

## **Brief Definitive Report**

### IMMUNOSUPPRESSIVE FACTOR(S) SPECIFIC FOR L-GLUTAMIC ACID<sup>50</sup>-L-TYROSINE<sup>50</sup> (GT) II. PRESENCE OF *I-J* DETERMINANTS ON THE GT-SUPPRESSIVE FACTOR\*

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The responses to the synthetic antigens, L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup> (GAT) and L-glutamic acid<sup>50</sup>-L-tyrosine<sup>50</sup> (GT) are controlled by genes in the *I* region of the mouse *H-2* complex (1-3). Preimmunization of mice bearing the *H-2<sup>p,q,s</sup>* nonresponder haplotypes with GAT stimulates the development of suppressor T cells that inhibit in vivo or in vitro antibody responses to GAT complexed to the immunogenic carrier, methylated bovine serum albumin (GAT-MBSA) (4). The copolymer GT is not immunogenic in any inbred mouse strain tested, and has a suppressive effect on the antibody responses to GT-MBSA in mouse strains bearing the *H-2<sup>d,f,k,s</sup>* haplotypes; suppressor T cells have been demonstrated to be responsible for specific GT suppression (3).

We have obtained specific suppressive extracts from thymus and spleen cells of GAT- or GT-primed suppressor strains (5, 6). The specific suppressive T-cell factors in the active extracts have been characterized (6, 7) and appear similar to the carrier-specific suppressor factor described by Tada and Taniguchi (8). These products belong to a family of newly identified molecules coded for by the *I* region of the *H-2* complex with affinity for antigen and helper (9, 10) or suppressive (5-8) regulatory activity on the immune response.

Recently, Tada et al. have reported that the keyhole limpet hemocyanin (KLH)-specific suppressor factor is coded for by the *I-J* subregion of the *H-2* complex (11). We now demonstrate also that a GT-specific suppressor factor extracted from the spleens and thymuses of B10.BR (*H-2<sup>k</sup>*) mice bears determinants controlled by the *I-J* subregion of the *H-2* complex.

#### Materials and Methods

*Mice.* B10.BR (*H-2<sup>k</sup>*) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c (*H-2<sup>d</sup>*) mice were obtained from the Health Research Laboratory, West Seneca, N. Y. Mice used in these studies were 2- to 8-mo old and were maintained on acidified-chlorinated drinking water and laboratory chow ad libitum.

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*Antigen.* GT was purchased from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. Preparation of GT-MBSA has been described (3).

*Preparation of the GT-Suppressive Extract.* B10.BR mice received 100  $\mu\text{g}$  of GT in Maalox (William H. Rorer, Inc., Fort Washington, Pa.) intraperitoneally 3 days before sacrifice. Single cell suspensions were prepared from spleen and thymus adjusted to  $6 \times 10^6$  cells/ml and sonicated as described previously (5). The lysate was then centrifuged for 1 h at 40,000  $g$  at 4°C. The supernate was collected and tested in vitro at concentrations designated in the experimental protocol. Some supernates were stored at -80°C until used. Control extracts were prepared in the same manner from the spleen and thymus of mice injected with Maalox alone.

*Assay of GT-Specific Suppressor T-Cell Factor (GT-T<sub>s</sub>F).* The in vitro assay of GT-T<sub>s</sub>F was made in Linbro plates (Model FB-16-24-TC; Linbro Chemical Co., Hamden, Connecticut). 0.5-ml cultures containing  $5 \times 10^6$  spleen cells per well were used according to a modification of the Mishell-Dutton system that will be described in detail in the forthcoming publication.<sup>1</sup> The antigen and the appropriate dilution of GT-T<sub>s</sub>F, in Mishell-Dutton medium, was added at culture initiation; 2.5  $\mu\text{g}$  of GT as GT-MBSA or  $10^6$  sheep erythrocytes (SRBC) were added per well. The lymphoid extracts were added at dilution  $\geq 1/400$ . In these conditions we never observed cytotoxic effect as measured by cell recovery and viability after the culture. The IgG PFC responses were measured after 5 days of culture using GAT-SRBC as indicator cells in a modified Jerne hemolytic plaque assay (2, 3).

*Antisera.* The different alloantisera were prepared as follows: anti-I-J<sup>k</sup> serum was obtained from B10.A(3R) mice immunized with B10.A(5R) lymphoid cells; anti-K<sup>k</sup> plus I-A<sup>k</sup> serum was made in (B10  $\times$  LP.RIII)F<sub>1</sub> mice immunized with B10.A(18R) lymphoid cells; and finally the anti-D<sup>d</sup> alloantiserum was made in B10.BR mice immunized with B10.A lymphoid cells. All sera were collected after six or more immunizations.

