GENETIC CONTROL OF IMMUNE RESPONSES IN VITRO*

II. CELLULAR REQUIREMENTS FOR THE DEVELOPMENT OF PRIMARY PLAQUE-FORMING CELL RESPONSES TO THE RANDOM TERPOLYMER L-GLUTAMIC ACID^{60}-L-ALANINE^{30}-L-TYROSINE^{10} (GAT) BY MOUSE SPLEEN CELLS IN VITRO*

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The immune response by inbred strains of mice to the random terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT)[1] is controlled by an immune response (Ir) gene(s) which maps within the *H-2* complex between the genes controlling expression of the major histocompatibility antigens, *H-2* (1–3). In the preceding paper (4), we demonstrated that IgG GAT-specific plaque-forming cell (PFC) responses developed in cultures of spleen cells from responder mice, C57Bl/6 (*H-2^b*), F$_1$(C57 × SJL) (*H-2$^{b/s}$*), and A/J (*H-2^a*), incubated for 5 days with 1–10 μg soluble GAT. No PFC specific for GAT developed in cultures of spleen cells from nonresponder mice, SJL (*H-2^s*), B10.S (*H-2^s*), and A.SW (*H-2^s*), after incubation with soluble GAT. However, cultures of spleen cells from both responder and nonresponder strains of mice developed IgG GAT-specific PFC responses after incubation with GAT complexed to methylated bovine serum albumin, an immunogenic carrier in *H-2^s* mice, (GAT-MBSA).

Since these data correlated precisely with data obtained by measuring the in vivo responses of mice to GAT and GAT-MBSA by serological techniques

(1, 2, 5, 6), we feel this in vitro system can effectively be used as a model to investigate the mechanism(s) by which histocompatibility-linked Ir genes regulate the antibody response. Data obtained with both in vivo (5, 6) and in vitro (4) experimental systems have demonstrated that nonresponder mice, like responder mice, have precursors of antibody-producing cells (B cells) capable of synthesizing GAT-specific antibodies if the appropriate thymus-derived cell (T cell) helper function is provided. These observations indicate that the genetic defect or the cell in which the Ir gene function is necessarily expressed is not the B cell. In addition to B cells, two accessory cells, the nonspecific macrophage, and the antigen-specific T cell, are also required for the development of primary PFC responses in vitro to most complex, multi-determinant antigens, such as sheep red blood cells (SRBC) (7) or hapten conjugates (8). In this communication, we describe experiments investigating the cellular requirements for the development of GAT-specific PFC responses to GAT and GAT-MBSA by spleen cells from responder and nonresponder mice in vitro.

Materials and Methods

Mice.—C57Bl/6J (H-2^b), SJL/J (H-2^s), AKR/J (H-2^k), and C3H/HeJ (H-2^k) mice were purchased from Jackson Laboratories, Bar Harbor, Me., B10.S (H-2^s) mice were a gift from Dr. D. Shreffler, University of Michigan and have been bred in our animal facilities. Mice used in these experiments were from 2 to 8-mo old and were maintained on acidified-chlorinated water and laboratory chow ad lib.

Antigens.—Two preparations of GAT were used in these studies: the first, molecular weight 55,000, was purchased from Pilot Chemicals, Division of New England Nuclear Corp., Boston, Mass.; the second, molecular weight 32,000, was purchased from Miles Laboratory, Kankakee, Ill. Preparations of the solutions of GAT, GAT complexed to MBSA, and SRBC for addition to spleen cell cultures were described in detail in the preceding paper (4).

Preparation of GAT-SRBC.—GAT was coupled to SRBC for use as indicator cells in the hemolytic plaque assay as described in the preceding paper (4).

Anti-Theta Serum.—Anti-theta serum was prepared in AKR/J mice by multiple injections of C3H/HeJ thymus cells according to the method of Raff (9) with modifications described previously (10). The serum used in these experiments had no cytotoxic activity against AKR/J thymus cells when tested by ^{51}Cr release assay (10). This serum was cytotoxic for 85–90% of thymus cells from C57Bl/6, B10.S, and SJL mice at final dilutions up to 1/120 in the presence of guinea pig serum (BBL, Division of BioQuest, Cockeysville, Md.) as a source of complement (C). At these same dilutions, this serum was cytotoxic for 20–27% of spleen cells from these strains of mice.

