

GENETIC CONTROL OF IMMUNE RESPONSES IN VITRO

V. STIMULATION OF SUPPRESSOR T CELLS IN NONRESPONDER MICE BY THE TERPOLYMER L-GLUTAMIC ACID⁶⁰-L-ALANINE³⁰-L-TYROSINE¹⁰ (GAT)*

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The immune response of inbred strains of mice to the random terpolymer L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT)¹ is controlled by a specific immune response (*Ir*) gene which maps in the I region of the *H-2* complex between the K and S regions (1-3). In previous reports in this series, we have described a technique for the detection of primary GAT-specific IgG plaque-forming cell (PFC) responses in mouse spleen cell cultures (4). Spleen cells from both responder (*H-2^{a, b, d, k}*) and non-responder (*H-2^{p, q, *}*) mice develop IgG PFC responses specific for GAT after incubation with GAT complexed to the immunogenic carrier methylated bovine serum albumin (GAT-MBSA), but only spleen cells from responder mice develop GAT-specific IgG PFC responses to GAT.

The immune responses to GAT and GAT-MBSA are thymus dependent (5). Furthermore, spleen cells from GAT-primed, irradiated responder mice were shown to have GAT-specific helper T-cell function, while no GAT-specific helper T-cell function could be demonstrated in spleen cells from GAT-primed, irradiated non-responder mice (5). These data suggest that the defect in genetic nonresponder mice is the failure of their T cells, after interaction with GAT, to provide appropriate helper T-cell function for the initiation of the B-cell response to GAT.

More recently, we have observed that injection of GAT not only fails to elicit a GAT-specific PFC response in nonresponder mice, but also specifically decreases the ability of nonresponder mice to develop a GAT-specific PFC response to a subsequent challenge with GAT-MBSA (6). Addition of soluble GAT to cultures of spleen cells from nonresponder mice also prevents development of the GAT-specific response

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¹ *Abbreviations used in this paper:* B cells, precursor of antibody-producing cell; FCS, fetal calf sera; GAT, random terpolymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰; GAT-MBSA, GAT complexed to methylated bovine serum albumin; Ig, immunoglobulin; IgG refers to IgG₁ and IgG_(2a+2b); *Ir* gene, specific immune response gene; MEM, completely supplemented Eagle's minimum essential medium; PFC, plaque-forming cell(s); T cell, thymus-derived helper cell; θ , surface alloantigen on T cells.

stimulated by GAT-MBSA. Thus, GAT is recognized by nonresponder mice, but this recognition leads to specific tolerance rather than immunity.

To analyze the mechanism involved in tolerance induced by GAT in nonresponder mice, we have investigated: (a) the immunocompetence of T and B cells from spleens of nonresponder mice previously rendered unresponsive by injection of GAT; and (b) the effects of such populations of T and B cells on the development of GAT-specific PFC responses by normal nonresponder spleen cells incubated with GAT-MBSA.

Materials and Methods

Mice

DBA/1 (*H-2^q*) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Mice used in these experiments were 2–8-mo old and were maintained on laboratory chow and acidified-chlorinated water ad libitum.

Antigens

GAT, mol wt 32,000, was purchased from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. Methylated bovine serum albumin (MBSA) was prepared according to the method of N. Sueoka, and Ts'ai-Ying Cheng (7). Preparations of SRBC, GAT, and GAT-MBSA for addition to cultures have been described previously (4). 10^7 SRBC or GAT-MBSA containing 5 μ g GAT and/or various amounts of soluble GAT were added to cultures according to the experimental protocol.

Immunization of Mice

Mice were injected intraperitoneally with 10 μ g GAT-MBSA in a mixture of Maalox (Wm. H. Rorer, Inc., Fort Washington, Pa.) and pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) as previously described (1). Mice were immunized with GAT as indicated in the experimental protocol.

Spleen Cell Cultures and PFC Assay

Suspensions of single spleen cells containing 10×10^6 nucleated cells in culture medium (MEM) were incubated according to the method of Mishell and Dutton (8) with modifications previously described (9). GAT coupled to SRBC with CrCl_3 were used as indicator cells in the hemolytic plaque assay as described previously (4).

