GENETIC CONTROL OF IMMUNE RESPONSES IN VITRO

IV. CONDITIONS FOR COOPERATIVE INTERACTIONS BETWEEN NONRESPONDER PARENTAL B CELLS AND PRIMED (RESPONDER X NONRESPONDER) F1 T CELLS IN THE DEVELOPMENT OF AN ANTIBODY RESPONSE UNDER Ir GENE CONTROL IN VITRO*

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The immune response of inbred strains of mice to the random terpolymer L-glutamic acid6°-L-alanine3°-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, 2). In preceding papers, a technique was described for the detection of primary GAT-specific IgG plaque-forming cell (PFC) responses in mouse spleen cell cultures. PFC responses to soluble GAT were obtained with spleen cells from the responder strains, C57BL/6 (H-2b) and A/J (H-2a), but not from the nonresponder strains, SJL, and the congenic resistant strains B10.S and A.SW, all of which are H-2s. Spleen cells from (C57BL/6 X SJL)F1 (H-2b) mice responded to GAT as well as spleen cells from the responder parental strain (3, 4). In spite of their inability to respond to GAT, H-2s spleen cells, like H-2b spleen cells, developed GAT-specific IgG PFC responses to GAT complexed to an immunogenic carrier, methylated bovine serum albumin (GAT-MBSA), demonstrating that nonresponder H-2s B cells are capable of producing antibody specific for GAT if appropriate helper T cells are activated (3).

In further studies, the cellular requirements for the development of GAT-specific PFC responses to GAT and GAT-MBSA by spleen cells from responder

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1 Abbreviations used in this paper: B cell, precursor of antibody-producing cell; GAT, random terpolymer of L-glutamic acid50-L-alanine30-L-tyrosine10; GLT, random terpolymer of L-glutamic acid17-L-lysine9-L-tyrosine5; GAT-MBSA, GAT complexed to methylated bovine serum albumin; GAT-Mφ, GAT adsorbed to peritoneal macrophages; Ig, refers to IgG1 and IgG2a + 2b; Ir gene, specific immune response gene; Mφ, macrophages; PFC, plaque-forming cell(s); T cell, thymus-derived helper cell; β, surface antigen on T cells.
and nonresponder mice were investigated (4). Macrophages are required to support the viability of lymphoid cells in culture and to present antigen to immunocompetent cells (5). Nanogram amounts of GAT bound to nonresponder H-2* macrophages were able to elicit anti-GAT PFC responses by responder splenic lymphoid cells equally as well as GAT bound to responder macrophages, indicating that the failure of H-2* spleen cells to respond to GAT is not the result of a defect in macrophage function. In contrast, GAT bound to responder macrophages could not elicit responses in cultures of nonresponder splenic lymphoid cells, showing that the genotype of the macrophage does not determine responder-nonresponder status in mice (4). We have also demonstrated that anti-GAT responses, both to GAT and to GAT-MBSA, are abolished by treatment of spleen cells with anti-theta (θ) serum and complement (C) before culture initiation and are, therefore, thymus dependent (4). Collectively, these data strongly suggested that the defect in genetic nonresponder spleen cells is the failure of their T cells, after interaction with soluble GAT, to provide appropriate helper function for initiation of the B-cell response.

In the recent studies by Kindred and Shreffler (6) and by our laboratory (7, 8), carrier specific T cells were shown to be unable to provide helper function for histoincompatible B cells. However, sharing of a single H-2 haplotype was nevertheless sufficient for successful T- and B-cell cooperation in the response to conventional antigens such as keyhole limpet hemocyanin. Thus, for these antigens, F1 T cells were able to provide efficient helper function for B cells from both parental strains. In contrast, if the response to the carrier moiety of the antigen is under Ir gene control, carrier-primed T cells from an F1 hybrid of responder and nonresponder strains, could provide effective helper function only for responder, but not for nonresponder, parental B cells in a double adoptive transfer system in vivo (9). This finding raised intriguing questions concerning a possible role of Ir genes in B cells.

In the present study, the analysis of the cellular requirements for the development of GAT-specific PFC responses was pursued to investigate further: (a) the conditions for successful T- and B-cell interaction in vitro; and (b) whether functional differences between responder and nonresponder B cells could be detected. More specifically, a study was made of the requirements for in vitro cooperative interactions between nonresponder B10.S B cells and spleen cells from primed irradiated responder (C57BL/6 × SJL)F1 mice, as a source of T cells, in cultures to which soluble GAT, GAT-MBSA, or GAT bound to F1 macrophages was added.

