ANTIGEN-SPECIFIC SUPPRESSOR T-CELL ACTIVITY IN GENETICALLY RESTRICTED IMMUNE SPLEEN CELLS*

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Splenic lymphocytes from responder mice that are immune to the random terpolymer of L-glutamic acid-L-alanine-L-tyrosine (GAT), an antigen under H-2-linked immune response (Ir) gene control, develop secondary plaque-forming cell (PFC) responses preferentially when stimulated in vitro with GAT-bearing macrophages (Mφ) syngeneic to the Mφ used to present GAT to the spleen cells during the immunization process in vivo (1–4). This preference or genetic restriction has been demonstrated with spleen cells from mice which are responders to GAT and which have been immunized with either syngeneic or allogeneic responder or nonresponder GAT-Mφ (2, 3). These restrictions are antigen-specific, operate at the level of interactions between GAT-Mφ and the immune helper T cell, and are mediated by products of the I-A subregion of the H-2 complex (4). Similar genetic restrictions have been observed in Mφ-T-cell interactions in other responses, such as the generation and functional expression of carrier-specific murine helper T cells in vitro (5), DNA synthetic responses to antigen by immune guinea pig (6) and murine (7) T lymphocytes, and the expression of delayed hypersensitivity reactions in mice (8). Primary IgG PFC responses to GAT are not governed by restrictions of Mφ-T-cell interactions (2, 3), however, genetic restrictions have been noted in primary IgM responses to other antigens under H-2-linked Ir gene control, such as poly-L-tyrosine, glutamic acid)-poly-D,L-alanine-poly-L-lysine (T,G)-A-L (9).

Although lymphoid cells from mice immune to GAT develop secondary PFC responses to GAT-Mφ syngeneic with the Mφ used for in vivo immunization, minimal PFC responses develop when these immune lymphoid cells are stimulated by GAT-Mφ allogeneic to those used for immunization. This is most apparent when C57BL/10 mice are immunized in vivo with allogeneic DBA/1 GAT-Mφ and develop secondary PFC responses in vitro when stimulated with DBA/1 GAT-Mφ, but fail to develop a significant response when stimulated with syngeneic C57BL/10 GAT-Mφ. It is unlikely that this failure to respond to syngeneic GAT-Mφ is due to an absence of virgin unrestricted GAT-specific helper T cells in spleens of these mice 2–8 wk after immunization.

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Abbreviations used in this paper: C, complement; GAT, random terpolymer of L-glutamic acid-L-alanine-L-tyrosine, Ig, immunoglobulin; Ir gene, immune response gene; Mφ, macrophage(s); PFC, plaque-forming cell(s); SRBC, sheep erythrocytes; (T,G)-A-L, poly-L-tyrosine, glutamic acid)-poly-D,L-alanine-poly-L-lysine; TNP, trinitrophenol.

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a single immunization with \(3 \times 10^8\) allogeneic GAT-Mq\(^+\) bearing \(100-150\) ng GAT. Thus, we investigated whether suppressor cells are present in these genetically restricted immune lymphoid cells and whether these suppressor cells may account for the failure of immune spleen cells to develop primary PFC responses to GAT-Mq\(^+\) allogeneic to the Mq\(^+\) used for the initial immunization in vivo. These investigations are the subject of this communication.

Materials and Methods

**Mice.** C57BL/10 (H-2b), C3H/He (H-2c), B10.D2 (H-2d), DBA/2 (H-2b), and DBA/1 (H-2b) mice were bred in the Animal Facility in the Yale Medical School at The Jewish Hospital of St. Louis. All mice were maintained on laboratory chow and water ad libitum and used at 10-20 wk old. In a single experiment, mice were sex- and age-matched. DBA/1 mice are nonresponders to GAT; all other mice are responder strains.

**Antigens.** GAT (Miles Laboratories Inc., Elkhart, Ind.; lot 4, mol wt \(45,000\)) was prepared as previously described for use as antigen in culture (10), for preparing GAT-Mq\(^+\) (2), and for coupling to sheep erythrocytes (SRBC) for use as indicator cells in the PFC assay (10). SRBC (Grand Island Biological Co., Grand Island, N. Y.) were prepared for use as antigen in culture and indicator cells in the PFC assay as previously described (11).

