460Id$^+$ Anti-TNP Response by Limiting Numbers of T Lymphocytes Recovered from MOPC 460-Coated Dishes

B cells (5×10^5) incubated with T cells		Anti-TNP PFC/culture ± SE		
T cells	Number	Total	In presence of anti-460Id	460Id$^+$
				%
—	0	290 ± 15	123 ± 4	58 ± 3
Untreated	5×10^5	199 ± 11	193 ± 16	3 ± 10
Adsorbed on E109	5×10^5	181 ± 2	172 ± 9	5 ± 5
Adsorbed on M460	5×10^5	283 ± 4	135 ± 8	53 ± 3
Recovered from	5×10^5	171 ± 14	176 ± 6	−3 ± 9
M460	2.5×10^5	242 ± 18	213 ± 7	12 ± 7
	1.0×10^5	252 ± 10	231 ± 25	8 ± 11
	0.1×10^5	250 ± 33	176 ± 8	30 ± 10
Recovered from E109	5×10^5	207 ± 16	126 ± 10	39 ± 5
	2.5×10^5	232 ± 15	118 ± 8	49 ± 5
	1.0×10^5	228 ± 18	126 ± 20	45 ± 10
	0.1×10^5	288 ± 11	149 ± 10	48 ± 4

B lymphocytes (5×10^5) from normal BALB/c mice were cultured alone or in the presence of BALB/c T lymphocytes. The latter were either untreated or separated by incubation on plastic dishes coated with E109 or MOPC 460 (M460). Cells which fail to adhere to E109 dishes (adsorbed on E109) or to M460 dishes (adsorbed on M460) and cells which were recovered from E109 dishes (recovered from E109) or from M460 dishes (recovered from M460) were used. The cell mixtures were cultured in sextuplicate with TNP-NWSM (3 μg/ml) for 3 d and the number of PFC on TNP-SRBC measured in the absence of or presence of anti-460Id. Each determination was done in triplicate. Results are presented as mean ± SEM. An approximate standard error of the mean for % 460Id$^+$ cells was calculated as follows:

$$\text{SE } 100\left(1-\frac{\bar{x}}{\bar{y}}\right)=\sqrt{\frac{\bar{x}^2}{\bar{y}^4}(\text{SE }\bar{y})^2+\frac{(\text{SE }\bar{x})^2}{\bar{y}^2}},$$

where $\bar{x}$ is the mean number of PFC in the presence of anti-460Id and $\bar{y}$ is the mean number of PFC with absence.

cell population. Because the cells which failed to adhere had little or no residual suppressive activity, it is likely that the majority of suppressor cells were removed on 460 plates. Thus, the actual number of recovered cells probably represents an upper limit for the frequency of the cells in the unseparated normal T-cell population. These data indicate that in lymphocyte populations there is a discrete but small subset of T cells specific for 460Id which controls the expression of clones potentially capable of secreting anti-TNP antibodies bearing 460Id. Suppressor cells specific for idiotype have previously been demonstrated by Owen et al. (10) in the anti-arsonate system. However, in such experiments the suppressor cells were found only after treatment of A/J mice with a rabbit anti-cross-reactive idiotype antibody.

Our results suggest that the 460Id, which had not previously been considered to be a major cross-reactive idiotype (IdX) of the anti-TNP system, is in fact such an IdX, the expression of which is normally suppressed by the action of T cells. In turn, this raises the possibility that in certain other systems in which idiotypes of antigen-binding myeloma proteins have not been found on antibody, natural suppression by T cells will also be found to occur.

In future work, we will present data which indicate that the idiotypic determinants

of T cells specific for 460Id are similar to those carried by antibodies specific for the 460Id (anti-460Id) and that the antibodies specific for idiotypes of these anti-460Id antibodies (anti-anti-460Id antibodies) interfere with the development or expression of the 460Id specific suppressive activity of T cells.

Summary

An idiotype of the dinitrophenyl-binding myeloma protein MOPC 460 was expressed on a small but significant proportion of anti-TNP antibodies which appeared after in vivo or in vitro immunization of BALB/c mice with three T-independent TNP antigens. In vitro experiments show that the depletion of T cells before culture increased significantly the number of plaques secreting anti-TNP antibodies bearing MOPC 460 idiotype (460Id). T cells from BALB/c mice, but not from C.B20 mice, exhibit this suppressor activity. Plate-binding experiments indicate that the suppressive action of the T-lymphocyte population depends on a cell which can bind to MOPC 460 myeloma protein. The possible role of these normally occurring, idiotype-specific T cells on expression of 460Id in the anti-TNP antibody response of BALB/c mice is discussed.

Received for publication 27 September 1978.

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