

IN VIVO EFFECTS OF ANTI-Ia ALLOANTISERA

I. Elimination of Specific Suppression by In Vivo Administration of Antisera Specific for I-J Controlled Determinants*

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The helper and suppressor regulatory activities exerted on antibody responses by T lymphocytes are associated with subregions of the *I* region of the murine *H-2* (major histocompatibility) complex. Using antisera defining Ia antigens, raised by immunization between congenic pairs of mice differing at the *I* region, specific suppressor T cells (1, 2) and their soluble and mediators (3-5) have been shown to bear *I-J* subregion coded determinants, whereas certain helper (enhancing) factors and possibly helper cells possess *I-A* coded determinants (6, 7).¹ Analysis of the effects of such specific anti-Ia antisera on immune responses can, therefore, provide information concerning the regulatory functions of the various *I* region products. This approach has been used in various model systems, and several investigators have reported marked inhibition of in vitro lymphocyte reactivity by distinct anti-Ia antisera (8-12). It was thus appropriate to determine whether such antisera would also alter in vivo immune responses. We reported recently (13) that microliter amounts of alloantisera against *I^k* and *I^d* gene products were able to potentiate primary IgM and IgG in vivo plaque-forming cell (PFC)² responses to suboptimal immunogenic doses of sheep erythrocytes (SRBC) in A/J and BALB/c mice, respectively. The same immunopotentiating activity was observed with an anti-I-J^k antiserum in A/J mice. Further studies revealed that in vivo administration of the same anti-I-J^k antiserum decreases tumor growth in A/J mice inoculated with a syngeneic methylcholanthrene-induced fibrosarcoma (14), known to elicit potent suppressor T-cell responses active in inhibiting anti-tumor immunity (15, 16). These observations raised the possibility that the potentiation of SRBC PFC responses observed with the anti-I-J^k antiserum,

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² Abbreviations used in this paper: GAT, random linear terpolymer of L-glutamic acid⁶⁰, L-alanine³⁰, and L-tyrosine¹⁰; GT, random linear copolymer of L-glutamic acid⁵⁰-L-tyrosine⁵⁰; HBSS, Hanks' balanced salt solution; i.p., intraperitoneally; i.v., intravenously; MBSA, methylated bovine serum albumin; M/P, Maalox pertussis; PFC, plaque-forming cells; SRBC, sheep erythrocytes.

and with the anti-I-J containing whole anti-I region antisera, could be attributed to an in vivo interference with the *I-J*-positive components (cells and/or factors) of an antigen-specific suppressor system.

The present investigation has been designed to test directly the possible action of anti-I-J antisera on the well-defined T-cell-dependent suppression characterizing the responses of certain nonresponder strains of mice to the copolymers L-glutamic acid⁶⁰, L-alanine³⁰, L-tyrosine¹⁰ (GAT) and L-glutamic acid⁵⁰, L-tyrosine⁵⁰ (GT), which have been extensively studied in this laboratory. The specific immune suppression in these systems can be summarized as follows (17). Nonresponder mice bearing the *H-2^{a,s}* haplotypes immunized with GAT develop specific suppressor T cells which suppress GAT-MBSA responses (18). Similarly, specific suppression of GT-MBSA responses can be detected after GT administration to nonresponder mice bearing the *H-2^{k,d,s,f}* haplotypes (19). In both cases, antigen-specific suppression can be transferred into normal syngeneic recipients by T-derived lymphocytes from such GAT or GT primed, unresponsive mice.

We report in this paper that in vivo treatment with anti-I-J^s and anti-I-J^k antisera: (a) potentiates the primary in vivo PFC response to suboptimal doses of SRBC in SJL and A/J mice; (b) induces direct responsiveness to GAT and GT in nonresponder suppressor SJL and CBA mice, respectively; and (c) prevents in these mice the development of detectable GAT and GT-specific suppressor T cells, as assayed by adoptive transfer experiments. The implications of these findings are discussed in regard to our knowledge of the suppressive mechanisms active in these systems.

Materials and Methods

Mice. SJL (*H-2^s*) and CBA (*H-2^k*) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine and used throughout this study at 8–12 wk of age. They were maintained in our animal facilities on standard laboratory chow and acidified chlorinated water ad lib. Either male or female were used, but all mice were age and sex matched in a single experiment.