**Materials and Methods**

*Mice.*—C57BL/6J (H-2^b), SJL/J (H-2^s), and DBA/1 (H-2^d) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. B10.S mice, a congenic-resistant strain of mice of the H-2^s haplotype on a C57BL/10 background were a gift from D. Shreffler, University of Michigan, Ann Arbor, Mich., and were bred in our animal facilities. F1 hybrid mice (H-2^bs) from C57BL/6 × SJL/J matings were produced in our animal facilities and have the same
H-2 haplotype as F1 mice from a C57BL/6 × B10.S (H-2^b/s) mating. Mice used in these experiments were 2- to 8-mo old and were maintained on laboratory chow and acidified chlorinated water ad libitum.

Antigens.—The synthetic linear random terpolymer GAT, mol wt 32,000, used in these studies was synthesized by Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. Preparation of GAT, GAT-MBSA, and GAT bound to (C57BL/6 × SJL)F1 peritoneal macrophages (GAT-Mφ) for addition to culture was previously described in detail (4, 5). Various amounts of soluble GAT, GAT-MBSA containing 5 μg GAT, or 7 × 10^4 macrophages bearing 1–2 ng of GAT were added to cultures according to the experimental protocol.

Spleen Cell Cultures and PFC Assay.—Techniques of spleen cell culture and the hemolytic plaque assay using GAT-SRBC as indicator cells have been described previously (3, 4).

Immunization of Mice.—(C57BL/6 × SJL)F1 mice were injected intraperitoneally with 10 μg GAT, or 10 μg GAT as GAT-MBSA, or 30 μg MBSA in a mixture of magnesium-aluminum hydroxide gel (Maalox, Wm. H. Rorer, Inc., Fort Washington, Pa.) and pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) as previously described (1, 2).

Preparation of B Cells.—B-bearing T cells were eliminated from normal spleen cell suspensions by treatment with AKR anti-θ C3H serum and C before culture initiation (4). As a control, a portion of the spleen cell suspensions were treated with C alone. After treatment, 10 × 10^6 cells in MEM were added to cultures with or without additional cells and with GAT, GAT-MBSA, or GAT-Mφ according to the experimental protocol.

Source of T Cells.—The helper cell function of antigen-primed T cells has been demonstrated to be radioresistant, whereas B cells and normal unprimed T cells are radiosensitive (10). Previous studies from our laboratories have established the conditions for using irradiated carrier-primed spleen cells as a source of helper T cells for cooperative antibody responses to soluble antigens in vitro (11). Therefore, F1 mice primed 1–4 mo earlier with GAT, GAT-MBSA, or MBSA were X irradiated with 700–800 R delivered by a General Electric Maximar 250 Type III X-ray therapy unit (250 kVp, 15 mA) at 36 R/min through 0.5 mm Cu, 1.0 mm Al filters (General Electric Co., Chemical & Medical Div., Schenectady, N. Y.). Within 3 h after X irradiation, the mice were sacrificed and single cell suspensions prepared from their spleens. 10 × 10^6 cells in MEM were added to cultures with or without additional cells and with GAT, GAT-MBSA, or GAT-Mφ according to the experimental protocol.

RESULTS

Failure of Nonresponder (H-2^b) B Cells to Develop PFC Responses to Soluble GAT In Vitro in the Presence of GAT-Primed (H-2^b/s) F1 T Cells.—In an initial series of experiments, we compared the ability of GAT-MBSA-primed irradiated (C57BL/6 × SJL)F1 spleen cells, as a source of specific T cells, to provide helper function for responder C57BL/6 or nonresponder B10.S B cells in responses to soluble GAT or GAT-MBSA. To this end, 10 × 10^6 normal C57BL/6 or B10.S B cells were mixed with an equal number of GAT-MBSA-primed irradiated (C57BL/6 × SJL)F1 spleen cells and incubated with 10 μg soluble GAT or 5 μg GAT as GAT-MBSA. The results of this experiment are shown in Fig. 1. C57BL/6 spleen cells developed GAT-specific IgG PFC responses to both soluble GAT and GAT-MBSA, whereas B10.S spleen cells developed GAT responses only to GAT-MBSA, not to GAT. Treatment with anti-θ serum and C abolished these responses by spleen cells from both B10.S and C57BL/6 mice. Antigen-primed irradiated T cells cultured alone did not respond to GAT. If, however, B10.S or C57BL/6 B cells were cultured with
irradiated GAT-MBSA-primed (C57BL/6 × SJL)F₁ spleen cells, the responses are restored; C57BL/6 B cells developed GAT responses to both GAT and GAT-MBSA, whereas B10.S B cells developed GAT-specific responses only to GAT-MBSA, not to soluble GAT.