Immunization of Mice.—Mice were immunized with 10 μg GAT or 10 μg GAT as GAT-MBSA as a suspension in a mixture of magnesium-aluminum hydroxide gels (Maalox, Wm. H. Rorer, Inc., Fort Washington, Pa.) and pertussis vaccine (Eli Lilly Co., Indianapolis, Ind.) as previously described (1).

Spleen Cell Cultures.—Spleen cells or fractionated spleen cell populations as suspensions of dispersed single cells were incubated at a density of 10 or 20 × 10^6 cells/ml in completely supplemented Eagle's minimum essential medium (MEM) according to the method of Mishell and Dutton (11) with modification previously described in detail (12). Experimental groups were duplicate cultures and each experiment contained cultures which were unstimulated or stimulated with 10^7 SRBC, 10 μg GAT, or 2.5–5.0 μg GAT as GAT-MBSA. PFC responses were assayed after 5 days incubation.

Cell Separation Techniques.—Suspensions of spleen cells were separated into adherent (mostly macrophages) and nonadherent (mostly lymphoid cells) populations by passage over plastic petri dishes according to the technique of Mosier (13) with modifications previously described (7). Briefly, 10×10^6 spleen cells in 1 ml of MEM were incubated for $1-1\frac{1}{2}$ h in 35 mm culture dishes at 37°C on a level stationary platform. The nonadherent cells were then gently resuspended and transferred to new culture dishes. After two additional incubation periods and transfers, nonadherent cells were collected by centrifugation and resuspended in MEM at 10×10^6 cells/ml. Cells adhering to the culture dishes during the first incubation period were washed extensively with Hanks' balanced salt solution (HBSS) to remove loosely adhering cells. The adherent and nonadherent cells were incubated with antigen either separately or after recombination.

Theta-bearing cells (T cells) were eliminated from spleen cell suspensions by treatment with AKR anti-theta and C. 0.3 ml of anti-theta serum per 100×10^6 cells was reacted with spleen cells at 4°C for 30 min. The spleen cells were then diluted with 50 ml of HBSS, collected by centrifugation and resuspended in guinea pig serum, as a source of C. The guinea pig serum, previously absorbed with normal C57Bl/6 spleen cells to remove nonspecific cytotoxicity, was diluted 1:3 in Medium L-15 (Microbiological Associated, Bethesda, Md.) containing 10 μg/ml deoxyribonuclease (Worthington Biochemical Corp., Freehold, N. J.) and 4 ml added per 100×10^6 spleen cells. After incubation at 37°C for 45 min, the cells were washed twice by centrifugation with 50 ml of HBSS and resuspended in MEM at 20×10^6 cells/ml MEM. 10×10^6 cells in 0.5 ml MEM were added to cultures with or without additional cells and antigen according to the experimental protocol.

Source of T Cells.—Antigen-primed mouse T cells are radioresistant in vivo whereas unprimed T cells and B cells are radiosensitive (14). Mice primed with 10 μg GAT or 10 μg GAT as GAT-MBSA in Maalox-pertussis 1-4 mo earlier were X-irradiated with 700 to 800 R delivered by a General Electric Maximar 250 Type III X-ray therapy unit (General Electric Co., Schenectady, N. Y.) (250 kVP, 15 mA) at 36 R/min through 0.5 mm Cu, 1.0 mm Al filters. Within 3 h of X-irradiation, single cell suspensions were prepared from the spleens of these mice and adjusted to 20×10^6 cells/ml in MEM. 10×10^6 cells in 0.5 ml MEM were added to cultures with or without additional cells and antigen according to the experimental protocol.

Peritoneal Exudate Cells.—Mice were injected intraperitoneally with 1.0 ml of sterile 10% proteose peptone broth (Difco Laboratories, Detroit, Mich.) 3 days before sacrifice. Mice were sacrificed by cervical dislocation and 3 ml HBSS containing 10 U/ml heparin was introduced aseptically into the peritoneal cavity. The cells were recovered by gentle aspiration of the HBSS and were washed four times by centrifugation. This procedure routinely yielded about $8-15 \times 10^6$ cells/mouse, approximately 85% of which were morphologically typical macrophages (15).

