ANTI-PHENYLTRIMETHYLAMINO IMMUNITY IN MICE

II. L-Tyrosine-*p*-Azophenyltrimethylammonium-induced Suppressor T Cells Selectively Inhibit the Expression of B-Cell Clones Bearing a Cross-Reactive Idiotype*

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It is well established that T cells play an important regulatory role on B-cell production of antibody (1). The functions ascribed to T cells include help (2), amplification (3), and suppression (4, 5) of B cell clones. Over the past several years much data have accumulated that indicate that the interactions between lymphoid cells are mediated by several regulatory components other than just antigen. These include antibody feedback (6), regulation by cell surface and soluble molecules encoded, in part, by the major histocompatibility complex (7–9), and, more recently, interactions mediated by idiotypic determinants (10, 11). Evidence for this network of interactions that is mediated by idiotypic determinants has gathered strength, particularly by the data that indicate that both T and B cells bear similar receptors for antigen as defined by anti-idiotypic antibody (12–15). Recent data have demonstrated restrictions between subpopulations of T-helper and B-cell clones, which appear to be governed, in part, by idiotypic (Id)[1] determinants located on antigen receptors (16, 17). So far these restrictions have not been reported between antigen-induced T-suppressor cells and responding B lymphocytes in the absence of exogenous Id antisera introduced into the system. To approach such a study, a model system in which specific probes, such as Id markers, is needed to follow the expression of certain B-cell clones.

We have recently described the response of A/J mice to the phenyltrimethylamino (TMA) hapten, which is characterized by cross-reactive idiotype(s) (CRI) (CRI-TMA).[2] An examination of the anti-TMA response for the appearance of the CRI in

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[1] *Abbreviations used in this paper:* anti-Id, idiotypic antiserum; Ars, arsanilic acid; BSA, bovine serum albumin; C′, guinea pig complement; CFA, complete Freund's adjuvant; CRI, cross-reactive idiotype(s); HBSS, Hanks' balanced salt solution; HGG, human gamma globulin; IBC, idiotype binding capacity; Id, idiotypic; KLH, keyhole limpet hemocyanin; NRS, normal rabbit serum; PBS, phosphate-buffered saline; PFC, plaque-forming cell(s); SRBC, sheep erythrocyte(s); T_h, T-helper cell; TMA, phenyltrimethylamino; TMA-BSA, azophenyltrimethylammonium BSA_d; T_s, suppressor T cell(s); T-S-T, [H-L-tyr(TMA)-NH-$(CH_2)_3$]2; tyr(ABA), L-tyrosine-azobenzenearsonate; tyr(TMA), L-tyrosine-*p*-azophenyltrimethylammonium.

[2] Alevy, Y. G., C. D. Witherspoon, C. A. Prange, and C. J. Bellone. Anti-TMA immunity in mice. I. The appearance of cross-reactive idiotype(s) to the trimethylammonium (TMA) hapten in A/J mice. *J. Immunol.* In press.

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a number of strains, plus the allotype congenic strain C57BL.Ig^e, suggests that the expression of the CRI is allotype linked. These findings made it attractive to investigate the possibility that T cells reactive to the same TMA determinant could be modulated by Id antisera used to define anti-TMA antibodies. To this end a helper T-cell assay specific for the small synthetic antigen L-tyrosine-*p*-azophenyltrimethylammonium [tyr(TMA)] was developed. It was shown in a preliminary report (18) that both T and B cells collaborated in a self-help system via TMA determinants on a bovine serum albumin (BSA) backbone. In lieu of the tyr(TMA) antigen, Id antisera induced TMA-specific T-helper cell activity, which suggested that receptors on T and B cells shared common structural features.

In this report we describe a system that assays for tyr(TMA) suppressor T cells that interact either directly or indirectly with anti-TMA B cells to produce self suppression. Examination of the Id profile in suppressed mice revealed that the suppression of the CRI$^+$-TMA was virtually complete, whereas the CRI$^-$-TMA clones were left intact. A/J mice primed with Id antisera rather than with the tyr(TMA) antigen similarly showed a marked suppression of anti-TMA B-cell formation that was characterized by the complete suppression of the CRI$^+$-TMA clones. These studies support the notion that T and B cells interact via an Id network, either directly or by an intermediate cell type(s).

Materials and Methods

Animals. Inbred A/J mice of both sexes (The Jackson Laboratory, Bar Harbor, Maine) were used throughout these experiments. All mice were 6–9 wk of age when the experiments were initiated.