This experiment demonstrates that B cells from nonresponder B10.S (H-2*) animals, in contrast to responder C57BL/6 (H-2b) B cells, cannot be stimulated by soluble GAT in spite of the presence of functional GAT-primed F₁ (H-2^{b') T cells bearing the H-2* haplotype of the B cell. The inability of B10.S B cells to respond to soluble GAT in this system cannot be ascribed to nonspecific suppressor activity generated in these cultures, because, as shown by the last two groups in Fig. 1, the addition of B10.S B cells to cultures of C57BL/6 B cells and the same primed F₁ T cells did not diminish the response to GAT.

Role of Macrophage Presentation of GAT in the Development of In Vitro PFC Responses by Nonresponder H-2* B Cells in the Presence of GAT-Primed H-2^{b') F₁ T cells.—Comparative studies of the susceptibility of responder C57BL/6 and nonresponder H-2* and H-2^b spleen cells to tolerance induction by GAT reported in the companion paper (12) have shown that nonresponder cells exposed to GAT in vivo or in vitro are rapidly rendered unresponsive to stimulation with GAT-MBSA, under conditions where spleen cells from responder animals develop normal GAT-specific IgG PFC responses. The possibility must
be considered, therefore, that the failure of soluble GAT to elicit responses by B10.S B cells in the presence of functional GAT-primed responder F1 T cells may reflect a greater susceptibility to tolerance induction of nonresponder B cells in the presence of what is less than optimal T-cell helper function for these B cells. This interpretation is also supported by findings that GAT presented on macrophages in very small amounts (1–2 ng) is strongly immunogenic for responder spleen cells (4) but not for nonresponder spleen cells (Table I). Furthermore, 1–2 ng of GAT in soluble form (12) or bound to macrophages, are unable to tolerize nonresponder spleen cells (Table I). As shown, the development of an immune response to GAT-MBSA by nonresponder, but not responder spleen cells was inhibited by 5 μg soluble GAT. 7 × 10^4 normal syngeneic macrophages

| ANTIGEN          | IgG GAT-specific PFC/culture |
|------------------|-------------------------------|
|                  | C57BL/6 | DBA/1      |
| 5 μg GAT         | 415     | <10        |
| GAT-MBSA         | 175     | 420        |
| GAT-MBSA + 5 μg GAT | 290  | 15         |
| GAT-MBSA + NMΦ* | 260     | 295        |
| GAT-ΜΦ*          | 410     | <10        |
| GAT-MBSA + GAT-ΜΦ* | 250  | 325        |
| GAT-ΜΦ†          | 335     |            |

* Syngeneic ΜΦ added to spleen cell cultures.
† DBA/1 GAT-ΜΦ added to C57BL/6 spleen cell cultures.

were added to one set of cultures stimulated with GAT-MBSA for comparison with cultures receiving 7 × 10^4 GAT-ΜΦ and cultures receiving GAT-MBSA plus 7 × 10^4 GAT-ΜΦ. GAT bound to macrophages did not inhibit the response to GAT-MBSA by either responder or nonresponder spleen cells. As a control for the effectiveness of GAT bound to DBA/1 macrophages, it can be seen that these cells were able to elicit a GAT-specific PFC response in cultures of C57BL/6 spleen cells but not in cultures of DBA/1 spleen cells.