Sources of B and T Cells

Populations of splenic T and B cells were prepared by two methods: column fractionation and depletion.

Column Fractionation.—

Preparation of immunoabsorbent columns: Mouse Fab was prepared from a pepsin digest of mouse Ig, emulsified in complete Freund's adjuvant, and used to immunize rabbits for the production of antimouse Fab. The resulting antisera were purified over Sepharose 4B mouse-Ig immunoabsorbent columns, concentrated, dialyzed, and stored at -20°C . The purified antibody was conjugated to cyanogen bromide-activated Sephadex G-200 by methods previously described (10). Approximately 0.3 mg of antibody was routinely bound/ml of activated Sephadex. 12-ml disposable syringes are fitted with polyethylene discs (Bel-Art Products, Pequannock, N. J.) and packed with 8–10 ml of the Sephadex conjugate. The columns were

washed with media 199 (Grand Island Biological Co., Grand Island, N. Y.) containing 5% fetal calf sera (FCS), 2.5 mM EDTA, 1% penicillin-streptomycin solution, incubated at 37°C for 60 min, and returned to 4°C for cell fractionation.

Cell preparation and fractionation: Mouse spleen cells were teased into the above media without EDTA, aggregates sedimented, and the resulting single cell suspension treated with iron carbonyl at 37°C for 30 min. The phagocytic cells were removed in a magnetic field as previously described (10). Cells were resuspended in EDTA containing media and placed on the column in a concentration of approximately 20×10^6 cells/ml. The cells were applied to the column at 4°C and collected by stepwise elution with 15-ml aliquots of EDTA containing starting media at a flow rate of 0.5 ml/min until the effluent was cell-free. The bound cells were eluted by competitive inhibition using 2×15 -ml aliquots of media containing 10% autologous mouse sera as a source of gamma globulin.²

Analysis of surface characteristics: Unfractionated, nonretained, and serum-eluted cell populations were studied with respect to surface Ig properties. The presence of surface Ig's was detected by using a direct fluoresceinated antibody technique. To approximately 2×10^6 cells was added 0.1 ml of fluoresceinated antimouse Fab and the reaction mixture incubated at 4°C for 30 min. The cells were then washed three times with cold PBS containing 10% FCS and suspended in a glycerol-phosphate-buffered saline buffer and viewed with a Zeiss Universal fluorescence microscope (Carl Zeiss, Inc., New York) with an Osram 100 watt ultraviolet light source and with phase contrast. Routinely 200 viable cells were counted on the phase contrast and percentage staining with fluoresceinated antisera enumerated. Control fluorescein reagents consisting of fluoresceinated rabbit gamma globulin were used and in these experiments stained less than 2% of the cells.

Depletion.—

Theta-bearing cells were depleted from normal spleen cell suspensions by treatment with appropriate concentrations of anti- θ C3H serum and a 1:3 dilution of guinea pig serum as a source of C before culture initiation. As a control, a fraction of the spleen cell suspension was treated with C alone. After treatment, 10×10^6 cells in MEM were added to cultures with or without additional cells and SRBC or GAT-MBSA or GAT according to the experimental protocol. The helper cell function of antigen-primed T cells has been demonstrated to be radioresistant, whereas B cells and normal T cells are radiosensitive (11, 12). Therefore, mice primed with GAT-MBSA 1-4 mo earlier were X irradiated with 700-800 R as previously described (5). Within 3 h after irradiation, the mice were sacrificed and single cell suspensions were prepared from their spleens. 10×10^6 cells in MEM were added to cultures with or without additional cells and GAT or GAT-MBSA as indicated in the experimental protocol.

RESULTS

Immunocompetence of Spleen Cells from DBA/1 Mice Primed with GAT.—The responsiveness of spleen cells from normal and GAT-primed DBA/1 mice to SRBC and GAT-MBSA was compared in vitro. The results (Table I) demonstrate that spleen cells from normal DBA/1 mice develop PFC responses to SRBC (group A) and to GAT-MBSA (group B). In contrast, spleen cells from mice injected with 10 μ g GAT in Maalox-pertussis 15 days before culture initiation fail to develop a GAT-specific PFC response to GAT-MBSA (group D), although they respond normally to SRBC (group C). Thus, removal of spleen

² Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations I. Quantitative isolation of human T and B cells and response to mitogens. J. Immunol. Manuscript submitted for publication.