Antigens. The monovalent antigens tyr(TMA) (19) and L-tyrosine-azobenzenearsonate [tyr(ABA)] (20) and the bivalent antigen [H-L-tyr(TMA)-NH-$(CH_2)_3]_2$ (T-S-T) (15) were prepared as previously described. In some cases tyr(TMA) was prepared commercially (Biosearch, San Rafael, Calif.) according to previously described procedures (19). The hapten-protein conjugate azophenyltrimethylammonium bovine serum albumin (TMA-BSA) was prepared according to the methods of Tabachnick and Sobatka (21). The conjugate ABA-keyhole limpet hemocyanin (KLH) (ABA-KLH) was prepared as described (21); by reacting 1 g of KLH with 1 mM (0.22 g) of the diazonium salt of trimethylaminoaniline (Bachem, Inc., Torrance, Calif.) for 3 h, 4°C, at pH 9.5. The reaction mixture was exhaustively dialyzed against water and then lyophilized. Twice-crystallized BSA (Sigma Chemical Co., St. Louis, Mo.) and KLH (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) were obtained commercially.

Immunizations. Both the small antigens tyr(TMA) and tyr(ABA), and the hapten-carrier conjugates TMA-BSA and ABA-KLH were emulsified with equal volumes of complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) before inoculation. For soluble booster injections, the lyophilized antigens were brought into solution with either 0.1 or 1.0 M NaOH, neutralized with HCl, and brought to the desired concentration with Hanks' balanced salt solution (HBSS). Sheep erythrocyte (SRBC) challenge consisted of 0.2 ml of a 10% suspension of thrice washed RBC. All other inoculations were given intraperitoneally in 0.1 vol. (See Results and Figs. 1 and 2 for the various immunization protocols.)

Irradiation Transfer Procedures. Mice were given 700 rad (116–122 rad/min) whole-body irradiation at 80 cm distance with a Gammatron S Colbalt 60 therapy unit (Siemans Corp., Federal Republic of Germany). Irradiated recipients received an adoptive transfer of syngeneic cells, 1.0–1.5 × 10^7 T cells and 1.0–1.5 × 10^7 B cells, by retro-orbital injection usually within 1 h after irradiation. Recipients were boosted intraperitoneally 16 h after cell transfer with the soluble antigen.

Production, Isolation of Antibody, and Preparation of anti-Id. Anti-TMA antibody was elicited in A/J mice with the bivalent antigen T-S-T according to the methods of Tung et al. (22). Anti-

TMA antibody from a single immune mouse was isolated by affinity chromatography with a TMA-Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) immunoadsorbent prepared according to Cheseboro and Metzger (23). After elution of the antibody with 10^{-3} M tyr(TMA), the protein was exhaustively dialyzed against borate-buffered saline. As determined by the OD_{280} using the $E^{1cm}_{1\%} = 14,500$ μg of the antibody was inoculated in CFA into each of several rabbits. Subcutaneous booster injections, with doses ranging from 100 to 300 μg in CFA, were given periodically and the rabbits were bled 10–20 d after the boost. Antisera from several bleeds were pooled and then adsorbed with normal A/J Ig coupled to Sepharose 4B (24) until the antisera no longer reacted with normal A/J Ig. As already described,[2] this antiserum showed the following characteristics with respect to Id specificity: (*a*) In an indirect radioimmunoassay (25), which used ~2 ng of labeled proband antibody, of which 30% was bound in the assay, 10–50 ng inhibited significant binding between the Id antiserum (anti-Id) and the ^{125}I-labeled proband antibody. No significant inhibition of this reaction was seen when as much as 20 μg of normal A/J mouse Ig or the mouse myeloma 104E was added. (*b*) This antiserum inhibited anti-TMA plaque-forming cells (PFC) in A/J mice, whereas it did not inhibit anti-ABA or SRBC PFC.

Normal rabbit serum (NRS) used as a control was absorbed on Sepharose-Ig in an identical manner to the anti-Id absorption procedures. Inoculation of mice with either 0.2 ml of anti-Id or NRS was given via the retro-orbital plexus. The idiotype binding capacity (IBC) for this absorbed serum was shown to be 10.7 μg/ml of serum and was determined by the indirect radioimmunoassay using a fixed volume of antiserum while varying the concentration of antigen. The IBC value was calculated by extrapolation to infinite antigen concentration.

Plaque Assays. Anti-TMA, ABA, and SRBC PFC were determined by a slide modification of the Jerne plaque assay as already described (26). The indicator SRBC, TMA-SRBC, and ABA-SRBC were prepared as follows: 0.5 ml of packed and saline-washed SRBC were resuspended in 8–10 ml of phosphate-buffered saline (PBS). To this suspension, 0.12 ml of a 0.05 M solution of the TMA or ABA diazonium salt was added and allowed to react for 10 min at 4°C. The cells were then washed three times with PBS and resuspended to a final 30% vol/vol concentration. Only indirect PFC were scored because no significant direct PFC have ever been observed. (See Table I for more details.)