Since GAT bound to macrophages was nontolerogenic for nonresponder spleen cells, experiments were carried out to ascertain whether GAT bound to macrophages from (C57BL/6 × SJL)F1 animals could stimulate GAT-specific responses in cultures of B10.S B cells and GAT-MBSA or GAT-primed F1 T cells. The results of these experiments are presented in Figs. 2 and 3. In these and all remaining experiments to be described, the same protocol used in the studies presented in Fig. 1 was employed. B-cell populations from responder C57BL/6 and nonresponder B10.S spleens were cultured with irradiated spleen cells from F1 mice primed to GAT, GAT-MBSA, or MBSA as a source of helper T cells. To these cell mixtures, antigen either in soluble form, or aggregated as GAT-MBSA, or bound to F1 macrophages was added.
The data presented in Figs. 2 and 3 demonstrate that nonresponder B10.S B cells can be induced to develop anti-GAT PFC responses in the presence of GAT-specific F1 T cells, if GAT in nanogram amounts is presented on (C57BL/6 × SJL)F1 macrophages. Similar results were obtained whether the (C57BL/6 × SJL)F1 T cells were primed to GAT-MBSA (Fig. 2) or to GAT (Fig. 3) and whether GAT was presented on (C57BL/6 × SJL)F1, C57BL/6, or SJL macrophages (not shown). As a further comparison of the ability of B10.S and C57BL/6 B cells to develop GAT-specific PFC responses when cultured with macrophage-bound GAT, these cells were incubated, in the presence of GAT-primed F1 T cells, with various numbers, 3.5 × 10³ to 7 × 10⁴, of F1 macrophages bearing GAT. Both responder and nonresponder B cells developed GAT-specific PFC responses when incubated with as few as 7 × 10⁴ GAT-bearing macrophages, whereas neither responder nor nonresponder B cells developed responses when cultured with 3.5 × 10³ macrophages bearing GAT. Thus, no difference between responder and nonresponder B cells in their ability to be stimulated by macrophage-bound GAT could be detected.
Fig. 3. The same general protocol as that in Figs. 1 and 2 was used. The source of T cells was from irradiated (C57BL/6 X SJL)F1 mice primed with GAT in Maalox-pertussis.

It is important to note that the response of B10.S B cells to macrophage-bound GAT required that responder (C57BL/6 X SJL)F1 T cells were primed to GAT. GAT-specific PFC responses could not be elicited by GAT bound to macrophages in the absence of GAT-primed responder F1 T cells. Thus, as shown in Fig. 4, when F1 T cells primed only to MBSA were used, GAT bound to macrophages did not stimulate anti-GAT PFC responses in cultures of either C57BL/6 or B10.S B cells. These cell populations responded adequately, however, to GAT-MBSA.

The addition of F1 macrophages and GAT separately to the cultures, as illustrated in Fig. 2, did not stimulate development of anti-GAT PFC responses in cultures of B10.S B cells and GAT-MBSA-primed F1 cells. Thus, F1 macrophages not bearing GAT when added to culture cannot stimulate a response, presumably because the soluble GAT tolerizes B10.S B cells before they are able to respond to any GAT which becomes bound to macrophages in culture (as will be shown in Fig. 5). In contrast, if GAT is added to the cultures as insoluble GAT-MBSA, B10.S B cells are able to cooperate effectively with GAT-primed F1 T cells and are able to develop GAT-specific PFC responses (Fig. 3) in the absence of MBSA-primed T cells. It is well established that insoluble
Fig. 4. See legend for Fig. 2 for protocol. In this experiment antigen-primed irradiated spleen cells were prepared from (C57BL/6 × SJL)F1 mice immunized with GAT-MBSA or MBSA in Maalox-pertussis. These cells cultured alone did not respond to GAT or GAT-MBSA. In this experiment 5 μg soluble GAT were added to designated cultures.

Aggregates, in contrast to soluble antigens, are very effectively taken up by macrophages (13). It is, therefore, very probable that the response of B10.S B cells observed in this experiment is explained by the presentation of GAT-MBSA on macrophages and the absence of free soluble GAT in sufficient amounts to tolerize the highly susceptible B10.S B cells.

**Inhibition of the Responses of B10.S B Cells and GAT-Primed H-2* F1 T Cells to Macrophage-Bound GAT by Soluble GAT.**—In the experiments that we have just described, nonresponder B10.S B cells were found to respond, in the presence of GAT-primed responder F1 T cells, only when GAT was bound to macrophages and not to concentrations of soluble GAT which stimulate responses by responder C57BL/6 B cells. It is relevant, therefore, to inquire what effect soluble GAT, added at the initiation of culture, had on the PFC responses stimulated by macrophage-bound GAT. The data presented in Fig. 5 demonstrate that soluble GAT almost completely inhibits the GAT-specific PFC
responses elicited by macrophage-bound GAT in cultures of B10.S B cells and GAT-MBSA-primed F1 T cells. In this experiment, the response of C57BL/6 B cells and the primed F1 T cells to macrophage-bound GAT was also inhibited somewhat by soluble GAT; the response approximated that stimulated by soluble GAT alone. However, the degree of inhibition of the anti-GAT response in cultures of C57BL/6 B cells was much smaller than that observed in cultures of B10.S B cells and was not observed in every experiment, whereas the nearly complete inhibition of anti-GAT responses by B10.S B cells by soluble GAT could always be demonstrated.