Preparation of Macrophage Bound GAT.—The preparation of GAT-macrophages was modified from the procedure of Katz and Unanue (15). Peritoneal exudate macrophages (2 ml containing 2×10^6 cells/ml MEM) from C57Bl/6 or SJL mice were reacted with 2.0, 20.0, or 200.0 μg GAT containing a small, known amount of GAT labeled with ^{125}I by a chloramine T method (16), (total volume of 2.2 ml) for 60 min at 4°C. The cells were then washed four times by centrifugation with 50 ml of HBSS at 4°C. The cells were resuspended to 7×10^5 cells/ml in MEM. The radioactivity in an aliquot of each macrophage preparation was counted in a Packard scintillation counter (Packard Instrument Co., Downers Grove, Ill.) and the amount of GAT bound to the macrophages was calculated. 7×10^4 macrophages in 0.1 ml MEM were added to cultures according to the experimental protocol. Under these conditions, the uptake of GAT was linearly related to the amount of GAT added to the reaction mixture. Macrophages used in these studies were exposed to 200 μg GAT and the average uptake by 7×10^4 macrophages was 1.2 ng GAT by C57Bl/6 macrophages and 1.6 ng GAT by SJL.

The solution containing 200 μg GAT was not depleted of absorbable GAT after incubation with 2 × 10⁶ macrophages. 2 × 10⁶ macrophages were incubated with the supernate from the first absorption and the process repeated a third time. The quantity of GAT absorbed by each of these preparations of macrophages was approximately equal.

Hemolytic Plaque Assay.—IgM and IgG PFC in spleen cell cultures were enumerated by a modification of the Jerne hemolytic plaque technique described previously (12). Spleen cell suspensions, after harvest from culture, were washed three times by centrifugation to remove any soluble GAT. Each spleen cell suspension was examined for IgM and IgG PFC against SRBC and GAT-SRBC indicator cells. The specificity of PFC on GAT-SRBC for GAT was verified by adding 10 μg of soluble GAT to the assay mixtures to inhibit anti-GAT PFC. GAT-specific PFC were calculated by subtracting the number of PFC uninhibited by soluble GAT on GAT-SRBC from the number of PFC detected on GAT-SRBC in the absence of soluble GAT. Data are expressed as GAT-specific PFC/culture.

RESULTS

Macrophage Requirement for Development of Primary PFC Responses to GAT In Vitro.—Spleen cells from responder, C57Bl/6, mice and nonresponder, SJL, mice were separated into adherent (macrophages) and nonadherent (lymphoid cells) populations. Cultures of unfractionated spleen cells, macrophages, nonadherent cells, and these latter two populations after recombination were stimulated with 10⁷ SRBC, 10 μg GAT, or 5 μg GAT as GAT-MBSA (Fig. 1). Although not shown here, but demonstrated in other reports (7, 12), the development of anti-SRBC PFC responses in cultures of nonadherent cells from both C57Bl/6 and SJL mice required macrophages. As shown, the development of primary IgG GAT-specific PFC responses by cultures of nonadherent cells from C57Bl/6 mice stimulated with GAT and GAT-MBSA and by cultures of nonadherent cells from SJL mice stimulated with GAT-MBSA required macrophages.

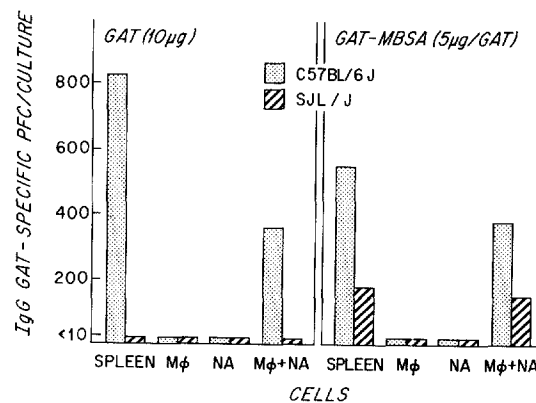
FIG. 1. Macrophage requirement for the development of GAT-specific PFC responses in cultures of spleen cells from C57Bl/6 mice stimulated with 10 μg/culture GAT or 5.0 μg/culture of GAT as GAT-MBSA and in cultures of spleen cells from SJL mice stimulated with 5.0 μg/culture of GAT as GAT-MBSA.