**Culture System and Hemolytic Plaque Assay.** Single-cell suspensions of spleen, at \(10^7\) cells/ml (or at other cell densities as indicated), in completely supplemented Eagle's minimal essential medium containing 10% fetal calf serum (Reheis Chemical Co., Division of Armour Pharmaceutical Co., Chicago, Ill.; lot M26362) were incubated as 1-ml cultures with GAT, GAT-Mq\(^+\), or SRBC for 5 days under modified Mishell-Dutton conditions (2, 11). Deviations from this protocol and separation and treatment of Mq\(^+\), T-cell, and B-cell populations are described below. IgM and IgG PFC responses to SRBC and IgG GAT-specific PFC responses were assayed using the slide modification of the Jerne hemolytic plaque assay (10, 11). Viable cell recovery was monitored using trypan blue dye exclusion and a Cytograf 6300A (Ortho Instruments, Westwood, Mass.).

**Preparation of GAT-Mq\(^+\) and Immunization of Mice.** The preparation of GAT-Mq\(^+\) using peptone-induced peritoneal exudate has been described in detail previously (2, 12). C57BL/10 mice were immunized by a single intraperitoneal injection of \(3 \times 10^8\) of the indicated GAT-Mq\(^+\) bearing \(20-30\) ng GAT/10\(^6\) cells as quantitated by 125I-GAT bound to Mq\(^+\) (12). Spleen cells from these mice were used in culture 21-35 days later. GAT-Mq\(^+\) for stimulation of PFC responses in vitro were prepared identically and 7 or 5 \(\times 10^4\) Mq\(^+\) bearing \(1-2\) ng GAT were added to cultures. In some experiments, peritoneal exudate Mq\(^+\) were treated with anti-Thy 1.2 sera and guinea pig complement (C) (lacking nonspecific cytotoxic activity on murine cells) to deplete T cells or 1,000 rads X-irradiation before pulsing with GAT. Other GAT-Mq\(^+\) were incubated after pulsing for 24 h at 37\(^\circ\)C in 5% CO\(_2\) in air, washed, and then used to stimulate PFC responses in culture (aged GAT-Mq\(^+\)). Mq\(^+\) content in the various GAT-Mq\(^+\) preparations was monitored by latex sphere ingestion. Freshly pulsed GAT-Mq\(^+\) preparations had a minimum of 85% phagocytic cells; anti-Thy 1.2 + C treated and aged GAT-Mq\(^+\) preparations had \(\geq 90\%\) and \(\geq 95\%\) phagocytic cells, respectively.

**Preparation of T- and B-Cell Populations.** Spleen cells from immunized mice were fractionated into T- and B-cell populations by a modification of the technique of Schlossman and Hudson (13). Immunoadsorbents were prepared by conjugation of the immunoglobulin (Ig) fraction of high titer, polyclonal rabbit anti-mouse Ig sera to CNBR-Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) according to the manufacturer's directions. Conditions were adjusted empirically to yield 2 mg protein/ml packed Sepharose. Spleen cells at \(2 \times 10^8\) cells/ml in L-15 (Microbiological Associates, Walkersville, Md.) containing 5% fetal calf serum, 2.5 mM EDTA and 50 U/ml each penicillin and streptomycin, were applied to the immunoadsorbent columns at 4\(^\circ\)C. The maximum capacity of the immunoadsorbent was \(10^9\) spleen cells/ml Sepharose. T cells were recovered from the column by addition of the EDTA medium until the effluent was free of cells. Mechanically trapped T cells and loosely adherent B cells were removed by gentle mixing of the Sepharose and washing with additional EDTA medium. The B cells adherent to the immunoadsorbent
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Table I
Suppressor Cell Activity in Immune Spleen Cells Restricted for Allogeneic GAT-Mφ

| C57BL/10 Spleen cells (× 10⁶)/culture | Day 5 IgG GAT-specific PFC/culture* |
|--------------------------------------|-----------------------------------|
| Virgin                               | C57BL/10 Mφ | DBA/1 Mφ | C3H/He Mφ |
| Experiment 1                         |            |          |           |
| 10                                   | —          | 430      | 695       | 755       |
| 12                                   | —          | 315      | 535       | 465       |
| 16                                   | —          | 600      | 465       | 475       |
| 10                                   | 10         | 50       | 545       | 10        |
| 10                                   | 2          | 770      | 605       | 310       |
| 10                                   | 6          | 170      | 710       | 150       |
| Experiment 2                         |            |          |           |
| 10                                   | —          | 880      | 780       | 930       |
| 12                                   | —          | 810      | 870       | 1,020     |
| 16                                   | —          | 620      | 620       | 690       |
| 10                                   | 10         | 80       | 780       | 20        |
| 10                                   | 2          | 50       | 640       | 10        |
| 10                                   | 6          | 30       | 510       | <10       |