PFC that produce antibodies bearing the CRI-TMA were determined by incorporating 100 μl of Id antisera into the plaquing medium. The percent inhibition of PFC was calculated according to the following equation: Percent inhibition = (1 − total PFC[anti-Id])/total PFC, in which the total PFC (anti-Id) represented the number of PFC in the presence of anti-Id over the number of PFC in the absence of the antisera. The percent inhibited were judged the CRI^+-TMA PFC and those remaining were designated the CRI^--TMA population.

Preparation of B Cells. To eliminate splenic T cells, spleen cell preparations were treated with a rabbit anti-A/J mouse brain serum prepared as described (27). 2 ml of the antiserum, adsorbed with A/J liver diluted 1:5 in HBSS, were added to 80×10^7 spleen cells in 5–8 ml total vol of HBSS, and the cells were incubated at 4°C for 30 min. At this time, guinea pig complement (C′), absorbed first with agarose (28) and then with a mixture of A/J liver and spleen, was added at a final 1:9 dilution and incubated at 37°C for 1 h. After washing, ~50% of the original live cells were recovered, of which 90% were Ig^+, as determined by immunofluorescence with a goat anti-mouse Ig reagent (Meloy Laboratories Inc., Springfield, Va.).

Preparation of T Cells. To obtain T cells, spleen cells were passed over nylon wool columns by the method of Julius et al. (29). Approximately 90% of the column-passed cells were killed by the rabbit anti-T-cell serum plus C′. Examination by immunofluorescence indicated that 90% of the column-passed cells were Ig^-.

Results

Because very little was known about the immunogenicity of the synthetic antigen tyr(TMA) in the mouse species, a protocol was first worked out to assay for suppressor T-cell activity. Our rationale was based on earlier observations that demonstrated that priming mice with the monovalent antigen tyr(TMA) for 1 wk and then challenging with TMA-BSA resulted in an enhanced anti-TMA PFC response

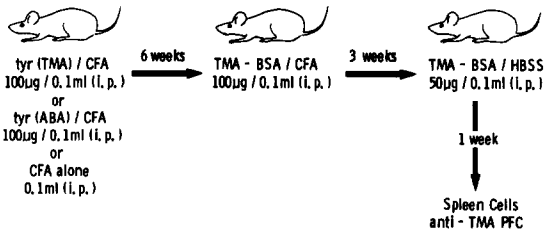

FIG. 1. Protocol for determining suppressor T-cell activity. See Results for detailed explanation.

TABLE I

Pretreatment with tyr(TMA) Induces Suppression of TMA Plaques

Antigen	Number of mice	PFC/10^8 cells ± SE
		*anti-TMA**
tyr(TMA)	38	8,761 ± 1,478‡ (66%)§
CFA	30	25,513 ± 2,201
tyr(ABA)	8	26,247 ± 4,077
		anti-ABA‖
tyr(TMA)	9	1,250¶ ± 149 (13%)
CFA	6	1,441 ± 310
		*anti-SRBC***
tyr(TMA)	5	216,200 ± 85,010 (−20%)
CFA	5	174,000 ± 31,761

* See Fig. 1 and text for immunization protocol used to generate anti-TMA PFC.

‡ Differences between the tyr(TMA) group and the controls, tyr(ABA), and CFA is significant, $P < 0.005$ (Student's t test). Only indirect PFC are recorded. All PFC data are expressed as the mean ± SE.

§ Numbers in parentheses are the percent inhibition of PFC in the tyr(TMA) group compared with the CFA group.

‖ 6 wk after priming with tyr(TMA) in CFA or CFA alone, both groups were inoculated with 100 µg ABA-KLH in CFA, boosted 4 wk later with ABA-KLH in HBSS, and plaqued 5 d after the final boost.

¶ The PFC numbers in these groups are significantly above background PFC determined by plaquing identical numbers of lymphoid cells against unsubstituted SRBC. Background PFC average 100 PFC/10^8 spleen cells.

** 6 wk after priming with tyr(TMA) in CFA or CFA alone, both groups were inoculated with 0.2 ml of a 10% vol/vol SRBC suspension and plaqued 5 d later.

compared with non-tyr(TMA)-primed mice. This was shown to be mediated by T-helper cells collaborating with B cells, both specific for the TMA determinant. We reasoned that if after 1 wk we could demonstrate a helper activity, then priming for longer periods of time may generate suppressor activity. According, as shown in Fig. 1, mice were inoculated with either tyr(TMA), tyr(ABA), or CFA alone, followed 6 wk later by TMA-BSA in CFA, and finally boosted 3 wk later with TMA-BSA in solution. 1 wk after the final boost all animals were plaqued for anti-TMA antibody formation. As seen in Table I, animals primed with monovalent tyr(TMA) showed a 66% reduction in the number of anti-TMA PFC compared with the tyr(ABA)- or CFA-primed groups.