TABLE I
Restoration of the GAT-Specific PFC Response in Cultures of Tolerant Spleen Cells by Irradiated GAT-MBSA-Primed Spleen Cells

Group	DBA/1 spleen cells			IgG PFC/culture*		
	10 ⁷ Normal	10 ⁷ GAT-primed†	10 ⁷ Irradiated GAT-MBSA primed‡	Antigen	SRBC	GAT
A	+	—	—	SRBC	1,240	—
B	+	—	—	GAT-MBSA	—	1,480
C	—	+	—	SRBC	1,540	—
D	—	+	—	GAT-MBSA	—	<10
<i>10⁷ Anti-θ and C treated</i>						
E	+	—	—	GAT-MBSA	—	<10
F	—	—	+	GAT-MBSA	—	60
G	+	—	+	GAT-MBSA	—	3,460
H	+	+	—	GAT-MBSA	—	180
I	—	+	+	GAT-MBSA	—	1,690

* Cultures harvested after 5 days incubation with 10⁷ SRBC or 5 μ g GAT as GAT-MBSA.

† Mice primed with 10 μ g GAT in Maalox-pertussis 15 days before culture initiation.

‡ Mice primed with GAT-MBSA containing 10 μ g GAT in Maalox-pertussis 32 days before X irradiation (800 R) and culture initiation.

cells from the environs of the tolerant mouse (6) does not reverse this GAT-specific unresponsiveness.

Normal B cells or spleen cells from GAT-MBSA-primed, irradiated DBA/1 mice (T cells) were added to cultures of spleen cells from GAT-primed DBA/1 mice to determine the immunocompetence of B and T cells from tolerant mice. B cells, prepared by treatment of normal spleen cells with anti- θ serum and C, and GAT-MBSA-primed, irradiated T cells did not develop GAT-specific PFC responses when cultured separately with GAT-MBSA (groups E and F), but developed a GAT-specific response when cultured together with antigen (group G) showing that both populations were functional. Addition of normal B cells to GAT-primed spleen cells (group H) failed to restore responsiveness to GAT-MBSA. Thus, the specific unresponsiveness of these spleen cells is not due merely to an inactivation or elimination of GAT-specific B cells by in vivo exposure to GAT. If that were the case, normal B cells should cooperate with carrier (MBSA)-specific T cells present in the spleen cells of GAT-primed mice and such cultures would be expected to develop a PFC response to GAT-MBSA.

Addition of 10⁷ irradiated GAT-MBSA-primed helper T cells to cultures of otherwise unresponsive spleen cells from GAT-primed mice did restore the GAT-specific immune response to GAT-MBSA (group I). Thus, B cells from unresponsive mice are not themselves unresponsive, but can be induced to develop a GAT response to GAT-MBSA provided additional MBSA helper T cells are used. We can conclude that priming with GAT renders T cells of the nonre-

sponder mice unable to provide adequate helper cell function for the B-cell response.

Suppressive Activity of Spleen Cells from GAT-Primed DBA/1 Mice.—Since it seemed improbable that GAT could render some carrier-specific helper T cells (MBSA-specific) inactive while leaving others (e.g., SRBC specific) intact, we examined spleen cells from GAT-primed mice for active suppressor cells. Spleen cells from such mice were added to cultures of spleen cells from normal DBA/1 mice and the immune response to GAT-MBSA measured. The results of two such experiments (Table II) show that spleen cells from GAT-primed mice not only failed to respond to GAT-MBSA in culture (group A) but also suppressed the development of a GAT-specific PFC response of normal DBA/1 spleen cells

TABLE II
Effect of Tolerant DBA/1 Spleen Cells on the Immune Response by Normal DBA/1 Spleen Cells In Vitro

Group	DBA/1 spleen cells		IgG GAT-specific PFC/culture*	
	10 ⁷ Normal	10 ⁷ GAT- primed‡	Exp. 1	Exp. 2
A	—	+	<10	80
B	+	—	560	1,203
C	+§	—	830	ND
D	+	+	110	400
E	+	+	330	1,000

* PFC response after 5 days in culture with 5 µg GAT as GAT-MBSA.