* The indicated numbers of virgin or immune C57BL/10 spleen cells were cultured with 7 × 10⁶ of the indicated GAT-Mφ bearing ≥2 ng GAT.
† C57BL/10 mice were immunized 28 (exp. 1) or 35 (exp. 2) days previously by intraperitoneal injection of 3 × 10⁶ DBA/1 GAT-Mφ bearing ≥30 ng GAT/10⁶ cells. At culture initiation, these spleen cells had <25 IgG GAT-specific PFC/10⁷ cells.

were eluted with EDTA medium containing 10% vol/vol mouse serum as a source of gamma globulin. This procedure routinely yielded recovery of 80-90% of the applied spleen cells; ≥35% were collected in the T-cell fraction, 45% in the B-cell fraction, and 20% in the intermediate fraction which was discarded. The T-cell fraction contained ≤2% Ig⁺ cells and the B-cell fraction was 75-85% Ig⁺ cells. In some experiments, immune spleen cells were treated with anti-Thy 1.2 serum and C to deplete T cells or 800 rads X-irradiation to inactivate B-cell function and determine radiosensitivity of T-cell functions.

Results
Demonstration of Suppressor Cells in Immune Spleen Cells whose PFC Responses are Restricted for the Immunizing Mφ. These experiments are based first on the observation that Mφ-lymphocyte interactions in primary PFC responses of virgin spleen cells are not governed by genetic restrictions (2, 3); syngeneic and allogeneic GAT-Mφ stimulate comparable primary IgG GAT-specific PFC responses by virgin spleen cells (Tables I-V). Second, immune spleen cells develop secondary PFC responses only when stimulated in vitro with GAT-Mφ syngeneic to those which presented the GAT during the immunization process in vivo, whether these Mφ are allogeneic (Table I) or syngeneic (Tables II-V) to the responding lymphoid cells. In these experiments, suppressor cell activity was assessed by adding graded numbers of these restricted immune spleen cells to 10⁷ unrestricted virgin spleen cells which were then stimulated by a panel of syngeneic and allogeneic GAT-Mφ. Suppressor cells were demonstrated in immune spleen cells restricted for both allogeneic (Table I) and syngeneic (Table II) GAT-Mφ. Thus, immune spleen cells responded to the GAT-Mφ used for priming in vivo, but had cells capable of suppressing primary PFC responses by virgin spleen cells stimulated with GAT-Mφ allogeneic to those used for priming. Generally, significant suppression was observed with 2 × 10⁶ immune spleen cells, however,
**Suppressor Cell Activity in Immune Spleen Cells Restricted for Syngeneic GAT-\(\text{M}\phi\)**

| Day 5 IgG PFC/culture* | GAT-specific vs. SRBC |
|------------------------|------------------------|
|                         | C57BL/10 GAT-M\(\text{M}\phi\) + SRBC | C57BL/10 GAT-M\(\text{M}\phi\) + SRBC |
| Virgin C57BL/10 GAT-M\(\text{M}\phi\) Primed† | C57BL/10 M\(\phi\) M\(\phi\) | DBA/1 M\(\phi\) M\(\phi\) | B10.D2 M\(\phi\) M\(\phi\) |
| 10 | 900 | 1,140 | 1,060 | 260 | 310 |
| 12 | 770 | 690 | 850 | 350 | 515 |
| 16 | 310 | 385 | 315 | 470 | 520 |
| 10 | 1,060 | 80 | 110 | 260 | 520 |
| 10 | 770 | 140 | 10 | 465 | 595 |

* The indicated number of virgin or immune C57BL/10 spleen cells were cultured with \(7 \times 10^4\) of the indicated GAT-M\(\text{M}\phi\) bearing \(2\) ng GAT, or GAT-M\(\text{M}\phi\) plus \(5 \times 10^6\) SRBC.
† C57BL/10 mice were immunized 28 days previously by intraperitoneal injection of \(3 \times 10^6\) C57BL/10 GAT-M\(\text{M}\phi\) bearing \(30\) ng GAT/10^6 cells. At culture initiation, these spleen cells had \(<25\) IgG GAT-specific PFC/10^7 cells.