Specificity of the antigen-induced suppressor activity was demonstrated at the afferent limb of this response, because suppression of anti-TMA PFC was noted only

TABLE II
Kinetics of Suppression by tyr(TMA)

Antigen	Number of mice	PFC/10^8 cells ± SE	Percent inhibition
		4 wk	
tyr(TMA)	9	21,688 ± 6,160*	41
CFA	9	36,755 ± 10,323	
		6 wk	
tyr(TMA)	38	8,761 ± 1,478	66
CFA	30	25,513 ± 2,201	
		9 wk	
tyr(TMA)	7	17,014 ± 4,282	−9
CFA	7	15,521 ± 5,128	
		12 wk	
tyr(TMA)	7	28,205 ± 4,950	−33
CFA	4	21,840 ± 3,179	

After priming with either tyr(TMA) in CFA or CFA alone, animals were challenged with TMA-BSA either 4, 6, 9, or 12 wk later to monitor anti-TMA suppression. See footnotes to Table I for details.

* The differences between the tyr(TMA) and the CFA group at 4 wk is significant at the $P < 0.05$ level (Student's t test).

with tyr(TMA) priming, whereas there was no difference in the anti-TMA response between CFA- and tyr(ABA)-primed mice. Furthermore, the suppression of anti-TMA PFC by priming with tyr(TMA) was specific at the efferent limb, because challenge of tyr(TMA)-primed animals with either ABA-KLH or with SRBC did not suppress the response to the ABA hapten or to SRBC when compared with CFA-primed animals challenged in an identical manner (Table I). It should be noted that all mice were challenged with the heterologous antigens 1 wk after priming, but the immunization protocols thereafter were different as a result of the kinetics of PFC formation for these antigens (Table I). A possible criticism of these heterologous antigen controls lies in the timing and numbers of PFC generated after challenge. Optimal anti-ABA PFC numbers were generated by boosting 6 wk after challenge, but it may be argued that suppression was overridden in this 6-wk interval. Thus we tried to keep the ABA-KLH protocol similar to the TMA-BSA schedule. The SRBC controls resulted in PFC numbers that were approximately eightfold higher than the anti-TMA PFC response. It might be argued that this magnitude of response can override suppression. Nevertheless, the above data indicate that the suppressor activity effect is specific for the TMA determinant.

The kinetics of this suppressor activity were then followed after priming with tyr(TMA). After inoculation with either tyr(TMA) in CFA or CFA alone, animals were challenged with TMA-BSA either 4, 6, 9, or 12 wk later to monitor anti-TMA suppression. As seen in Table II, at 4 wk there was, on the average, a 41% inhibition of anti-TMA PFC, this suppression peaked at 6 wk (66% inhibition), and no detectable suppressor activity was detected by the 9th or 12th wk after tyr(TMA) priming.

To establish which cell, T or B, was responsible for the suppressed anti-TMA PFC response, irradiation transfer experiments were employed. The protocols used in the transfer experiments are shown in Fig. 2. To examine the T-cell compartment, animals were primed with tyr(TMA) in CFA and 6 wk later inoculated with TMA-BSA in CFA. 3 wk thereafter, T cells from these mice were prepared by passage of spleen cells

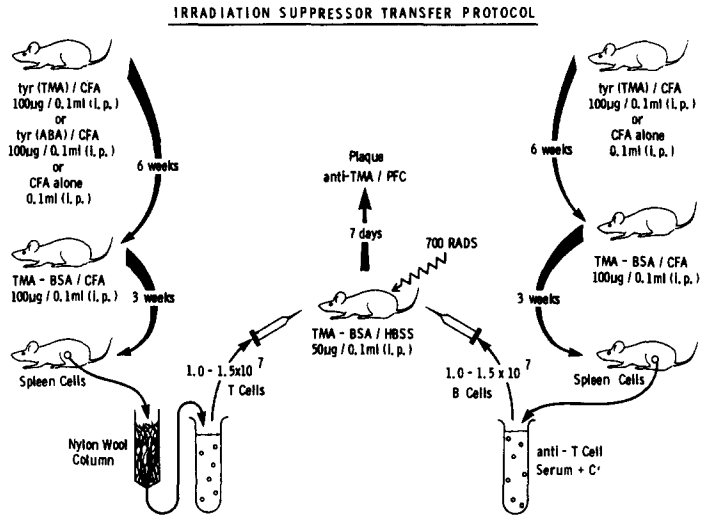

FIG. 2. Protocol to monitor either T or B cells for suppression of anti-TMA PFC. See Results for detailed explanation.