‡ Mice injected with 10 µg GAT in Maalox 3 days (exp. 1) or 4 days (exp. 2) before culture initiation.

§ 2 × 10⁷ normal spleen cells.

|| Treated with anti-θ serum and C.

stimulated with GAT-MBSA (compare groups B and D). It is unlikely that this suppression is due to a detrimental effect of increased cell density since 2 × 10⁷ normal DBA/1 spleen cells (group C) responded even better than 1 × 10⁷ spleen cells (group B). Thus, the failure of GAT-primed spleen cells to respond to GAT-MBSA involves an active suppression. Treatment of spleen cells from GAT-primed DBA/1 mice with anti-θ serum and C before culture initiation significantly reduced but did not abolish the suppressive activity which these cells exerted on the response of normal spleen cells (group E).

Radiosensitivity of Suppressor T Cells.—The ability of spleen cells from GAT-primed DBA/1 mice exposed to 800 R X irradiation 3 h before sacrifice to suppress the normal DBA/1 spleen cell response to GAT-MBSA was compared to the suppressive ability of spleen cells from unirradiated GAT-primed mice. The results (Table III) demonstrate that suppressor cell activity is radiosensitive 11 days after GAT priming (group G vs. H).

Analysis of Splenic T- and B-Cell Populations from GAT-primed DBA/1 Mice

TABLE III
Radiation Sensitivity of Suppressor T Cells

Group	DBA/1 spleen cells			Antigen	IgG GAT-specific PFC/culture§
	10 ⁷ Normal	10 ⁷ GAT-primed*	10 ⁷ X irradiated GAT-primed‡		
A	+	—	—	GAT	<10
B	+	—	—	GAT-MBSA	525
C	—	+	—	GAT	15
D	—	+	—	GAT-MBSA	10
E	—	—	+	GAT-MBSA	<10
F	+	—	—	GAT-MBSA	625
G	+	+	—	GAT-MBSA	<10
H	+	—	+	GAT-MBSA	655

* Spleen cells from mice immunized with 10 μ g GAT in Maalox, 11 days before sacrifice.

‡ Same as above, except mice given 800 R X irradiation 3 h before sacrifice.

§ Cultures harvested after 5 days with 5 μ g GAT or 5 μ g GAT as GAT-MBSA.

|| 2 \times 10⁷ normal DBA/1 spleen cells.

Fractionated on a Dextran Column Specific for Fab of Mouse Ig.—To directly assess the properties of T and B cells from GAT-primed DBA/1 mice, spleen cells were fractionated by passage over a Sephadex G-200 column to which rabbit antimouse Fab antibodies had been coupled. Cells which do not bear membrane Ig's are not bound to such a column. The cell fraction which passes through the column contains all the θ -bearing lymphocytes as well as other non-Ig-bearing cells (10). Ig-bearing (B) cells are retained and may be subsequently eluted from the column by washing with medium containing normal mouse serum. The cell recovery from these columns is excellent (95–100%) and these cells are functional as shown by their activity in culture.

The T and B cells obtained by this column fractionation procedure were cultured and their immunocompetence determined (Table IV). Groups A through D are controls showing that anti- θ and C-treated normal spleen cells, irradiated GAT-MBSA-primed spleen cells, and column-purified T and B cells did not develop GAT-specific PFC responses to GAT-MBSA when cultured separately. The B- and T-cell sources used for reconstitution are shown to be competent in group E. Column purified T and B cells cultured together developed a PFC response to SRBC (group F) but failed to develop a significant GAT-specific PFC response to GAT-MBSA (group G).

Column-purified T cells from GAT-primed mice were unable to provide helper function to normal B cells (group H). However, B cells from GAT-primed mice responded very well to GAT-MBSA when cultured with carrier-primed helper T cells (group I). This experiment again demonstrates that unresponsiveness induced by injection of GAT in vivo is not due to B-cell tolerance, but rather to a failure of the T cells to provide helper function for the B-cell response.