Occasionally larger numbers of cells were required to achieve significant suppression (Table I, exp. 1). Suppressor cell activity was always observed with third party GAT-M\(\text{M}\phi\) (C3H/He M\(\phi\) in Table I and B10.D2 M\(\phi\) in Table II) unrelated by H-2 haplotype to either the responding spleen cells or the GAT-M\(\text{M}\phi\) used for in vivo priming. The suppression was antigen-specific; primary IgM (data not shown) and IgG PFC responses to SRBC were not suppressed by the immune spleen cells in the presence of GAT-M\(\text{M}\phi\) which induced suppression of the GAT-specific primary response (Table II).

**Characterization of the Suppressor Cell.** The suppressor cells in genetically restricted immune spleen cells were characterized as T cells by two methods. First, immune spleen cells were separated into T and B cells by adsorption to and elution from a rabbit anti-mouse Ig Sepharose 4B column (Table III). Purified T cells, but not B cells, suppressed responses of virgin spleen cells stimulated by GAT-M\(\text{M}\phi\) allogeneic to those used to prime the restricted immune spleen cells. Here, C57BL/10 GAT-M\(\text{M}\phi\) were used for immunization and DBA/1 GAT-M\(\text{M}\phi\) elicited suppressor cell activity. Second, the suppressive activity in restricted immune spleen cells was abrogated by treatment with anti-Thy 1.2 serum plus C demonstrating, by a subtractive procedure, that the suppressor cells are T cells (Table IV). Lastly, suppressor T-cell function was radiosensitive, because 800 rads X-irradiation abolished their activity (Table IV). Thus, suppressor cell activity in restricted immune spleen cells is a property of radiosensitive, antigen-specific T cells.

**Allogeneic T Cells Contaminating the GAT-M\(\text{M}\phi\) are not Responsible for the Suppression.** Suppressor T cells in immune spleen cells that are restricted for the allogeneic GAT-M\(\text{M}\phi\) are stimulated by syngeneic GAT-M\(\text{M}\phi\) in vitro (Table I); here, exposure to allogeneic cells is limited to the in vivo immunization process. However, suppressor T cells in immune spleen cells restricted for syngeneic GAT-M\(\text{M}\phi\) are activated by allogeneic GAT-M\(\text{M}\phi\) in vitro (Table II) where the exposure to allogeneic cells is during the culture and the contribution of allogeneic T cells contaminating the M\(\phi\) preparation to the suppressive activity is important to determine. Thus, M\(\phi\)
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Suppressor Cell Activity in Restricted Immune Spleen Cells is a Function of T Cells

| C57BL/10 Spleen cells (× 10⁶)/culture | Day 5 IgG GAT-specific PFC/culture* |
|---------------------------------------|------------------------------------|
| **Virgin**                            | **C57BL/10 GAT-Mφ primed‡**        |
|                                       | **C57BL/10 Mφ** | **DBA/1 Mφ** |
| Spleen Cells                          | T Cells | B Cells |                |
|                                       |         |         |                |
| 10                                    | —       | —       | —              |
| 12                                    | —       | —       | —              |
| 10                                    | 10      | —       | —              |
| 2                                     | 2       | —       | —              |
| 10                                     | — — 10 | 10      |                |
| 10                                     | — — 5  | 5       |                |
| 10                                     | — 2    | —       |                |
| 10                                     | — — 2  | —       |                |

* The indicated numbers of virgin or immune C57BL/10 spleen cells or purified T or B cells were cultured with 7 × 10⁶ C57BL/10 or DBA/1 GAT-Mφ bearing ≥2 ng GAT.
‡ C57BL/10 mice were immunized 35 days previously by intraperitoneal injection of 3 × 10⁶ GAT-Mφ bearing ≥30 ng GAT/10⁶ cells. At culture initiation, spleen cells from these mice had <30 IgG GAT-specific PFC/10⁶ cells. T and B cells were prepared as described in Materials and Methods.
§ PFC responses to SRBC: Spleen cells-IgM-2,550, IgG-290; T cells-IgM-300, IgG-0; B cells-IgM-330, IgG-0; T cells + B cells-IgM-1,830, IgG-210.
|| ND, not done.