Ability of Allogeneic Macrophages to Support Development of Primary PFC Responses to GAT In Vitro.—Cosenza and Leserman have previously shown that macrophages will support the development of PFC responses by histo-incompatible nonadherent cells stimulated in vitro with SRBC (17). In the next experiment (Fig. 2), we compared the PFC responses developed by nonadherent cells incubated with allogeneic macrophages to those which developed with syngeneic macrophages. Macrophages and nonadherent cells in this experiment were from the same preparations of cells used in the experiment described in Fig. 1.

SJL macrophages supported not only development of anti-SRBC PFC responses, but also development of IgG GAT-specific PFC responses by C57Bl/6
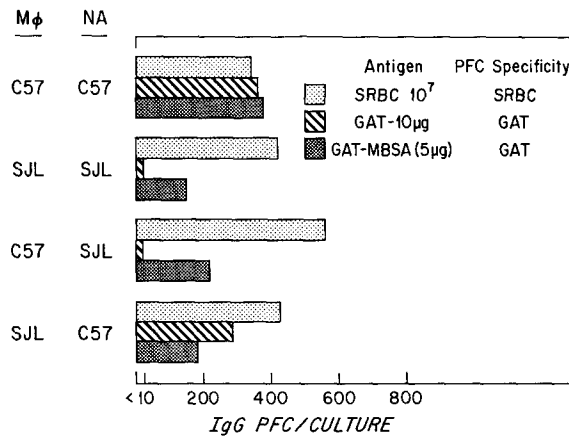

FIG. 2. Comparison of the ability of syngeneic and allogeneic macrophages to support the development of PFC responses by nonadherent spleen cells stimulated with 10^7 SRBC, 10 µg/culture GAT, or 5.0 µg/culture GAT as GAT-MBSA. The macrophages and nonadherent cells used in this experiment were from the same preparations as those used in the experiment described in Fig. 1.

nonadherent cells incubated with GAT or GAT-MBSA. This demonstrates that macrophages from nonresponder mice are not defective with regard to their ability to support responses to soluble GAT. C57Bl/6 macrophages supported development of PFC responses by SJL nonadherent cells to SRBC and GAT-MBSA, but not to soluble GAT. Thus, the ability to develop a GAT-specific PFC response after stimulation with soluble GAT is determined by the genotype of the nonadherent cells, not the macrophages.

Ability of GAT Bound to Macrophages to Support Development of Primary PFC Responses In Vitro.—The elimination of macrophages from nonadherent cell populations by adherence techniques is not absolute. In the preceding experiment, it is possible to argue that SJL macrophages support the viability and survival of the C57Bl/6 nonadherent cells, but that it is the possible con-

taminating C57Bl/6 macrophages which actually interact with and present GAT in a manner which stimulates development of GAT-specific PFC response by the C57Bl/6 nonadherent cells.

We have carried out a series of experiments demonstrating that SJL macrophages are, indeed, able to support the development of IgG GAT-specific PFC responses by C57Bl/6 nonadherent cells. Small amounts of antigen bound to peritoneal exudate macrophages can effectively stimulate development of PFC responses in cultures of spleen cells from mice previously immunized with the antigen (15). Peritoneal exudate macrophages from responder and nonresponder mice were reacted with GAT as described in Methods and the amount of GAT associated with the macrophages was determined. C57Bl/6 macrophages bearing 0.4 ng GAT and SJL macrophages bearing 0.9 ng GAT were added to cultures of nonadherent cells from unprimed C57Bl/6 mice without additional soluble GAT. Soluble GAT, 10 μg, 0.4 ng, or 0.9 ng, was added to cultures of C57Bl/6 or SJL macrophages and C56Bl/6 nonadherent cells as indicated in Fig. 3. The subnanogram quantities of GAT associated with either C57Bl/6 or SJL macrophages stimulated GAT-specific PFC responses by C57Bl/6 nonadherent cells which approximated the responses in cultures to which 10 μg soluble GAT was added. When the equivalent subnanogram quantities of GAT which were associated with macrophages were added to cultures as soluble GAT, no significant PFC response developed.