Antisera. The antisera used in this study were raised in mice from our breeding colony by repeated intraperitoneal inoculation of the appropriate lymphoid cells. The following strain combinations were used: (a) anti-I-J^k, B10.A(3R) anti-B10.A(5R); (b) anti-I-J^s, [B10.A(3R) × B10.S(9R)]F, anti-B10.HTT. As a result of the strain combinations used to produce the latter serum, it may contain contaminating antibodies directed to the *I-C^k*, *S^k*, and *G^k* gene products, although such antibodies have not been observed. The cytotoxic titer of these sera by standard dye exclusion against donor spleen cells was <1/10.

Antigens. Sheep blood in Alsever's solution was purchased from Grand Island Biological Co., (Grand Island, N. Y.). Before using, the SRBC were washed three times in sterile Hanks' balanced salt solution (HBSS), the buffy coat being removed after the first centrifugation. The random linear copolymer GAT, average mol wt 38,000 and GT, average mol wt 32,000 were purchased from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. Stock solutions (10 mg/ml) were prepared in saline containing 1% NaCO₃ at pH 9.0–9.5. Methylated bovine serum albumin (MBSA) was purchased from Calbiochem, San Diego, Calif. The copolymers GT and GAT were complexed to MBSA as previously described (20, 21).

Immunizations. SRBC, adjusted to the appropriate concentrations in 0.5 ml of HBSS, were mixed with various amounts of alloantisera and injected in the lateral tail vein of mice. Control groups received the same inoculum containing SRBC alone. Mice were immunized either with 100 μg GT, 10 μg GT as GT-MBSA, 10 μg GAT or 10 μg GAT as GAT-MBSA intraperitoneally (i.p.) in a vol of 0.2 ml. The adjuvants were either a 5% solution of magnesium and aluminum hydroxides (Maalox, William H. Rorer, Inc., Fort Washington, Pa.) or a mixture of Maalox (5%) and pertussis (25%) (Eli Lilly and Co., Indianapolis, Ind.) (M/P).

Antiserum Treatment. 10 μ l of the appropriate antiserum was diluted in 0.25 ml of HBSS and injected intravenously into mice immediately after immunization with the copolymer. The same treatment was repeated the 2 following days. Control groups received either 10 μ l of an irrelevant antiserum or 10 μ l of HBSS alone in the same manner.

Adoptive Transfer. Mice were primed on day 0 either with GAT or GT in Maalox, or with Maalox (5%) alone. They received three daily intravenous (i.v.) injections of 10 μ l of antiserum diluted in HBSS (days 0, 1, and 2). 3 days after the priming, the mice were sacrificed and the spleens were teased in sterile HBSS. The spleen cells were washed twice in HBSS and adjusted to 40×10^6 viable cells per ml. 10×10^6 primed cells were injected intravenously into normal recipients which were immediately challenged with GAT-MBSA or GT-MBSA in M/P i.p. at the doses detailed above.

PFC Assay. The anti-SRBC IgM and IgG were enumerated 6 days after immunization by a modification of the Jerne hemolytic plaque technique (22). A goat anti- μ antiserum (the gift of R. Asofsky, the National Institutes of Health) was used to inhibit IgM PFC, and a rabbit anti-mouse IgG was used to develop IgG PFC. The antibody responses to GAT, GT, GAT-MBSA, or GT-MBSA were assayed 7 days after immunization, using GAT-coated SRBC, which detect the highly cross-reactive anti-GT antibodies as well (20). All results are expressed as PFC per spleen. The cell recovery per spleen did not vary significantly between groups.

Statistical Analysis. All data were analysed for significance by using a two-tailed Student's *t* test, performed on a Wang 700 calculator.