TABLE III

tyr(TMA) Induces Suppressor T Cells

Source of		Number of mice	PFC/10^8 cells ± SE
T cells	B cells		
tyr(TMA)	CFA	18	1,358 ± 424* (77%)
CFA	CFA	12	5,828 ± 1,172
CFA	tyr(TMA)	8	9,765 ± 2,291

See Results and Fig. 2 for immunization schedule and explanation of transfer protocols. Only indirect PFC are recorded. All PFC data are expressed as the mean ± SE.

* Differences between the T_T + B_{CFA} group and the others is highly significant, $P < 0.001$ (Student's t test).

over nylon wool (designated T_T cells), and, along with 1–1.5 × 10^7 B_{CFA} cells, they were transferred into lethally irradiated syngeneic recipients. After 24 h the recipients were inoculated with 50 µg of soluble TMA-BSA, and 7 d later their spleens were plaqued. To assess the contribution of B cells in tyr(TMA)-primed spleen cells, T cells were prepared from CFA-primed donors (T_{CFA}), and B cells were prepared from tyr(TMA)-primed spleen cells (B_T), and this combination was transferred into irradiated recipients, challenged with soluble TMA-BSA, and plaqued 7 d later. When assessing the cellular compartments (T or B) for the suppressive activity, either protocol was always run in parallel with T and B cells prepared from animals primed initially with CFA only, (T_{CFA} + B_{CFA}). As seen in Table III, there is a significant suppression (77%) where tyr(TMA)-primed T cells were transferred along with the B_{CFA} population. The suppression of anti-TMA PFC did not appear to be mediated by the B_T, because transfer of this population along with T_{CFA} cells did not suppress the PFC when compared with the T_{CFA} + B_{CFA} control. Despite the low number of PFC, when calculated by the usual PFC per 10^6 spleen cells, these were bonafide anti-TMA-secreting cells that were determined by plaquing with large numbers of spleen cells (1–3 × 10^6 spleen cells/slide). They were then calculated by subtracting the total

TABLE IV

Anti-Id Serum Substitutes for tyr(TMA) in Inducing Self-suppression to anti-TMA PFC

Mice injected with	Number of mice	PFC/10^8 cells ± SE
		*anti-TMA**
0.2 ml anti-Id	10	6,897 ± 2,168‡ (62%)
0.2 ml NRS	8	18,150 ± 4,653
		anti-ABA§
0.2 ml anti-Id	8	42,300 ± 8,030 (−7%)
0.2 ml NRS	9	39,555 ± 8,576
		anti-SRBC‖
0.2 ml anti-Id	6	281,000 ± 42,419 (12%)
0.2 ml NRS	6	320,000 ± 38,049

* Same protocol as in Table I except that 0.2 ml anti-Id and NRS substitute for tyr(TMA) and CFA.

‡ Difference between the anti-Id and NRS group is significant at the $P < 0.001$ level (Student's t test). All PFC are expressed as the mean ± SE.

§ These mice were given 100 μg of ABA-KLH in CFA 6 wk after priming with either anti-Id or NRS followed 5 wk later by 50 μg of ABA-KLH in HBSS and plaqued for anti-ABA PFC 4 d after the final boost. Note that this change from the ABA-KLH immunization scheme given in Table I resulted in a 30-fold increase in the PFC number to ABA.

‖ Same immunization protocol as described in Table I after priming with anti-Id or NRS.

PFC minus the PFC number in a nonsubstituted SRBC layer (average of 100 PFC/10^8). Furthermore, these anti-TMA PFC were blocked with an Id antiserum that has been previously described.[2] It should be noted that anti-TMA PFC numbers generated in the intact A/J mice were relatively modest (between 200 and 300 PFC/10^6 spleen cells) when compared with other reported anti-hapten responses in vivo.

Induction of tyr(TMA) Suppressor Activity by Id Antisera. Attempts were made to stimulate suppressor reactivity with Id antiserum for the following reasons: (*a*) we have recently demonstrated CRI on anti-TMA antibodies in A/J mice,[2] and (*b*) Id antisera directed against the CRI-TMA can induce T-helper cells in vivo specific for the TMA determinant (18). These data made it attractive to explore the possibility that Id antisera directed to the CRI-TMA could induce TMA-specific suppressor activity. The characteristics of the Id antiserum used in these experiments have been described in Materials and Methods. To stimulate suppressor activity by Id antiserum, the basic protocol, as outlined in Fig. 1, was used except that priming with tyr(TMA) or CFA only was substituted with anti-Id or (NRS), respectively. As seen in Table IV, mice primed with anti-Id showed a 62% decrease in the number of PFC when compared with NRS controls. Specificity of the anti-Id-induced suppression is also shown in Table IV. Groups of mice primed with either anti-Id or NRS were challenged 6 wk later with either ABA-KLH or SRBC. The ABA-KLH-primed mice were boosted with soluble ABA-KLH 5 wk later and their spleens plaqued 4 d after the final boost. Plaquing to SRBC was done 5 d after a single SRBC inoculation. There were no significant differences in the number of PFC to either the ABA hapten or SRBC when comparing the anti-Id and NRS-primed animals. Thus the anti-Id appeared to mimic the tyr(TMA) antigen insofar as 6 wk after priming there was a marked suppressor activity with specificity for the TMA determinant.