T Cell Requirement for Development of GAT-Specific PFC Responses In Vitro.—The T cell requirement for the development of GAT-specific PFC responses in cultures of spleen cells from responder and nonresponder mice was determined by depletion of theta-bearing cells from the spleen cell suspensions. Spleen cells were incubated with anti-theta serum and C before culture
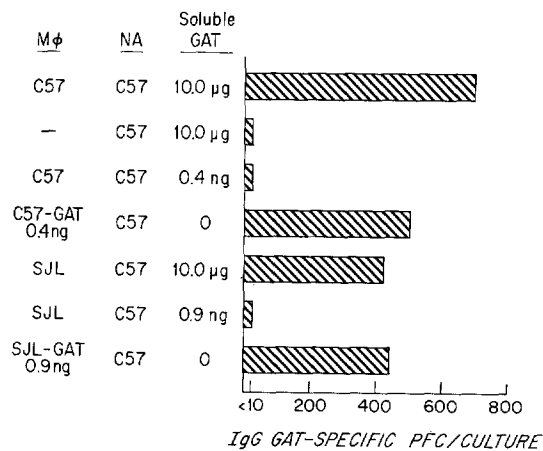

FIG. 3. Comparison of the ability of GAT bound syngeneic and allogeneic macrophages to support the development of GAT-specific PFC responses by cultures of nonadherent spleen cells from responder, C57Bl/6, mice in the absence of free soluble GAT.

initiation. In addition, 10×10^6 spleen cells from mice which had been primed with GAT or GAT-MBSA in Maalox-pertussis 1–4 mo earlier and X-irradiated (720 R) before sacrifice were incubated with antigen either alone or in combination with 10×10^6 anti-theta-treated spleen cells. Spleen cells from C57Bl/6 mice treated with anti-theta serum and C failed to develop PFC responses to SRBC, GAT and GAT-MBSA (Fig. 4). Spleen cells from X-irradiated, GAT or GAT-MBSA primed C57Bl/6 mice also failed to develop GAT-specific PFC responses when incubated alone with GAT-MBSA. However, when these spleen cells were added to cultures of anti-theta-treated C57Bl/6 spleen
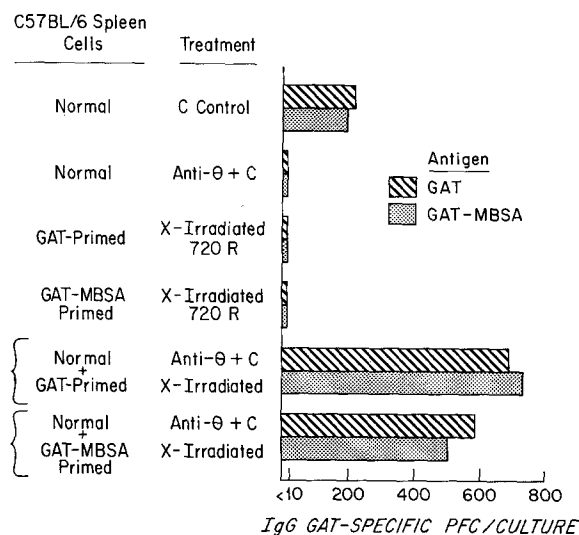

FIG. 4. Requirement for thymus-derived helper cells for the development of GAT-specific PFC responses by cultures of spleen cells from responder, C57Bl/6, mice to 10 μg/culture GAT or 5.0 μg/culture GAT as GAT-MBSA.

cells the GAT-specific PFC responses to GAT and GAT-MBSA were fully reconstituted. The same experiment was performed with spleen cells from normal and primed nonresponder B10.S mice which differ from C57Bl/6 mice only at the *H-2* complex (Fig. 5). The results in Fig. 5 demonstrate that the development of GAT-specific PFC responses by spleen cells from nonresponder mice stimulated with GAT-MBSA is also T cell dependent. Helper T cell function in the response to GAT-MBSA was restored by spleen cells from X-irradiated B10.S mice primed with GAT-MBSA but not by spleen cells from X-irradiated B10.S mice primed with GAT.

DISCUSSION

These experiments demonstrate that macrophages are required for the development of primary GAT-specific PFC responses in vitro by nonadherent spleen cells from responder mice stimulated with GAT or GAT-MBSA and by