Results

Potentiation of the Primary In Vivo Response to SRBC by Anti-I-J Alloantisera in SJL and A/J Mice. We previously reported that a [B10.A(3R) \times DBA/2]F₁ anti-B10.A(5R) antiserum (anti-I-J^k) was able to potentiate the IgM and IgG primary PFC responses to suboptimal doses of SRBC in A/J mice (13). Experiments were designed to verify that the same potentiation could also be observed in the same strain using another anti-I-J^k antiserum. A/J mice were immunized with 3.5×10^5 SRBC alone or with the same dose of SRBC mixed with 1 μ l of [B10.A(3R) anti-B10.A(5R)] antiserum (anti-I-J^k). A twofold increase in the IgM and IgG PFC responses (groups V and VI, Table I) was observed in the group receiving the antiserum treatment. Thus, two different anti-I-J^k antisera share the same immunopotentiating activity on SRBC responses in A/J mice. In preliminary titration experiments in SJL mice, 2×10^6 SRBC were found to be the antigen dose giving a response just above the background. Groups of mice were then immunized with this number of SRBC together with 5 or 10 μ l of anti-I-J^s antiserum, and the PFC responses on day 6 were compared to those of the control groups receiving 2×10^6 SRBC alone. The results of several experiments of this type are summarized on the top of Table I. 5 or 10 μ l of anti-I-J^s antiserum induced a significant (twofold) increase of both IgM and IgG responses, whereas no significant increase was observed with 10 μ l of normal mouse serum. It can be concluded, therefore, that this potentiating activity on SRBC responses is a common property of both anti-I-J^k and anti-I-J^s antisera.

Responsiveness to GT in "Suppressor" CBA Mice after In Vivo Treatment with an Anti-I-J^k Antiserum. The nonresponder status to GT in suppressor strains is related to a predominant specific suppression after immunization with this antigen. This conclusion is based in large measure on the observation that GT suppressor mice can respond to GT after appropriate cyclophosphamide treatment (23) which eliminates suppressor responses (24). Experiments were,

TABLE I
Effect of Anti-I-J Antisera on the Primary In Vivo Anti-SRBC Responses in A/J and SJL Mice

Group	Strain	No. of mice per group	SRBC*	Antiserum†	IgM PFC per spleen Mean ± SE	P‡	IgG PFC per spleen Mean ± SE	P‡
I	SJL (H-2 ^b)	14	2 × 10 ⁶	None	6,060 ± 483	—	2,457 ± 182	—
II	SJL (H-2 ^b)	10	2 × 10 ⁶	Anti-I-J ^s (10 μl)	12,280 ± 1,148	<0.001	5,315 ± 622	<0.001
III	SJL (H-2 ^b)	5	2 × 10 ⁶	Anti-I-J ^s (5 μl)	13,540 ± 3,286	0.001	4,850 ± 1,387	0.01
IV	SJL (H-2 ^b)	4	2 × 10 ⁶	NMS§ (10 μl)	8,200 ± 895	NS¶	3,087 ± 37	NS
V	A/J (H-2 ^a)	4	3.5 × 10 ⁶	None	3,060 ± 207	—	887 ± 202	—
VI	A/J (H-2 ^a)	5	3.5 × 10 ⁶	Anti-I-J ^s (1 μl)	5,980 ± 919	0.02	2,540 ± 395	0.01

* The SRBC were injected i.v. in 0.5 ml of Hanks' solution.

† Anti-I-J^s: (B10.A(3R) × B10.S(9R))F₁; anti-B10.HTT; anti-I-J^k: B10.A(3R) anti-B10.A(5R).

‡ Calculated according to the Student's *t* test.

§ NMS, normal mouse serum.

¶ NS, not significant (>0.05).

TABLE II
Effects of Anti-I-J Antisera on Primary In Vivo Responses to GT and GAT in CBA and SJL Mice

Group	Strain	No. of Mice per group	Immunization* (Day 0)	Antiserum treatment‡ on day 0, 1, and 2	IgG Specific PFC per spleen on day 7 ± SE	P Value§
I	CBA (H-2 ^b)	15	GT M/P	None (HBSS)	440 ± 86	—
II	CBA (H-2 ^b)	17	GT M/P	Anti-I-J ^s	5,245 ± 578	<0.001
III	CBA (H-2 ^b)	4	GT M/P	Anti-I-J ^k	525 ± 375	0.5
IV	SJL (H-2 ^b)	8	GAT M/P	None (HBSS)	407 ± 129	—
V	SJL (H-2 ^b)	8	GAT M/P	Anti-I-J ^s	4,176 ± 428	<0.001

* 100 μg of GT or 10 μg of GAT i.p. in M/P.