DISCUSSION

In previous reports from this laboratory, some of the genetic requirements for cooperative interactions among T and B cells in the mouse were defined (7–9). Helper T cells and B cells must share one H-2 haplotype for successful interactions to occur in the response to conventional thymus-dependent antigens and their hapten conjugates. However, when the response to the carrier moiety of the antigen is under control of a specific Ir gene, as is the response to the random terpolymer of L-glutamic acid\(^{a}\), L-lysine\(^{b}\), and L-tyrosine\(^{c}\) (GLT),
GLT-specific (responder X nonresponder)F1 T cells could provide helper function only for B cells of the responder parent bearing the relevant Ir gene for GLT in the development of a response to DNP-GLT and not for B cells of the nonresponder parent lacking the Ir gene for GLT. These experiments could be interpreted to indicate that the specific Ir genes are necessarily expressed in B cells as well as in T cells where Ir gene function has been conclusively demonstrated (14). Alternatively, the conclusion could be reached that a close spatial relationship exists between the products of the genes in the H-2 complex controlling T- and B-cell cooperation and the products of the Ir genes and, therefore, that those products of that H-2 haplotype, where the relevant Ir gene is located, are activated in T cells for optimal cooperative interaction with the corresponding products on the B cell coded for by the same haplotype (9).

The present investigations of the cellular requirements for the response to GAT in vitro have permitted an analysis of these possibilities and present an explanation for the absence of cooperative interaction between primed (responder X nonresponder)F1 T cells and nonresponder B cells in this system. The same restrictions observed in the DNP-GLT system in vivo were indeed observed with the GAT system in vitro. Nonresponder B10.S B cells did not develop GAT-specific PFC responses when exposed to soluble GAT in the presence of GAT-primed responder (C57BL/6 X SJL)F1 T cells, whereas responder C57BL/6 B cells developed excellent responses under these conditions. The failure of the B10.S B cells to respond in this system was clearly shown not to be attributable to the generation of nonspecific suppressor substances in the cultures since B10.S B cells added to the mixtures of C57BL/6 B cells and GAT-primed (C57BL/6 X SJL)F1 spleen cells did not diminish the response to GAT in these cultures.

However, B10.S B cells were shown to cooperate with GAT-primed F1 T cells and to develop a response to GAT when the polymer was presented on macrophages. This mode of presentation of antigen is required to avoid the greater tolerogenic property of soluble GAT for nonresponder (H-2b) B cells than for responder (H-2h) B cells in the presence of GAT-primed H-2N* T cells. Macrophage-bound GAT, although added to spleen cultures in nanogram amounts, was shown to be both strongly immunogenic for responder spleen cells and not capable of inducing B-cell tolerance in nonresponder cells. It is not inconsistent, therefore, that anti-GAT responses developed in cultures of nonresponder B10.S B cells and irradiated GAT-primed responder (C57BL/6 X SJL)F1 spleen cells to which GAT bound to macrophages was added, irrespective of the source of the macrophages used, responder, nonresponder, or F1. This response could be clearly inhibited if 10 μg of soluble GAT were added to the culture.

Several important issues are raised by these findings: (a) Are the results obtained in vitro with the GAT system applicable to the GLT system in vivo? Or can successful cooperation be observed in vivo between GLT-primed (re-
sponder × nonresponder)F1 T cells and nonresponder hapten-specific B cells if the hapten conjugate of GLT is administered on macrophages? This question has been asked in a double adoptive transfer experiment using irradiated F1 recipients reported elsewhere (15). Macrophages which bound DNP-GLT failed to stimulate DNP-specific nonresponder parental B cells in the presence of irradiated GLT-primed F1 T cells, although they were able to stimulate anti-DNP responses from DNP-primed parental responder B cells. Thus, confirmation of the results whereby macrophage-bound GAT could induce a response by B10.S B cells in vitro with the help of responder F1 T cells could not be duplicated in the GLT system in vivo.