BIO.S Spleen
Cells   Treatment

Normal   C Control

Normal   Anti-θ + C

GAT Primed   X-Irradiated
               720 R

GAT-MBSA   X-Irradiated
Primed   720 R

⎰ Normal   Anti-θ + C
⎱ GAT Primed   X-Irradiated

⎰ Normal   Anti-θ + C
⎱ GAT-MBSA   X-Irradiated
  Primed

Antigen
▧ GAT
▦ GAT-MBSA

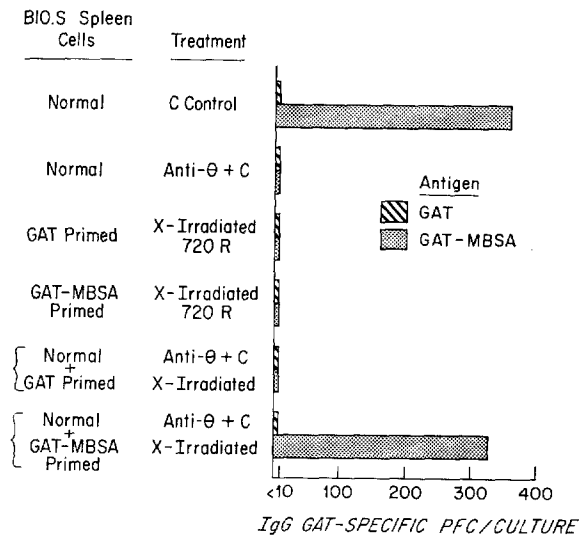<10   100   200   300   400

IgG GAT-SPECIFIC PFC/CULTURE

FIG. 5. Requirement for thymus-derived helper cells for the development of GAT-specific PFC responses by cultures of spleen cells from nonresponder, B10.S, mice to 5.0 μg/culture GAT as GAT-MBSA. The data in this graph represent the arithmetic mean of two separate experiments.

nonadherent spleen cells from nonresponder mice stimulated with GAT-MBSA. Macrophages from responder, C57Bl/6, mice and from nonresponder, SJL, mice supported equally the development of PFC responses by allogeneic nonadherent spleen cells in cultures stimulated with SRBC and GAT-MBSA. However, macrophages from C57Bl/6 mice did not confer the ability to develop GAT-specific PFC responses to nonadherent nonresponder spleen cells in cultures stimulated with GAT. Thus, the responder status to GAT is determined by the genotype of the nonadherent cells, not by the genotype of the macrophages. Equally important is the observation that nonresponder macrophages are not defective in their ability to support development of GAT-specific PFC responses by nonadherent responder spleen cells in cultures stimulated with GAT. Not only did nonresponder macrophages support development of GAT-specific PFC responses by nonadherent responder spleen cells in cultures stimulated with soluble GAT but, like responder macrophages, they stimulated comparable responses by nonadherent responder spleen cells when the only GAT in the cultures was provided in subnanogram amounts bound to the macrophages. Thus, macrophage function with regard to GAT is the same in cultures of spleen cells from responder and nonresponder mice and the macrophage is not responsible for the failure of nonresponder spleen cells to develop PFC responses to soluble GAT. One additional conclusion can be drawn from these data. That is: in the primary PFC response to soluble antigens in vitro, one function of macrophages is definitely antigen presentation. Our results and the earlier experiments

of Katz and Unanue (15) are in apparent contradiction with the recent findings of Rosenthal and Shevach[2] that in guinea pigs, antigen-bearing histoincompatible macrophages are much less efficient in stimulating DNA synthesis by sensitized T cells than syngeneic or semi-syngeneic macrophages. This discrepancy could be attributed to a species difference in macrophage function or to the possibility that specific helper T cells are not the limiting factor in antibody responses in vitro. According to this concept, a sufficient number of helper T cells could be stimulated by allogeneic macrophages. This issue can be resolved experimentally and does not invalidate our conclusion that the Ir gene need not be expressed in macrophages.

We have also shown that treatment of spleen cells from responder mice with anti-theta serum and C before culture initiation abolishes the development of PFC responses to SRBC, GAT, and GAT-MBSA. Similar treatment of spleen cells from nonresponder mice abolishes the development of PFC responses in cultures stimulated with SRBC and GAT-MBSA. Spleen cells from responder mice which had been primed with GAT or GAT-MBSA and X-irradiated before addition to the cultures reconstituted GAT-specific PFC responses to both GAT and GAT-MBSA by anti-theta-treated spleen cells from responder mice. Similarly, spleen cells from X-irradiated B10.S mice previously primed with GAT-MBSA, but not those primed with GAT, reconstituted GAT-specific PFC responses to GAT-MBSA, but not GAT, by anti-theta-treated B10.S spleen cells. Thus, the development of GAT-specific PFC responses in spleen cell cultures are dependent upon the presence of thymus-derived "helper" cells expressing the relevant Ir gene.