† Anti-I-J^s antiserum: B10.A(3R) anti-B10.A(5R); anti-I-J^k antiserum: (B10.A(3R) × B10.S(9R))F₁; anti-B10.HTT.

‡ Calculated according to Student's *t* test. Comparisons made to groups receiving HBSS alone.

therefore, designed to investigate whether in vivo treatment with an anti-I-J^k antiserum could induce responsiveness in CBA mice. Groups of mice were immunized with 100 μg of GT in M/P i.p. and received three daily i.v. injections of 10 μl of either an anti-I-J^k antiserum or an I-J^s antiserum, with the control group receiving the same inoculum of HBSS. The results from four experiments summarized on the top of Table II indicate that the anti-I-J^k antiserum allows CBA mice to respond to GT. This effect appears to be specific, as indicated by the fact that an anti-I-J^s antiserum (which genetically cannot cross-react with the products of the I-J^k subregion but potentially could have reacted with the *I-C^k*, *S^k* or *G^k* region gene products) or an irrelevant [(B10.BR × A.SW)F₁ anti-SJL] antiserum (data not shown) failed to induce responsiveness to GT in CBA mice. Thus, both cyclophosphamide and in vivo treatment with anti-I-J^k antiserum induce responsiveness to GT in suppressor nonresponder strains. These data are consistent with an inactivation of GT-specific suppression by the anti-I-J^k treatment.

Nonresponder "Suppressor" SJL Strain Responds to GAT after In Vivo Treatment with an Anti-I-J^s Antiserum. The next series of experiments investigated whether in vivo treatment with anti-I-J^s antiserum would lead to GAT responses in SJL mice. These nonresponder mice normally fail to give a GAT PFC response to GAT itself, due to the development of a strong suppressor

T-cell response. Two groups of mice were immunized with 10 μ l of GAT in M/P i.p., and one group received three daily injections of 10 μ l of an anti-I-J^s antiserum. The control group received the same inoculum of HBSS alone. The GAT specific IgG PFC 7 days later show (as for GT responses in CBA mice after I-J^k treatment) a moderate but very significant response in the group treated with the anti-I-J^s serum (Table II, bottom).

Lack of Transferrable Specific Suppression from GT-Primed CBA Mice and from GAT-Primed SJL Mice after In Vivo Treatment with Anti-I-J Antiserum. The preceding observations suggested that the GT and GAT-specific suppressor pathways in nonresponder suppressor mice were affected by appropriate anti-I-J treatment. Previous studies revealed that complete suppression of GAT-MBSA or GT-MBSA responses could be achieved by transfer of low numbers of Thy 1.2-positive spleen cells from GAT or GT-primed mice into normal syngeneic recipients (17). To investigate the ability of anti-I-J antisera to interfere with the generation of active suppressor cells in such GAT or GT-primed mice, adoptive transfer experiments were performed. Mice were primed on day 0, either with GT (CBA) or with GAT (SJL), or as a control, with Maalox alone. On days 0, 1, and 2, 10 μ l of the appropriate anti-I-J antiserum was injected intravenously into the primed mice. The control groups received the same inoculations of HBSS alone. 3 days after priming, 10×10^6 spleen cells from the different groups were transferred to normal syngeneic recipients, which were immediately challenged with either GT-MBSA (CBA) or GAT-MBSA (SJL). 7 days later, specific IgG PFC per spleen were enumerated. Fig. 1 summarizes the results from several experiments in CBA mice. 10×10^6 transferred GT-primed cells completely suppressed the control response to GT-MBSA, whereas no suppression was observed with the same number of Maalox-primed cells or with 10×10^6 spleen cells from GT-primed mice treated with anti-I-J^k antiserum. In other experiments (data not shown), a higher number of transferred spleen cells was used with similar results: 20×10^6 GT-primed spleen cells from control mice injected only with HBSS completely suppressed the control response, whereas the same number of GT-primed cells from anti-I-J^k antiserum treated mice did not affect the GT-MBSA response of syngeneic recipients. Moreover, 20×10^6 GT-primed cells from CBA mice treated with an anti-I-J^s antiserum still suppressed the control response, further providing evidence for the specificity of the anti-I-J effect. Fig. 2 summarizes the data concerning the SJL mice, which showed the same pattern as CBA mice. 10×10^6 GAT-primed spleen cells suppressed the control GAT-MBSA response, but this suppression was no longer detectable with cells from mice treated with anti-I-J^s antiserum. These data, therefore, indicate that 3 days after both antigen priming and appropriate anti-I-J antiserum treatment, the nonresponder suppressor CBA and SJL mice do not possess GT or GAT-specific suppressor cells detectable by standard adoptive transfer protocols.