TABLE V

Antigen and Anti-Id Suppress the Major CRI$^+$-TMA PFC

Mice primed with	Number of mice	PFC/10^6 cells ± SE		Percent CRI$^+$-TMA PFC
		No serum	Anti-Id (100 μl)	
tyr(TMA)	21	11,300 ± 2,400 (49%)	11,000 ± 2,300	3
CFA	17	21,900 ± 2,270	12,000 ± 1,620	46
0.2 ml anti-Id	5	29,200 ± 2,000 (49%)	28,900 ± 4,700	1
0.2 ml NRS	6	56,800 ± 13,000	26,900 ± 5,500	53

For immunization schedules refer to Table I. Only indirect anti-TMA PFC are recorded. All PFC data are expressed as the mean ± SE. Percent CRI$^+$-TMA PFC was calculated as follows: PFC + anti-Id/PFC (no serum) × 100. Numbers in parentheses represent the percent inhibition of the tyr(TMA) group as compared with the CFA group.

TABLE VI

Suppression of CRI$^+$ PFC by T Cells

Source of		Number of mice	PFC/10^6 cells ± SE		Percent CRI$^+$ PFC
T Cells	B Cells		No serum	Anti-Id (100 μl)	
tyr(TMA)	CFA	6	2,910 ± 983* (56%)‡	2,590 ± 962§	11%
CFA	CFA	3	6,673 ± 1,147	2,973 ± 702‖	55%

For immunization schedules and transfer protocols refer to Table III. Only indirect anti-TMA PFC are recorded. All PFC data are expressed as the mean ± SE.

* Differences between the T_T + B_{CFA} and T_{CFA} + B_{CFA} groups is significant at the $P < 0.025$ level (Student's t test).

‡ Numbers in parentheses represent the overall percent suppression.

§ The differences between the PFC numbers in the presence and absence of anti-Id is not significantly different.

‖ The differences between the PFC numbers in the presence and absence of anti-Id is significant at the $P < 0.025$ level (Student's t test).

Antigen- and Anti-Id-induced Suppression Results in the Elimination of the CRI$^+$-TMA PFC. Based on the proposed network theory (30) and the more recent work on Id restrictions between collaborating T-helper and antibody-producing cells (16, 17), we investigated the possibility of whether these same restrictions might be operating between T-suppressor and B lymphocytes in the antigen-induced suppressor protocol. Because the anti-TMA PFC response is characterized by CRI, comprising ~50% of the total PFC response when employing TMA-BSA as the immunogen, this marker made it possible to follow the fate of this subpopulation as well as the non-CRI-bearing clones. Selective elimination of one population over another would indicate restrictions among suppressor T-cell (T_S) clones and specific antibody-producing cells.

The Id profile in both the suppressed and nonsuppressed animals was examined by incorporating the Id antiserum into the plaquing medium just before plating. As seen in Table V, the response to TMA-BSA resulted in ~50% of the PFC that bears the major CRI-TMA in the nonsuppressed groups. In both the tyr(TMA) and anti-Id-primed animals the total PFC response was suppressed by 49% and could be accounted for by the almost complete and exclusive elimination of the CRI$^+$-TMA PFC. The CRI$^-$-TMA PFC were left intact (Table V) when comparing the PFC number in the presence and absence of anti-Id in both the tyr(TMA)- and the anti-Id-suppressed groups.

To demonstrate that tyr(TMA)-induced T_s cells could mediate the selective elimination of the CRI^+ anti-TMA PFC, transfer protocols (as outlined in Fig. 2) were employed. As seen in Table VI, the transferred T cells in this experiment inhibited the total anti-TMA PFC by 56%, which can be accounted for by the almost total elmination of the CRI^+ PFC.