Macrophages from nonresponder mice can bind soluble GAT and stimulate nonadherent cells from responder mice to develop GAT-specific PFC responses. Precursors of antibody-producing cells from nonresponder mice can be stimulated to synthesize GAT-specific antibody under the appropriate experimental circumstances. The development of GAT-specific PFC responses is dependent upon thymus-derived cells. These pieces of information eliminate macrophages and B cells as the site of the defect in genetic nonresponder mice and establish that nonresponder mice lack GAT-specific thymus-derived "helper" cell function. These experiments, however, do not rule out the possibility that in responder animals the Ir gene products need to be expressed also in B cells for physiological T and B cell co-operation.

A direct test of the issue would be to demonstrate that responder T cells, in the presence of GAT, can stimulate development of GAT-specific PFC responses by nonresponder B cells. This question has been investigated in vivo by one of us (B. Benacerraf) in collaboration with Doctors Katz, Hamaoka, Dorf, and Maurer (18)

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[2] Rosenthal, A. S., and E. M. Shevach. The function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. Manuscript submitted for publication.

in another genetically controlled response. The experimental system utilized an immune response gene controlling antibody responses to the random terpolymer of L-glutamic acid-L-lysine-L-tyrosine (GLT) and its hapten-conjugates to which A/J mice (H-2^a) are nonresponders and BALB/c (H-2^d) and (BALB/c $\times$ A)F_1 (CAF$_1$) mice are responders. These studies demonstrated that GLT primed T cells from CAF$_1$ mice can provide helper function for DNP-specific responder BALB/c B cells, but not for nonresponder A/J B cells, in the development of secondary anti-DNP responses to the DNP conjugate of GLT. Preliminary experiments using the immune response to GAT in vitro support these observations, and will be the subject of a separate communication. These data might, at first glance, be interpreted to indicate the need for both T and B cells to express the relevant, specific Ir gene. However, these results must be interpreted in light of other observations demonstrating the importance of the H-2 complex in T and B cell interactions (19). It has, indeed, been established that T and B cells are required to share a common H-2 allele for efficient physiological co-operation. If the Ir gene product in T cells is concerned with antigen presentation to B cells, and if effective T and B cell interactions involve the histocompatibility molecules of the H-2 complex, then the data discussed above simply indicate that an Ir gene product expressed in F_1 T cells dictates the H-2 allele required for effective co-operation and that the Ir gene product and the histocompatibility molecules coded for on the same H-2 allele are expressed in close relationships on the cell surface of thymus-derived cells. This interpretation is compatible with the report that in vitro blast transformation of sensitized guinea pig T cells by antigens under Ir gene control are inhibited by alloantisera specific for histocompatibility antigens (20, 21).

The in vitro system described in these papers can be used effectively to resolve these issues and more specifically to study the effects of alloantisera directed against products coded for in the Ir region on immune responses under the control of histocompatibility-linked Ir genes.

SUMMARY

The cellular requirements for the development of primary IgG GAT-specific PFC responses in cultures of spleen cells from responder, C57Bl/6, mice stimulated with GAT and GAT-MBSA and in cultures of spleen cells from nonresponder, SJL and B10.S, mice stimulated with GAT-MBSA were investigated. Macrophages were required for development of responses to GAT and GAT-MBSA in cultures of spleen cells from responder mice and for responses to GAT-MBSA in cultures of spleen cells from nonresponder mice. Macrophages from nonresponder mice supported the development of responses to GAT by nonadherent responder spleen cells, indicating that the failure of nonresponder mice to respond to GAT is not due to a macrophage defect. Furthermore, responder macrophages supported the responses of nonadherent, nonresponder spleen cells to SRBC and GAT-MBSA, but not to GAT. This indicates that the capacity to respond to GAT is a function of the nonadherent population which is composed of thymus-derived (T) helper cells and precursors of antibody-

producing cells. Treatment of spleen cells with anti-theta serum and comple-
ment before culture initiation abolished PFC responses to GAT and GAT-
MBSA thus establishing the requirement for T cells in the development of PFC
responses to these antigens.

Since precursors of antibody-producing cells in nonresponder mice are capable
of synthesizing antibody specific for GAT after stimulation with GAT-MBSA
and since the response to GAT is thymus-dependent, it appears that non-
responder mice lack GAT-specific helper T cell function.