Discussion

The data reported here provide further evidence for potent in vivo effects of anti-I-J antisera on immune responses. First, the immunopotentiating activity of [B10.A(3R) \times DBA/2]F₁ anti-B10.A(5R) antiserum (anti-I-J^k) on the primary in vivo anti-SRBC response in A/J mice, which we recently reported (14), was

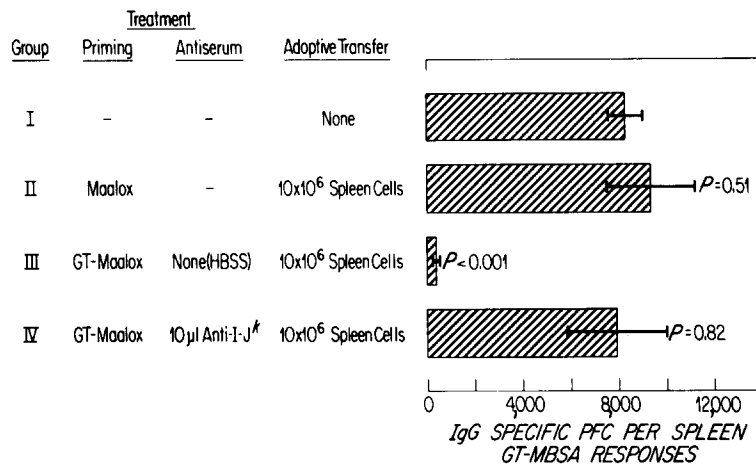


FIG. 1. In vivo effects of anti-I-J^k antiserum on the transfer of GT-specific suppression in CBA (H-2^k) mice. Groups of mice (three mice per group) were primed on day 0 with 100 μg of GT in Maalox, or with Maalox alone, i.p. Group III received on days 0, 1, and 2, 0.25 ml of HBSS i.v., and group IV received in the same manner 10 μl of B10.A(3R) anti-B10.A(5R) antiserum (anti-I-J^k) diluted in 0.25 ml of HBSS. On day 3, 10×10^6 viable spleen cells from each group were transferred i.v. into normal recipients (four per group) which were immediately challenged with 10 μg of GT as GT-MBSA in M/P, i.p. 7 days later, the specific IgG PFC per spleen were enumerated. The results from three experiments (12 mice per group) are expressed as arithmetic mean of PFC per spleen \pm standard error (bars). *P* value calculated according to Student's *t* test.