*Preparation of the Immunoabsorbents.* All sera were heat-inactivated at 56°C for 30 min and the globulin fractions of the different sera were prepared by precipitation with 18% sodium sulfate. The precipitates were dissolved and extensively dialyzed against 0.1 M NaHCO<sub>3</sub> containing 0.5 M NaCl. The globulin fraction was then coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), as described previously (7). The efficiency of the coupling was determined by measuring the optical density at 280 nm of the original globulin fraction and the wash fluids. The amount of globulin attached to the Sepharose was calculated using an extinction coefficient of  $E_{1\%}^{1\text{cm}} = 15$  and the coupling conditions were adjusted to obtain 2 mg of protein/ml of packed Sepharose. The coupled beads were stored at 4°C in phosphate-buffered saline (PBS) containing 0.02% NaN<sub>3</sub>.

*Coupling of GT and BSA to Sepharose-4B.* GT and BSA, labeled with <sup>125</sup>I (New England Nuclear, Boston, Mass.) using the chloramine-T method (12), were used as tracer to follow the efficiency of coupling to amino-hexyl-Sepharose obtained from Pharmacia Fine Chemicals, Inc. The method of coupling has been described (7). The conditions of the coupling were empirically adjusted so that 1-2 mg of antigen were coupled/ml of packed Sepharose.

*Use of Immunoabsorbent Columns.* The immunoabsorbent columns were prepared by packing 2 ml of Sepharose beads into the barrels of 5-ml syringes. Before use, the beads were extensively washed with PBS. 1 ml of cell extract diluted 1/10 was applied to the columns incubated at 4°C for 3 h and then eluted with PBS. Various dilutions of the eluates were then assayed in vitro on the GT-MBSA responses of BALB/c mice.

## Results

Our previous studies of the suppressive factors were made with extracts obtained from GAT-primed DBA/1 (*H-2<sup>a</sup>*) (7) or GT-primed BALB/c (*H-2<sup>d</sup>*) mice.<sup>1</sup> Because of the lack of appropriate recombinants and alloantisera in these haplotypes, we could not investigate the presence of determinants controlled by the *I-J* subregion in these factors. However, we have already reported the

<sup>1</sup> Thèze, J., C. Waltenbaugh, R. Germain, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic acid<sup>50</sup>-L-tyrosine<sup>50</sup> (GT). IV. *In vitro* activity and immunochemical properties of the GT-specific suppressive factor. Manuscript in preparation.

TABLE I  
Specificity of the B10.BR GT-Specific Factor\*

	GT-specific IgG PFC response per 10 <sup>7</sup> cells‡			SRBC PFC response per 10 <sup>7</sup> cells§				
	No extract	Final dilution of extract		No extract	Final dilution of extract			
		1/400	1/1,600	1/4,800		1/400	1/1,600	1/4,800
Control response	1,094				2,400			
Untreated extract		<10	<10	1,064		1,800	2,300	2,100
Extract adsorbed on GT-Sepharose column¶		1,394	1,814	ND				
Extract adsorbed on BSA-Sepharose column¶		0	264	ND				

\* Effect of lymphoid cell extracts from B10.BR mice preimmunized with GT on the PFC responses to SRBC and GT-MBSA of normal BALB/c spleen cells in vitro. Extracts were added at culture initiation at the indicated dilutions.

‡ GT-specific, IgG responses after 5 days in culture.

§ IgG plus IgM SRBC responses after 5 days in culture.

|| Mean PFC response from duplicate cultures.

¶ Untreated extract diluted 1/10 was incubated with GT or BSA-Sepharose for 3 h at 4°C.

suppressive properties of a GT-T<sub>s</sub>F obtained from lymphocytes of B10.BR (*H-2<sup>k</sup>*) mice (6). Since alloantisera directed against the *I-J* subregion of the *H-2<sup>k</sup>* haplotype are available, experiments were initiated to investigate the B10.BR GT-specific suppressor factor for the presence of *I-J* determinants. We have previously shown that GT-T<sub>s</sub>F extracted from B10.BR (*H-2<sup>k</sup>*) lymphoid cells suppresses the GT-MBSA responses of BALB/c (*H-2<sup>d</sup>*) mice (6). We have accordingly assayed the B10.BR GT-T<sub>s</sub>F on the in vitro IgG plaque-forming cell (PFC) response of BALB/c mice spleen cell to GT-MBSA.

Table I shows the specificity of GT-T<sub>s</sub>F made from lymphocytes of B10.BR mice preimmunized 3 days earlier with 100 μg of GT. The suppression observed is specific and only affects the GT-MBSA response; no significant suppression of the SRBC response is observed. The suppressive activity on the GT-MBSA response disappears at a dilution of 1/4,800 but is still complete at a 1/3,200 dilution (data not shown). Control extracts from B10.BR mice injected 3 days earlier with Maalox only did not show any significant effect either on the GT-MBSA or the SRBC responses (data not shown). This result confirms in vitro the observations already made in vivo that a B10.BR GT-T<sub>s</sub>F may suppress a GT-MBSA BALB/c response and demonstrates the absence of an allogeneic restriction in the activity of the suppressive factors in the GT system.