Column-purified T and B cells from GAT-primed DBA/1 mice were also examined for suppressive activity on cultures of spleen cells from normal DBA/1 mice (Table V). As demonstrated earlier, the spleen cells from GAT-primed mice failed to respond to GAT-MBSA in vitro (group C) and suppressed the immune response of normal spleen cells to GAT-MBSA (group B compared to group D). Suppressor cell activity was present in the fraction containing T cells (group F), but was not present in the B-cell fraction (group G).

Antigen Specificity of Suppressor T Cells.—Data presented in the experiments thus far have shown that the generation of suppressor cells in nonresponder mice

TABLE IV
Characterization of Column-Purified B and T Cells from GAT-Primed DBA/1 Mice

Group	DBA/1 spleen cells				Antigen	IgG PFC/culture*			
	10 ⁷ Normal treated with anti- θ and C	10 ⁷ X-irradiated GAT-MBSA primed‡	GAT-primed column-purified§			Exp. 1		Exp. 2	
			5 × 10 ⁶ B	5 × 10 ⁶ T		SRBC	GAT	SRBC	GAT
A	+	—	—	—	GAT-MBSA	—	<10	—	<10
B	—	+	—	—	GAT-MBSA	—	35	—	<10
C	—	—	+	—	GAT-MBSA	—	<10	—	<10
D	—	—	—	+	GAT-MBSA	—	<10	—	<10
E	+	+	—	—	GAT-MBSA	—	1,080	—	1,375
F	—	—	+	+	SRBC	585	—	1,000	—
G	—	—	+	+	GAT-MBSA	—	50	—	40
H	+	—	—	+	GAT-MBSA	—	15	—	<10
I	—	+	+	—	GAT-MBSA	—	1,255	—	785

* Cultures harvested after incubation with 10⁷ SRBC or 5 μ g GAT as GAT-MBSA for 5 days.

‡ Mice primed with GAT-MBSA containing 10 μ g GAT 60 days before irradiation (800 R) and sacrifice.

§ Mice primed with 10 μ g GAT in Maalox 3 days before sacrifice. Column-purified splenic B cells: exp. 1, 85% Ig bearing and exp. 2, 96% Ig bearing. Column-purified splenic T cells: exp. 1, 7% Ig bearing and exp. 2, 0% Ig bearing.

TABLE V
Suppressive Activity of Column-Purified T Cells from GAT-Primed DBA/1 Mice

Group	DBA/1 spleen cells				Antigen	IgG GAT-specific PFC/culture*	
	10 ⁷ Normal	10 ⁷ GAT-primed‡	GAT-primed column-purified§			Exp. 1	Exp. 2
			5 × 10 ⁶ B	5 × 10 ⁶ T			
A	+	—	—	—	GAT	<10	<10
B	+	—	—	—	GAT-MBSA	515	600
C	—	+	—	—	GAT-MBSA	15	35
D	+	+	—	—	GAT-MBSA	105	10
E	—	—	+	+	GAT-MBSA	50	40
F	+	—	—	+	GAT-MBSA	<10	35
G	+	—	+	—	GAT-MBSA	935	725

* Cultures harvested after incubation with 5 μ g GAT or 5 μ g GAT as GAT-MBSA for 5 days.

‡ Mice primed with 10 μ g GAT in Maalox 3 days before sacrifice.

§ Column-purified cells from the same cell suspension as described in (‡) and as described in Table IV.

depends upon previous exposure to GAT. The findings that mice injected with GAT fail to respond to a subsequent challenge with GAT-MBSA, but respond normally to a subsequent injection of SRBC (6) and that cultures containing GAT-induced suppressor cells are nevertheless able to respond to SRBC suggests, but does not prove, that the suppression is antigen specific. Since the demonstration of suppression involves exposure to GAT-MBSA, it is possible that GAT-MBSA may be required to induce active suppressors in cultured spleen cells from mice previously primed with GAT. Therefore, the specificity of the suppressive activity must be examined by the addition of both GAT-MBSA and SRBC to cultures containing normal and GAT-primed spleen cells. Such an experiment is presented in Table VI. All spleen cell cultures responded