Discussion

In this report we have described an antigen-specific suppressor system in which both T and B cells recognize the same antigenic determinant, self suppression. Evidence is presented to show that the monovalent antigen tyr(TMA) in A/J mice induced a T_s population; and further, Id antisera directed to the CRI on anti-TMA antibodies could stimulate suppressor activity of the same specificity. In either mode of induction suppression was highly specific for B-cell clones that bear the major CRI-TMA. This was evident when 6-wk tyr(TMA)-primed animals were challenged with TMA-BSA in CFA and plaqued after an intervening boost. In nonsuppressed groups, challenge with TMA-BSA elicited a response that was comprised of ~50% CRI^+ and 50% CRI^- anti-TMA PFC. Challenge in the tyr(TMA)-primed group resulted in a 50% suppression of the total anti-TMA PFC, which was accounted for by the elimination of the CRI^+ PFC population. Examination of the remaining 50% of the PFC revealed that they were not inhibited by the anti-Id and, therefore, CRI^-. These data suggest the T_s population neglected the CRI^- B cells in vivo, and to our knowledge this is the first report of a specific antigen-induced T cell-mediated suppression that is Id specific.

What might be the mechanisms involved in this very restricted Id suppression? Such a mechanism is offered by the network hypothesis (30), which envisions a series of regulatory interactions between cells bearing Id determinants, both T and B, and cells bearing complementary anti-Id receptors. The demonstration of both T and B cells bearing similar idiotypes (12–15) and the existence of anti-Id clones have been reported (10, 31, 32). With respect to the suppressor system, this network might operate as follows: A first set of idiotypes on the surface of antigen-specific T_s is stimulated by the antigen. This is followed by a second set of complementary anti-Id clones, which in turn act directly on the Id^+ B cells or on T-helper cell (T_h) clones, assuming the T_h cells bear similar idiotypes to the T_s, which then cease to deliver the appropriate signals to the B cell and PFC formation shuts down. An alternative to this proposed pathway is the possibility that the same T cell recognizes antigen and the idiotype. However, evidence to date would suggest that the former scheme is the more likely (11, 33). In any case, the shut down of restricted numbers of Id-bearing clones would depend upon a restricted number of first-set idiotypes activated.

To strengthen our contention that the Id network is operating in the TMA system, evidence is needed that both T and B cells share similar, if not identical, Id determinants. To date we do not have conclusive evidence that this is the case but, we offer several arguments, albeit indirect, that TMA receptors on T and B cells may share some structural similarity. We have preliminary evidence that the monovalent antigen tyr(TMA) induces a T_h population and further, Id antisera directed to the CRI on anti-TMA antibodies could stimulate T_h of the same specificity (18). In addition, recent experiments have shown that tyr(TMA)-induced T_h can be functionally blocked by pretreatment with anti-Id before transfer into irradiated recipients

(Y. G. Alevy and C. J. Bellone. Unpublished observation.). In these reported experiments we have been able to trigger suppressor activity with anti-Id in lieu of the antigen, which opens the possibility that T_s bears similar idiotypes along with T_h and B cells. This evidence is not conclusive. Nevertheless, tyr(TMA) does induce T cell-mediated idiotype-specific suppression (Tables V and VI) and it could be argued that at least some T-cell subpopulation, either an antigen-specific T_s or anti-Id-specific T clone, is involved in the Id^+ B-cell suppression. We argue against Id^+ B cells mediating this suppression because we have not been able to trigger suppression with tyr(TMA)-primed B cells in transfer protocols. We also have never detected any anti-TMA PFC after single or multiple injections with monovalent tyr(TMA) (Y. G. Alevy and C. J. Bellone. Unpublished data.). Thus, anti-TMA B-cell clones do not appear to be activated to a stage of differentiation or expansion by tyr(TMA) that would allow them to mediate suppression or produce detectable amounts of antibody that might trigger an anti-Id clone.

A somewhat similar finding was reported by Benjamin and Teale (34) using tolerogenic doses of arsanilic acid (Ars) coupled to human gamma globulin (HGG) (Ars HGG). In this report it was shown that tolergenic doses followed by immunogenic levels of Ars HGG resulted in significant decreases of total anti-Ars. This was accounted for by the selective loss of the major CRI found on anti-Ars antibodies in the A/J strain. However, they had no evidence of a idiotype-specific T_s operating in this system, although this idea was entertained in their discussion.

Evidence for idiotype suppression, in which anti-Id is introduced in vivo, has been observed in adult mice with small doses of anti-Id (31), in adult mice with large doses of anti-Id together with administration of antigen (35), and in newborn mice (36). In the first two cases suppression was shown to be mediated by T cells. Further, Owen et al. (10) have shown a T-cell population in the suppressed mouse that binds Id^+ anti-Ars antibodies and, when isolated, these T cells mediate idiotype-specific suppression (35). Recent transfer experiments from mice suppressed to the CRI (Ars) indicate that the cell responsible for the suppression bears $Ly\text{-}2^+\text{-}3^+$ antigens (37). Thus, the evidence for Id-specific suppression and/or the existence of T anti-Id clones is clear, but whether Id-specific suppression or these anti-Id clones play a role in the normal immune response to Ars is unclear.