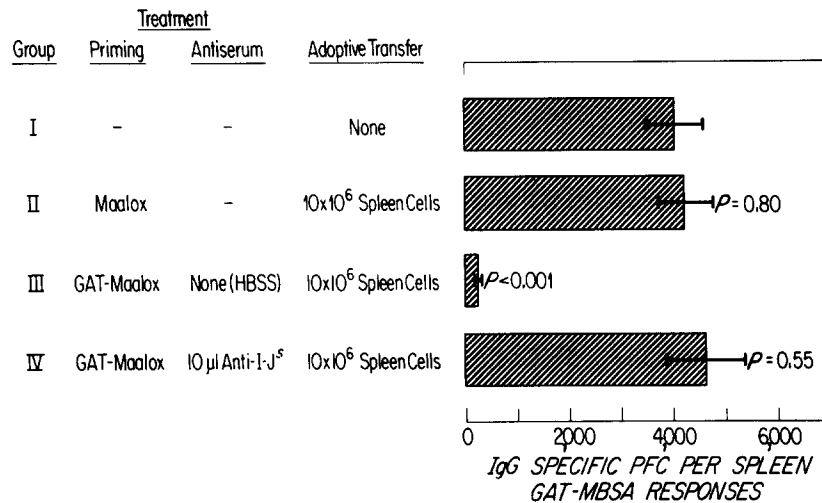


FIG. 2. In vivo effects of anti-I-J^s antiserum on the transfer of GAT-specific suppression in SJL (H-2^s) mice. Groups of mice (three mice per group) were primed on day 0 with 10 μg of GAT in Maalox or with Maalox alone, i.p. One group of mice (III) received on days 0, 1, and 2, 0.25 ml of HBSS i.v., and another group of mice (IV) received in the same manner 10 μl of [B10.A(3R) \times B10.S(9R)]F₁ anti-B10.HTT antiserum (anti-I-J^s) diluted in 0.25 ml of HBSS. On day 3, 10×10^6 viable spleen cells from each group were transferred i.v. into normal recipients (four per group) which were immediately challenged with 10 μg of GAT as GAT-MBSA in M/P, i.p. 7 days later the specific IgG PFC per spleen were enumerated. The results from two experiments (8 mice per group) are expressed as arithmetic mean of PFC per spleen \pm standard error (bars). *P* values calculated according to the Student's *t* test.

also observed with another anti-I-J^k antiserum, B10.A(3R) anti-B10.A(5R), and in SJL mice with an anti-I-J^s antiserum, [B10.A(3R) × B10.S(9R)]F₁ anti-B10.HTT. This adjuvant effect on anti-SRBC responses appears, therefore, to be a common property of such anti-I-J reagents, and represents a simple, convenient, and sensitive method for testing putative anti-I-J antisera for activity. The ability to perform the assay using mice not congenic at the *I* region, as required in the MLR assay for I-J activity reported recently (25), provides this method with certain advantages.

The fact that I-J coded determinants have to date been considered to be expressed selectively on suppressor T cells and T-derived suppressor factors (1-5) raised the possibility that the potentiating activity of these sera could be related to an *in vivo* interference with these I-J-positive components of antigen-induced specific suppression. The observation that an anti-I-J^k antiserum decreased tumor growth in A/J mice inoculated with a methylcholanthrene-induced fibrosarcoma (14) supported this possibility. In this model suppressor T cells play an important role in regulating tumor growth (15, 16), and preliminary experiments indicated that anti-I-J^k antiserum treatment reduces suppressor cell activity in tumor-bearing mice. The GT or GAT-specific suppression characterizing some nonresponder strains, such as CBA for GT or SJL for GAT, was, therefore, an appropriate model for testing the hypothesis that anti-I-J antisera mediated potentiation of immunity is due to reduction of suppressor T-cell responses. In a first series of experiments, it was established that 10 μ l of anti-I-J^k antiserum per day for 3 days allowed CBA mice to respond to GT, an antigen toward which this strain normally develops strong specific suppression. Similarly, responsiveness to GAT was observed after treatment with an anti-I-J^s antiserum, in the SJL strain. The earlier observation that responsiveness to GT could be induced in BALB/c by cyclophosphamide (23) appears very similar to these results. It is, therefore, reasonable to assume that the anti-I-J antisera acts similarly to cyclophosphamide, preventing *in vivo* development of efficient T-cell-mediated suppression, thus permitting GAT or GT-specific T helper cells to differentiate and/or to act (26). The second series of experiments strengthened this interpretation by establishing that *in vivo* anti-I-J antiserum treatment prevents the development of detectable antigen-specific suppressor cells in GT-primed CBA mice or GAT-primed SJL mice, as assessed by an adoptive transfer protocol. However, since both helper and suppressor activity can be identified in nonresponder strains (23, 27), it cannot be definitively excluded at this time that the anti-I-J antisera does not provide a positive inductive signal to I-J-bearing helper T cells, which are then able to act despite the presence of the usually predominant suppressor cells. Support for this possibility, however, awaits clear demonstration of the presence of I-J-coded products on helper cells, particularly for the antigens GAT and GT. At least one "helper" factor for GT is known to carry I-A, and not I-J subregion coded determinants, but the presence of I-J coded antigens on other helper factors or on helper cells for either antigen has not been excluded at present.

The effects of the anti-I-J antisera appear to be specific, since: (a) anti-I-J^s antiserum does not induce responsiveness to GT in CBA (I-J^k) mice; and (b) anti-I-J^s antiserum does not interfere with the development of GT-specific

suppression in CBA (I-J^k) mice. However, absorption experiments have not been performed to prove directly that the effects observed were related to anti-I-J antibodies contained in these sera. One can infer, however, from such absorptions performed in previous studies, that antibodies to I-J region determinants are the active material in these reagents (13, 14).