TABLE VI
Antigen Specificity of Suppressor T Cells

Group	DBA/1 spleen cells		Antigen	IgG PFC/culture*	
	10 ⁷ Normal	10 ⁷ GAT-primed†		SRBC	GAT
A	+	-	GAT-MBSA	-	525
B	-	+	GAT-MBSA	-	10
C	+	+	GAT-MBSA	-	<10
D	+	-	SRBC	250	-
E	-	+	SRBC	530	-
F	+	+	SRBC	640	-
G	+	-	SRBC and GAT-MBSA	470	-
H	+	+	SRBC and GAT-MBSA	635	-

* Cultures harvested after 5 days incubation with 10⁷ SRBC or GAT-MBSA containing 5 µg GAT or both.

† Mice primed with 10 µg GAT in Maalox 11 days before sacrifice and culture initiation.

to SRBC and GAT-primed spleen cells did not inhibit the normal spleen cell response to SRBC (group F). Addition of GAT-MBSA to cultures of normal spleen cells stimulated by SRBC did not inhibit the development of anti-SRBC PFC responses (group G) nor was this response inhibited if GAT-primed spleen cells were present (group H). Therefore, the suppressor cells elicited by GAT inhibit only GAT-specific B-cell responses.

DISCUSSION

The data in these experiments demonstrate that:

(a) Treatment of nonresponder DBA/1 mice with GAT renders their spleen cells unable to develop a GAT-specific response when incubated with GAT-MBSA, although their response to SRBC is equivalent to that of spleen cells from normal DBA/1 mice. B cells from GAT-primed mice can respond to GAT-MBSA in vitro in the presence of added carrier-primed (GAT-MBSA) helper T cells. Earlier data (6, 18) indicated that, in contrast to the responder B

cell, the GAT-specific response of nonresponder B cells in the presence of (responder \times nonresponder) F_1 GAT-primed T cells to GAT-MBSA and macrophage-bound GAT could be inhibited if soluble GAT was added to the cultures. Thus, nonresponder B cells, in the presence of GAT, are less able than responder B cells to cooperate with F_1 helper T cells. However, it has been shown that interaction with GAT does not permanently inactivate these B cells. Once removed from the presence of soluble GAT, these B cells become responsive again, perhaps by capping and endocytosing bound GAT and regenerating membrane receptors. This possibility is currently being investigated. Thus, the failure of GAT-primed spleen cells to respond to GAT-MBSA in vitro cannot be attributed to B-cell tolerance. This unresponsiveness is rather the result of impaired carrier-specific helper T-cell function in GAT-primed spleen cell suspensions.

(b) Spleen cells from GAT-primed DBA/1 mice specifically suppress the development of GAT-specific PFC responses by spleen cells from normal DBA/1 mice incubated with GAT-MBSA. This suppression is prevented by treatment of GAT-primed spleen cells with anti- θ serum and C or by exposure to 800 R X irradiation. The activity of suppressor T cells can be overcome by the addition of adequate numbers of carrier-primed helper T cells. This suggests that competition may exist between the activities of helper and suppressor T cells.

(c) The activity of suppressor T cells induced by GAT is antigen specific. The specificity was determined by the observation that addition of spleen cells from GAT-primed DBA/1 mice and GAT-MBSA to cultures of normal DBA/1 spleen cells did not inhibit the PFC response stimulated by SRBC but did inhibit the GAT-specific response to GAT-MBSA. These findings are in agreement with the hypothesis of Gershon et al. (13), which postulated the generation of suppressor T cells in nonresponder animals injected with GAT.

An important question to resolve concerns the mechanism and the site of action of suppressor T cells in the GAT system. In several other systems where they have been demonstrated, suppressor T cells have been shown to exert their effects on the generation of helper T cells or on T cells which mediate delayed hypersensitivity reactions (14, 15). The results of our experiments do not permit us to distinguish whether the suppressor T cells generated by GAT in DBA/1 mice act on GAT-specific B cells or on MBSA (carrier)-specific helper T cells which by binding GAT-MBSA could be susceptible to GAT-specific suppression. This issue may be resolved by investigating whether the suppressor T cells generated by GAT can also suppress the antibody response to MBSA in GAT-MBSA-immunized animals.