Furthermore, the suppressive activity can be adsorbed by GT-Sepharose columns and not by BSA-Sepharose columns, thus indicating that the suppressive material has affinity for GT, also confirming in vivo observations already reported (6). The suppressive activity was recovered from GT-Sepharose by elution with glycine-HCl buffer, pH 2.5 (data not shown). By these two criteria, specificity of suppression of GT-MBSA PFC response and affinity of the suppressive material for the antigen we may conclude that the B10.BR extract contains a factor that has identical properties to the previously characterized GAT-T<sub>s</sub>F and GT-T<sub>s</sub>F factors (6, 7).

In Table II are reported two representative experiments in which the B10.BR GT-T<sub>s</sub>F was passed through immunoabsorbents directed against different subregions of the *H-2<sup>k</sup>* haplotype. The immunoabsorbent directed against the *I-J<sup>k</sup>*

TABLE II  
Adsorption of B10.BR GT-Specific T-Cell Factor with Various Alloantisera\*

Treatment of the extract†	GT-specific IgG PFC response per 10 <sup>7</sup> cells‡			
	No extract	Final dilution of extract		
		1/400	1/1,600	1/4,800
Exp. I				
Control response	948			
None		<10	<10	1,064
Anti-I-J <sup>k</sup>		960	875	
Anti-K <sup>k</sup> + I-A <sup>k</sup>		<10	<10	
Anti-D <sup>d</sup>		35	<10	
Exp. II				
Control response	610			
None		<10	<10	820
Anti-I-J <sup>k</sup>		380	810	860
Anti-K <sup>k</sup> + I-A <sup>k</sup>		<10	<10	760

\* Effect of lymphoid cell extracts from B10.BR mice (*H-2<sup>k</sup>*) preimmunized with GT on the IgG PFC response of BALB/c spleen cells to GT-MBSA.

† Extract diluted 1/10 has been incubated in the various immunoabsorbents: anti-I-J<sup>k</sup>, B10.A (3R) anti-B10.A (5R); anti-K<sup>k</sup> + I-A<sup>k</sup>, (B10 × LP.RIID)F, anti-B10.A (18R); anti-D<sup>d</sup>, B10.BR anti-B10.A.

‡ GT-specific IgG responses after 5 days in culture.

subregion removed all the suppressive activity, whereas the immunoabsorbent directed against the *K<sup>k</sup>* plus *I-A<sup>k</sup>* subregions had no effect on the titer of the suppressive material. An additional control is shown in exp. I: the suppressive activity is not affected by passage of the extract through an immunoabsorbent made with an irrelevant alloantiserum directed against the *D* subregion of the *H-2<sup>d</sup>* haplotype. The suppressive activity adsorbed on the *I-J<sup>k</sup>* column could be eluted by a glycine-HCl buffer, pH 2.5 (data not shown).

### Discussion

It has been established already that GAT-T<sub>s</sub>F and GT-T<sub>s</sub>F factors are products of the *I* region of the *H-2* complex of mice (reference 6 and footnote 1). In this report, we have demonstrated that GT-T<sub>s</sub>F extracted from B10.BR (*H-2<sup>k</sup>*) mice, immunized with GT, bears determinants controlled by the *I-J* subregion. This result adds additional information on the genetic mechanism concerned with the regulation of the suppression in the GT system. At least three genes localized in the *I* region of the *H-2* complex are now known to be implicated: (a) two complementing genes tentatively localized in the *I-C* or *S* and *I-A* or *I-B* subregions, respectively, are responsible for the development of GT-specific suppressor cells (13); (b) a third gene(s) mapping in the *I-J* subregion is involved in the coding of the specific suppressor factor. The presence of *I-J* determinants extends to another system the result reported by Tada et al. for the KLH-carrier-specific factor (11). Furthermore, as shown in a companion paper (14) the factor described originally by Asherson and Zembala (15) which suppresses contact sensitivity to picryl chloride also bears determinants controlled by the *I-J* subregion. It is tempting to generalize these findings and to consider that the *I-J* subregion is specialized to code for suppressive molecules. On the other hand, the specific helper factors described by Taussig et al. (16) and Isac et al. (17), appear to be coded by the *I-A* subregion, stressing again the specialization of different *I* subregions of the *H-2* complex and the molecular distinction between helper and suppressor materials.

Different *I* subregions therefore appear to be specialized in coding for the specific helper or suppressor factors recently described. Since these molecules are able to bind antigen (6-10) and in the case of GAT-T<sub>s</sub>F with a specificity and an avidity comparable to that of specific antibody (7), it will be of interest to determine if they share idiotypic determinants with the corresponding specific antibodies.

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