(b) Why then, do (responder × nonresponder)F1 T cells provide what appears to be inadequate helper function for parental nonresponder B cells and excellent helper function for parental responder B cells when stimulated with soluble GAT compared to the adequate helper cell function for both responder and nonresponder B cells when the antigen was administered in very small amounts on macrophages or in insoluble form as GAT-MBSA that is easily bound to macrophages? Or to put it in other terms, why are nonresponder B cells rendered tolerant so much more readily than responder B cells by soluble GAT in culture or in vivo (12)? At this time, it cannot be ruled out that this difference between responder and nonresponder B cells reflects the expression of the Ir gene product in responder B cells in the same way as it was demonstrated in responder T cells (14). However, if this is the case, specific Ir genes would not be clonally expressed in B cells and, therefore, could not reasonably be concerned with specificity since all Ir genes would be expressed in each B cell. This conclusion is not consistent with the remarkable specificity exhibited by the recognition which different specific Ir genes control (14). Another hypothesis may also account for the available data. Results from several laboratories indicate that B cells are very easily rendered tolerant by multivalent antigens in the absence of activated functional helper T cells (16). Thus, hapten conjugates of heterologous carriers render hapten-specific B cells unresponsive to subsequent challenge with the conjugate used for primary immunization because of what would appear to be an insufficient number of helper T cells specific for the second carrier for the number of primed B cells when this hapten-carrier conjugate is administered (17). The data presented in the companion paper where tolerance is readily induced in nonresponder B cells in the presence of what appears to be less than adequate helper function for nonresponder B cells may be another example of the crucial role played by the signal contributed by activated T cells in preventing the induction of B-cell tolerance.

(c) If indeed this interpretation is correct, the issue to be considered, then, is why are (responder × nonresponder)F1 T cells very ineffective in providing helper function for nonresponder B cells but quite effective in providing helper function for responder B cells for antigens under Ir gene control when antigen is soluble? This state of affairs may be related to the possibility, hypothesized
previously (9), that the gene product(s) concerned with T-B-cell interaction which might be activated by these antigens in responder F1 T cells is that of the responder allele, whereas effective stimulation of the nonresponder B cell might require the activation of the allelic gene product coded for in the nonresponder $H-2$ haplotype common to the nonresponder B cell. The fact that macrophage-bound antigen is able to remedy this apparent restriction is very informative and shows that the T-cell signal which is unable to stimulate nonresponder B cells incubated with soluble GAT is received by these B cells if the GAT is presented in a nontolerogenic form on the surface of macrophages.

A fundamental difference exists, therefore, between the ability of responder and nonresponder B cells to cooperate with F1 T cells in the presence of soluble GAT. In the presence of F1 T cells, responder B cells can be stimulated by soluble GAT or GAT bound to macrophages, whereas nonresponder B cells can be stimulated only if GAT is bound to macrophages.

This raises the issue of whether antigen bound to macrophages is particularly efficient in stimulating B cells as well as T cells as compared with free soluble antigen which is much more tolerogenic. In our experiments, however, such interactions between antigen bound to macrophages and B cells did not obviate the necessity for the essential contribution of GAT-primed T cells for the development of anti-GAT responses by B10.S B cells.

Lastly, the experiments reported herein demonstrate that genetic disparity at $Ir$ loci is not an absolute barrier to T-B-cell cooperative interactions in the response to antigens under $Ir$ gene control which may indeed explain the observations of Bechtol et al. (18), that allophenic mice derived from responder and nonresponder strains may produce antibodies of nonresponder allotype under conditions of hyperimmunization.

**SUMMARY**

The conditions for cooperative interactions between nonresponder B10.S B cells and GAT-primed irradiated (C57BL/6 X SJL)$F_1$ T cells in the response by cultures of mouse spleen cells to GAT were investigated. GAT-specific antibody responses could be elicited by soluble GAT in cultures of GAT-primed irradiated (C57BL/6 X SJL)$F_1$ T cells with C57BL/6 B cells but not with B10.S B cells.

In contrast, when GAT was presented to the cultures on F1 macrophages or as aggregates of GAT with MBSA, GAT-specific PFC responses were observed with both B10.S or C57BL/6 B cells. Irradiated GAT-primed T cells were nevertheless essential for the development of these responses. The GAT-specific response of B10.S B cells in these cultures was inhibited by the addition of soluble GAT at culture initiation. These results indicate that genetic disparity at $Ir$ loci is not an absolute barrier to T-B-cell cooperative interactions in the response to antigens under $Ir$ gene control. The significance of these data for the function of $Ir$ gene products in immunocompetent cells is discussed.

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