Radiosensitivity of Suppressor T Cells in Restricted Immune Spleen Cells

| C57BL/10 Spleen cells (× 10⁶)/culture | Day 5 IgG GAT-specific PFC/culture‡ |
|---------------------------------------|------------------------------------|
| **Virgin**                            | **C57BL/10 GAT-Mφ primed***        |
|                                       | **C57BL/10 Mφ** | **DBA/1 Mφ** |
| Spleen cells                          | Anti-Thy 1 + C treated | X-irradiated |
|                                       |                   |                |
| 10                                    | —       | —       | —              |
| 12                                    | —       | —       | —              |
| 10                                     | 10      | —       | —              |
| 2                                     | 2       | —       | —              |
| 10                                     | — — 10 | 10      |                |
| 10                                     | — — 5  | 5       |                |
| 10                                     | — 2    | —       |                |
| 10                                     | — — 2  | —       |                |

* C57BL/10 mice were immunized 35 days previously by intraperitoneal injection of 3 × 10⁶ C57BL/10 GAT-Mφ bearing ≥25 ng GAT/10⁶ cells. At culture initiation, spleen cells from these mice had <30 IgG GAT-specific PFC/10⁶ cells. Spleen cells were treated with anti-Thy 1 + C or irradiation as described in Materials and Methods.
‡ The indicated numbers of virgin or immune C57BL/10 spleen cells, anti-Thy 1 + C treated, or X-irradiated (800 rads) spleen cells were cultured with 5 × 10⁶ C57BL/10 or DBA/1 GAT-Mφ bearing ≥1 ng GAT.
§ ND, not done.
populations were treated with anti-Thy 1.2 serum plus C before pulsing with GAT to deplete T cells under the same conditions used in Table IV to deplete T cells from immune spleen cell populations. The resulting Mφ preparations were ~90% phagocytic by latex bead ingestion and bound approximately equivalent amounts of GAT as normal control Mφ, i.e., ~2 ng/10^6 cells. Normal and T-cell-depleted C57BL/10 and DBA/2 GAT-Mφ stimulated comparable primary PFC responses by virgin C57BL/10 spleen cells, secondary responses by immune cells restricted for syngeneic C57BL/10 GAT-Mφ, and suppressor cell activity in primary responses when these immune cells were stimulated with allogeneic DBA/2 GAT-Mφ (Table V). Identical results were obtained with Mφ preparations X-irradiated (1,000 rads) before pulsing with GAT (data not shown). Another more indirect approach to this question used GAT-Mφ which had been aged after pulsing. The T-cell depletion in this situation is by attrition, because the GAT-Mφ are incubated in medium without serum in 5% CO₂ in air for 24 h. Few cells are lost by adherence to the plastic culture tubes and the recovered cells are routinely 95% phagocytic. However, ~90% of the GAT originally associated with the Mφ is released into the supernate as nonacid precipitable radioactivity and the Mφ retain ~0.2 ng GAT/10^6 cells (14). Nevertheless, these aged GAT-Mφ behaved identically to freshly pulsed or T-cell-depleted GAT-Mφ in terms of their ability to stimulate primary responses by virgin spleen cells, restricted responses by immune cells, and suppressor T-cell activity (Table V). Thus, T cells contaminating the allogeneic peritoneal exudate Mφ preparations used in the various protocols do not appear to contribute either to the restrictions on Mφ-immune T-lymphocyte interactions in secondary PFC responses or the activation of suppressor T cells in these immune spleen cells by an inappropriate GAT-Mφ. Moreover, these results have been derived using whole unfractionated spleen cells rather than rigorously Mφ-depleted lymphoid cell populations, because depletion of Mφ does not alter the results. Further, the experiments described in Tables III–V have been carried out
with identical results using spleen cells from mice immunized with allogeneic GAT-MΦ where secondary responses are restricted to those allogeneic GAT-MΦ, and suppressor cells are stimulated by syngeneic GAT-MΦ (data not shown).