Bona and Paul (38) have recently demonstrated naturally occurring idiotype-specific suppressor T cells directed to the idiotype(s) associated with the trinitrophenol-binding mouse myeloma protein MOPC 460. No introduction of antigen and/or anti-Id in vivo was necessary to detect their activity. Their results, along with the data in the TMA system, support the concept of idiotype-specific T cells playing a general physiological role in immune regulation.

Support for Id restrictions playing a role in the immune response has recently come from two laboratories in which they present evidence for idiotype-specific T_h populations (16, 17). Woodland and Cantor (16) have demonstrated that T_h specific for the Ars idiotype are essential in the expression of Ars-specific B cells bearing the CRI (Ars). Surprisingly, they found Id restrictions between KLH-specific Ly-1-helper cells and Ars-specific Id^+ B cells in this classical carrier-hapten protocol. However, Hetzelberger and Eichmann (17) were unable to show Id preferences between T and B cells that recognize different determinants on a carrier-hapten complex. They did show apparent restriction between Id^+ T and B cells when both were specific for the

same group A carbohydrate determinant. In both antigen systems, the introduction of Id antisera in vivo was necessary to either amplify (17) or block (16) the Id^+ T and B clones to detect Id restrictions between these cells types. Our results would indirectly support these (16, 17) findings by extending Id restrictions to T_s and B-cell interactions induced by antigenic stimulation only. Apparently, our synthetic antigen can dissect out these Id-restricted interactions by triggering a very restricted number of clones, possibly all bearing similar or identical Id determinants.

Is the immediate target of the TMA-specific T_s a T_h that can then no longer collaborate with Id^+ B clones or the B cells that are directly turned off by the Id-specific T_s population? Because we monitor suppression by challenge with TMA-BSA, much of the helper activity for the CRI^+ and CRI^- B cells can be supplied by BSA-specific T_h populations. Unless BSA activates Id-specific T_h, as in the Ars system (16), then it is likely that CRI^+ B cells are the direct target of the T_s population.

Many questions remain to be answered in this antigen system. Is there an antigen-specific Ly-2^+-3^+ T_s that bears the CRI-TMA determinants that in turn trigger idiotype-specific T_s? Are both these T_s populations mandatory in suppression of the anti-TMA response, or can an antigen-specific Id^+ T_s directly and preferentially interact with Id^+ B cells? The possibility of two identical receptors complexing to mediate cell-cell interactions has been suggested in other systems (39). We are currently investigating these problems, as well as testing the stringency of these apparent Id restrictions by making use of the Id^+ C57.Ig^e allotype congenic strain and the Id^- C57BL/6 partner.

Despite these unanswered questions, the tyr(TMA) antigen system in mice should prove a valuable tool in dissecting out the physiological role of idiotypes in the immune response.

Summary

The anti-phenyltrimethylamino (TMA) response in A/J mice is characterized by a cross-reactive idiotype(s) (CRI) that appears linked to the Ig-I^e allotype.[2] These findings made it attractive to look for a CRI on T cells reactive to the same TMA determinant. Thus a suppressor T-cell (T_s) assay specific for L-tyrosine-*p*-azophenyltrimethylammonium [tyr(TMA)] was developed.

A/J mice were primed with either tyr(TMA) in complete Freund's adjuvant (CFA), L-tyrosine-azobenzenearsonate [tyr(ABA)] in CFA, or with CFA alone. 6 wk later all mice were inoculated with TMA-bovine serum albumin (BSA) in CFA, boosted with soluble TMA-BSA 3 wk later, and plaqued 7 d after the soluble boost. Priming with tyr(TMA) in CFA resulted in 66% suppression of anti-TMA plaque-forming cells (PFC) as compared with control groups primed with tyr(ABA) in CFA or CFA alone. The suppression was shown to be mediated by T_s, as only T cells but not B cells from suppressed animals transfer the suppression in adoptive cell transfer experiments into lethally irradiated recipients. The profile of the anti-TMA PFC in the suppressed and nonsuppressed animals was examined via incorporation of anti-idiotypic sera (specific for CRI-TMA) into the plaquing medium. The results of these experiments indicate that the suppression of the major CRI^+-TMA PFC was virtually complete, whereas the CRI^--TMA PFC are left intact.

When A/J mice were primed with idiotypic antisera (anti-Id) or normal rabbit serum (NRS) rather than with the antigen on CFA alone, and the same protocol was

followed thereafter, the anti-Id-inoculated mice were suppressed by 63% when compared with the NRS-primed controls. Again the suppression could be accounted for by the exclusive elimination of CRI^+ anti-TMA PFC.

The possibility that the antigen-induced idiotype suppression may result from idiotypic restrictions between interacting CRI^+-T_s and CRI^+-B cells will be discussed.

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