The conversion by anti-I-J sera of nonresponder mice to responders, as demonstrated above, suggests the presence in nonresponder mice of helper T-cell precursors capable of responding to GAT or GT presented by conventional routes of immunization. This further implies that it is the net balance of (helper and suppressor) regulatory T-cell responses rather than absolute defects in one or the other pathway which determines whether or not immunity is seen after challenge with GAT or GT in suppressor nonresponder strains. Such a hypothesis predicts that transfer of lymphocytes from anti-I-J-treated, antigen-primed (GAT or GT) nonresponder mice into normal syngeneic recipients might yield detectable helper activity able to more than counterbalance the usual suppressor response to a GAT or GT challenge in such recipients. Preliminary evidence indicates that this can, in fact, be observed in the GT system (M. Pierres, unpublished observations).

Finally, it is becoming clear that I-J subregion-coded determinants are present on both suppressor cells and suppressor factors (1-5), and that the pathway from antigen to final suppressive action requires at least two distinct suppressor T cells and a minimum of one factor which mediates interaction between these two T cells (28, 29). The suppressor factor and the T suppressor cell that it induces have already been typed as I-J positive (2, 4),³ and it is likely that the factor-producing T cell is an I-J-bearing cell also. This raises questions as to the site(s) of action of *in vivo* administered anti-I-J antisera, and studies are currently underway to independently assess potential anti-I-J activity on each of the known steps in the suppressor and helper pathways.

Summary

The *in vivo* effects of intravenous administration of alloantisera directed to I-J subregion coded determinants were investigated. In confirmation and extension of our previous results, anti-I-J^k [B10.A(3R) anti-B10.A(5R)] and anti-I-J^s ([B10.A(3R) × B10.S(9R)]F₁ anti-B10.HTT) antisera, when administered in 1 to 10 μ l amounts at the time of immunization, led to twofold increases in the IgM and IgG plaque-forming cells (PFC) responses to suboptimal doses of sheep erythrocytes in A/J (I-J^k) and SJL (I-J^s) mice, respectively. To assess whether this immunopotential was due to a decrease in specific suppression, experiments were carried out using the polypeptide antigens random linear terpolymer of L-glutamic acid⁶⁰, L-alanine³⁰, and L-tyrosine¹⁰ (GAT) and random linear copolymer of L-glutamic acid⁵⁰-L-tyrosine⁵⁰ (GT), since administration of GAT to the nonresponder strain SJL, or GT to the nonresponder strain CBA fails to induce a primary PFC response and stimulates specific suppressor T cells able to prevent PFC responses to subsequent challenge with the immunogens GAT-methylated bovine serum albumin (MBSA) or GT-MBSA, respectively. The

³ R. N. Germain. 1977. Manuscript in preparation.

current study demonstrates that CBA (I-J^k) mice given 100 μ g GT in Maalox-pertussis adjuvant on day 0, and 10 μ l anti-I-J^k antiserum i.v. on days 0, 1, and 2, develop a significant primary specific PFC response on day 7. A similar responsiveness to 10 μ g GAT is found in SJL mice treated with 10 μ l anti-I-J^s antiserum for 3 days. This same active anti-I-J^s antiserum does not permit CBA mice to respond to GT, demonstrating the specificity of the anti-I-J effect.

These data suggest that anti-I-J antiserum treatment at the time of antigen administration reduces suppressor responses to GAT or GT, permitting primary PFC responses. To directly demonstrate such an effect on suppressor activity, SJL or CBA mice treated, respectively, with GAT or GT to induce suppressor cells active on GAT-MBSA or GT-MBSA responses after adoptive transfer to normal syngeneic recipients were also given anti-I-J antisera (10 μ l/day) for 3 days, at which time their spleen cells were tested for suppressive activity upon transfer. Cells from such treated mice failed to show detectable suppressive activity upon transfer to syngeneic recipients challenged with GAT-MBSA or GT-MBSA, confirming the hypothesis of an in vivo effect of anti-I-J antiserum on suppressor activity.

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