Discussion

These experiments further characterize the immunological consequences of immunizing responder mice with GAT bound to syngeneic or allogeneic MΦ. As shown previously, virgin responder spleen cells develop comparable primary PFC responses to syngeneic or allogeneic GAT-MΦ, whereas immune spleen cells develop secondary PFC responses only when stimulated with GAT-MΦ which are syngeneic with the MΦ that presented GAT to the virgin lymphocytes during the immunization process in vivo (2, 3). In this system, no difference between responder and nonresponder MΦ, with regard to their ability to present GAT in an immunogenic form to lymphoid cells, has been detected. A primary objective of the present experiments was to determine whether suppressor T cells are involved in these genetic restrictions in secondary responses and account for the failure of immune spleen cells, which should contain virgin unrestricted GAT-specific helper T cells, to develop primary PFC responses to GAT-MΦ allogeneic to MΦ used for in vivo immunization.

Cultures containing small numbers of spleen cells primed with syngeneic or allogeneic GAT-MΦ plus virgin spleen cells develop responses only to GAT-MΦ syngeneic to the priming MΦ and have suppressed primary responses to GAT-MΦ allogeneic to the priming MΦ. Thus, these primed spleen cells contain helper T cells, whose function is genetically restricted for the priming MΦ, and suppressor cells, whose function is apparently not restricted, that inhibit the responses of virgin spleen cells. The suppression is specific for GAT because no suppression of responses to SRBC is observed in the presence of GAT-MΦ which activate the suppressor cells. The suppression is mediated by T cells from GAT-MΦ primed mice and this suppressor T-cell function is relatively radiosensitive. Lastly, the possibility that a negative allogeneic effect (15) mediated by T cells contaminating the MΦ preparations causing either the restriction in secondary responses or the suppression in primary responses has been ruled out.

These experiments also provide an explanation for the previous failure to demonstrate GAT-specific suppressor T cells in responder mice using experimental protocols which readily induce suppressor T cells in mice which are nonresponders to GAT (14, 16, 17). First, in nonresponder mice soluble GAT induces suppressor T cells which inhibit primary GAT-specific responses to GAT-MBSA in the presence of syngeneic MΦ; GAT-MΦ are ineffective activators of these suppressor T cells, but can induce GAT-specific helper T cells (14, 18). In responder mice, GAT-MΦ and soluble GAT induce both restricted helper T cells and suppressor T cells which are only activated by GAT-MΦ allogeneic to the MΦ present during the in vivo priming. Second, the kinetics of development of suppressor T cells differs in responder and nonresponder mice; suppressor T cells are detected from 3 to 28 days after the injection of GAT in nonresponder mice (14, 16), but only 21 or more days after the injection of GAT-MΦ in responder mice (C. W. Pierce, and J. A. Kapp, unpublished observations). Thus, GAT-specific suppressor T cells in responder and nonresponder mice appear to differ in both their mode and kinetics of activation. However, their mechanism of action may be similar, because they inhibit activation of virgin, but not immune, helper T
Further studies comparing the mechanism(s) of action of suppressor T cells in responder and nonresponder mice are currently in progress.

GAT-specific suppressor T cells induced in responder mice by immunization with GAT-Mφ have not yet been characterized by Ly phenotype. Based on the observations that GAT-specific suppressor T cells in nonresponder mice express the Ly 1− 23+ phenotype, it might be predicted that suppressor cells in responder mice will express the same Ly phenotype. However, the recent report by Eardley et al. (19) demonstrating that antigen-primed Ly 1+ T cells function as helper cells for B-cell responses and also act with Ly 123+ T cells to initiate a feedback-suppressor cell loop, make the determination of the Ly phenotype on these suppressor T cells mandatory. Moreover, the investigation of feedback suppressor loops and regulatory interactions among T cells of the various Ly phenotypes in responder and nonresponder mice may be revealing with regard to the precise nature of the Ir gene defect in nonresponder mice.