With respect to mechanism of action, the suppressor T cells may act by direct cell contact or through the release of an active product. If the latter is true, the demonstration that the suppressor activity is antigen-specific implies that this product must combine both suppressor and specificity properties.

The demonstration that nonresponder mice injected with GAT, the response to which is under *Ir*-gene control, do not develop an antibody response to GAT

and become specifically unresponsive to a subsequent challenge with GAT-MBSA and that this unresponsiveness is the result of an active suppressive process mediated by T cells, raises important questions concerning the mechanism of *Ir*-gene regulation of the immune response. The first relevant issue is whether the generation of suppressor T cells in nonresponder mice is unique to this system or may be generalized to other systems and species where the response to the antigen is under the control of histocompatibility-linked *Ir* genes.

What are the implications of the demonstration of GAT-specific suppressor T cells in nonresponder animals for the function of *Ir* genes and their products? There are at least three interpretations of the role of *Ir* genes which could account for these observations. First, one could postulate that both responder and nonresponder mice can develop both helper and suppressor T cells, but that the sensitivity of B cells to these T-cell activities is inherently different in responder and nonresponder mice. Nonresponder B cells would be more sensitive to suppressor T-cell activity, thus any GAT-specific helper T-cell activity in these mice would be undetectable. However, in data to be published: (a) we have not detected GAT-specific helper function in spleens of irradiated GAT-primed nonresponder mice for responder F₁ B cells exposed to GAT-MBSA; (b) nor have we been able to demonstrate that T cells from GAT-primed, irradiated nonresponder animals could cooperate with syngeneic nonresponder B cells in a response to macrophage-bound GAT. However, evaluation of the effect of various ratios of helper to suppressor T cells cultured with responder or nonresponder B cells should permit this possibility to be directly explored.

The second possibility is that suppressor T cells are a normal component of all immune responses, as suggested by the data of Tada and Takemori (16, 17) but that *Ir* genes determine the presence or absence of helper T cells specific for the antigen and are not involved in the regulation of suppressor cells. However, since GAT specifically induces suppressor T cells in nonresponders, it is then necessary to postulate two separate, antigen-specific recognition systems for helper and suppressor T cells, respectively, the former one under *Ir*-gene control.

The third model to be considered also assumes that suppressor T cells are a normal component of the immune system. Rather than proposing two different T-cell populations and/or two different antigen-recognition systems as in the second model, we can consider that responder T cells have the potential of developing into helper or suppressor cells. The manner of interaction of antigen with the GAT-specific T-cell receptor would select the pathway to helper or suppressor cell activity. On the other hand, interaction of antigen with the GAT-specific T-cell receptor on nonresponder T cells results in development of only suppressor cells. Thus, *Ir* genes would behave as regulator genes, perhaps coding for specific antigen receptors and through these receptors determining the function of T cells after interactions with antigen or perhaps by controlling the production of T-cell factors which enhance or suppress the B-cell response.

SUMMARY

In recent studies we have found that GAT not only fails to elicit a GAT-specific response in nonresponder mice but also specifically decreases the ability of nonresponder mice to develop a GAT-specific PFC response to a subsequent challenge with GAT bound to the immunogenic carrier, MBSA. Studies presented in this paper demonstrate that B cells from nonresponder, DBA/1 mice rendered unresponsive by GAT in vivo can respond in vitro to GAT-MBSA if exogenous, carrier-primed T cells are added to the cultures. The unresponsiveness was shown to be the result of impaired carrier-specific helper T-cell function in the spleen cells of GAT-primed mice.

Spleen cells from GAT-primed mice specifically suppressed the GAT-specific PFC response of spleen cells from normal DBA/1 mice incubated with GAT-MBSA. This suppression was prevented by pretreatment of GAT-primed spleen cells with anti- θ serum plus C or X irradiation. Identification of the suppressor cells as T cells was confirmed by the demonstration that suppressor cells were confined to the fraction of the column-purified lymphocytes which contained θ -positive cells and a few non-Ig-bearing cells. The significance of these data to our understanding of *Ir*-gene regulation of the immune response is discussed.

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