REFERENCES

1. Martin, J. W., P. H. Maurer, and B. Benacerraf. 1971. Genetic control of im-
mune responsiveness to a glutamic acid, alanine, tyrosine copolymer in mice.
J. Immunol. **107**:715.

2. Merryman, C. F., and P. H. Maurer. 1972. Genetic control of immune responses
to glutamic acid, alanine, tyrosine copolymers in mice. I. Association of re-
sponsiveness to H-2 genotype and specificity of the response. *J. Immunol.*
108:135.

3. Dunham, E. K., M. E. Dorf, D. C. Shreffler, and B. Benacerraf. Mapping the
H-2-linked genes governing respectively the immune responses to a glutamic
acid-alanine-tyrosine copolymer and limiting doses of ovalbumin. *J. Immunol.*
In press.

4. Kapp, J. A., C. W. Pierce, and B. Benacerraf. Genetic control of immune re-
sponses in vitro. I. Development of primary and secondary plaque-forming cell
responses to the random terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}
(GAT) by mouse spleen cells in vitro. **138**:1107.

5. Dunham, E. K., E. R. Unanue, and B. Benacerraf. 1972. Antigen-binding and
capping by lymphocytes of genetic nonresponder mice. *J. Exp. Med.* **136**:403.

6. Gershon, R. K., P. H. Maurer, and C. F. Merryman. 1973. A cellular basis for
genetically controlled immunologic-unresponsiveness in mice: Tolerance in-
duction in T cells. *Proc. Natl. Acad. Sci. U.S.A.* **70**:250.

7. Pierce, C. W. Immune responses *in vitro*. VI. Cell interactions in the development
of primary IgM, IgG and IgA plaque-forming cell responses in vitro. *Cell.
Immunol.* In press.

8. Bluestein, H. G., and C. W. Pierce. 1973. Cellular requirements for development
of primary anti-hapten antibody responses *in vitro*. *J. Immunol.* **3**:137.

9. Raff, M. C. 1969. Theta isoantigen as a marker of thymus-derived lymphocytes
in mice. *Nature (Lond.).* **224**:378.

10. Pierce, C. W., S. M. Solliday, and R. Asofsky. 1972. Immune responses in vitro.
IV. Suppression of primary γM, γG, and γA plaque-forming cell responses in
mouse spleen cell cultures by class-specific antibody to mouse immunoglobulins.
J. Exp. Med. **135**:675.

11. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell
cultures from normal mice. *J. Exp. Med.* **126**:423.

12. Pierce, C. W., B. M. Johnson, H. E. Gershon, and R. Asofsky. 1971. Immune responses in vitro. III. Development of primary γM, γG, and γA plaque-forming cell responses in mouse spleen cell cultures stimulated with heterologous erythrocytes. *J. Exp. Med.* **134:**395.

13. Mosier, D. E. 1967. A requirement for two cell types for antibody formation *in vitro*. *Science (Wash. D.C.).* **158:**1575.

14. Katz, D., and B. Benacerraf. 1972. Regulatory influences of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15:**1.

15. Katz, D. H., and E. R. Unanue. 1973. Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. *J. Exp. Med.* **137:**967.

16. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. *Biochem. J.* **89:**114.

17. Cosenza, H., and L. Leserman. 1972. Cell interactions *in vitro*. I. Role of the third cell in the *in vitro* response of spleen cells to erythrocyte antigens. *J. Immunol.* **108:**418.

18. Katz, D. H., T. Hamaoka, M. E. Dorf, P. H. Maurer, and B. Benacerraf. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (Ir) gene in the control of lymphocyte interactions in responses controlled by the gene. *J. Exp. Med.* **138:**734.

19. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. III. Demonstration that the H-2 gene complex determines successful physiologic lymphocyte interactions. *Proc. Natl. Acad. Sci. U.S.A.* **70.** In press.

20. Shevach, E. M., W. E. Paul, and I. Green. 1972. Histocompatibility-linked immune response gene function in guinea pigs. Specific inhibition of antigen induced lymphocyte proliferation by alloantisera. *J. Exp. Med.* **136:**1207.

21. Bluestein, H. G. 1973. Specific suppression of GA responses in lymphocytes from Hartley guinea pigs by an anti-strain 2 alloantiserum. *Fed. Proc. Abst.* **32:**985.