The observation that the development of genetic restrictions governing Mφ-immune helper T-cell interactions correlates with the development of specific suppressor T cells in GAT-Mφ primed responder mice raises the issue of whether GAT-Mφ stimulate a population of genetically restricted helper T cells and a population of antigen-specific, but genetically unrestricted, suppressor T cells, or, whether the genetic restrictions in helper T-cell function are mediated by the suppressor T cells. The former possibility suggests first, that GAT-Mφ induce expansion of the clones of T cells that recognize GAT in the context of I-A subregion products on the Mφ membrane and that function as restricted helper cells only when re-exposed to those GAT-Mφ. This concept makes no prediction about the nature of the T-cell receptor in terms of dual recognition vs. altered self or complex antigenic determinant models. Second, suppressor T cells appear to be activated by the GAT alone and not by GAT in the context of a macrophage membrane complex. This concept is supported by observations that suppressor T cells are induced in vitro by soluble antigen in rigorously Mφ-depleted T cell cultures (20), and that the suppressor T cells described here are activated by any GAT-Mφ which are allogeneic to the priming Mφ. Thus, the restricted immune helper T cells would be resistant to the effects of the suppressor T cells which only inhibit the activation of virgin helper cells. Alternatively, the latter possibility suggests primed helper T cells are not themselves restricted, but appear restricted because the suppressor T cells inhibit the function of those helper T cells activated by all GAT-Mφ other than those used for immunization. Experiments to distinguish between these two alternatives are currently in progress.

Restrictions mediated by products of the H-2 gene complex governing T-cell responses or activities have been demonstrated in both humoral and cellular immune responses. There are experimental systems in which restrictions between helper T cells and Mφ in the development of antibody responses in vitro are different from those in the GAT system. For some antigens, such as ovalbumin and keyhole limpet hemocyanin, no requirement for syngenicity between Mφ and carrier-primed helper T cells in the development of secondary antibody responses to hapten-carrier conjugates by hapten-primed B cells has been demonstrated (21). In contrast to primary responses in the GAT system where Mφ-T-cell interactions are not restricted, primary IgM anti-TNP PFC responses to trinitrophenol (TNP)-(T,G)-A-L by lymphoid cells from

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2 J. Kapp. Manuscript in preparation.
3 J. Kapp, et al. Manuscript in preparation.
(responder × nonresponder)F₁ mice developed only in the presence of responder, but not nonresponder, parental Mφ (9). These results suggest expression of Ir gene control at the level of the Mφ. This observation is also in contrast to the GAT system, where responder and nonresponder parental GAT-Mφ stimulate comparable primary IgG PFC responses by (responder × nonresponder)F₁ lymphoid cells (22). Further secondary anti-TNP responses in cultures containing (T,G)-A-L-primed (responder × nonresponder)F₁ T cells and TNP-primed responder parental B cells develop only in the presence of responder parental Mφ (23). Thus, development of both primary and secondary responses to TNP-(T,G)-A-L appear to involve genetic restrictions governing Mφ-T-cell interactions. Whether the differences in results in the GAT and (T,G)-A-L systems reflect different culture and assay procedures or differences in Mφ function with respect to Ir gene product expression awaits further investigation.

Summary

Virgin spleen cells develop comparable primary antibody responses in vitro to syngeneic or allogeneic macrophages (Mφ) bearing the terpolymer L-glutamic acid⁶₀-L-alanine⁴⁰-L-tyrosine¹⁰ (GAT), whereas immune spleen cells primed with syngeneic or allogeneic GAT-Mφ develop secondary responses preferentially when stimulated with GAT-Mφ syngeneic to the GAT-Mφ used for priming in vivo. These restrictions are mediated by products of the I-A subregion of the H-2 complex and are operative at the level of the GAT-Mφ-immune helper T-cell interactions. To investigate why these immune spleen cells fail to develop a significant antibody response to GAT-Mφ other than those used for in vivo immunization and determine the mechanism by which the restriction is maintained, spleen cells from virgin and syngeneic or allogeneic GAT-Mφ-primed mice were co-cultured in the presence of GAT-Mφ of various haplotypes. Antibody responses to GAT developed only in the presence of GAT-Mφ syngeneic to the Mφ used for in vivo priming; responses in cultures with GAT-Mφ allogeneic to the priming Mφ, whether these Mφ were syngeneic or allogeneic with respect to the responding spleen cells, were suppressed. The suppression was mediated by GAT-specific radiosensitive T cells. Thus, development of GAT-specific suppressor T cells appears to be a natural consequence of the immune response to GAT in responder as well as nonresponder mice. The implications of stimulation of genetically restricted immune helper T cells, and antigen-specific, but unrestricted, suppressor T cells after immunization with GAT-Mφ in vivo are discussed in the context of regulatory mechanisms in antibody responses.

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