SUPPRESSOR T-CELL ACTIVITY IN RESPONDER × NONRESPONDER (C57BL/10 × DBA/1)F1 SPLEEN CELLS RESPONSIVE TO L-GLUTAMIC ACID\textsuperscript{50}-L-ALANINE\textsuperscript{30}-L-TYROSINE\textsuperscript{10}*

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Antibody responses of mice to the terpolymer of L-glutamic acid\textsuperscript{50}-L-alanine\textsuperscript{30}-L-tyrosine\textsuperscript{10} (GAT)\textsuperscript{*} are controlled by an H-2-linked immune response (Ir) gene(s). The lack of response in nonresponder mice (H-2\textsuperscript{p,q}) immunized with soluble GAT appears to be due to a preferential stimulation of GAT-specific suppressor T cells which inhibit activation of helper T cells (1–3). However, GAT-specific helper T cells can be demonstrated in nonresponder mice immunized with GAT complexed to methylated bovine serum albumin (GAT-MBSA) or GAT-bearing macrophages (GAT-M\textsuperscript{p}) (2–4). Several observations indicate that the Ir gene defect is not expressed in M\textsuperscript{p}, at least in responses to GAT. First, responder and nonresponder M\textsuperscript{p} bind comparable amounts of GAT in vitro (5, 6). Second, allogeneic nonresponder and syngeneic responder GAT-M\textsuperscript{p} stimulate comparable primary IgG plaque-forming cell (PFC) responses by responder lymphoid cells in vitro (6). Third, nonresponder GAT-M\textsuperscript{p} stimulate GAT-specific helper T cells in responder mice in vivo that function in vitro only when stimulated with GAT on M\textsuperscript{p} syngeneic with the M\textsuperscript{p} used for initial immunization (6, 7).\textsuperscript{2} In addition to these genetically restricted immune helper T cells, genetically unrestricted, but antigen-specific, radiosensitive suppressor T cells, which suppress primary PFC responses stimulated by M\textsuperscript{p} that are genetically unrelated to those which induced the restricted helper T cells, are present in these immune T-cell populations (8).

By contrast, Ir gene products may be involved in mediating genetic restrictions in M\textsuperscript{p}-T cell interactions in other systems. (Responder × nonresponder)F\textsubscript{1} guinea pig T cells develop DNA synthetic responses to antigens under Ir gene control when antigen is presented on responder, but not nonresponder, parental M\textsuperscript{p} (9). Similar observations have been made with antigens under Ir gene control in murine delayed hypersensitivity responses in vivo (10) and primary and secondary PFC responses in vitro (11, 12). These observations led to the hypothesis that the Ir gene product is

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1 Abbreviations used in this paper: C, complement; GAT, random terpolymer of L-glutamic acid\textsuperscript{50}-L-alanine\textsuperscript{30}-L-tyrosine\textsuperscript{10}; GAT-MBSA, GAT-complexed to methylated bovine serum albumin; GAT-M\textsuperscript{p}, GAT-pulsed macrophage(s); Ir gene, immune response gene(s); M\textsuperscript{p}, macrophages; PFC, plaque-forming cell(s).

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functionally expressed in Mφ, and nonresponder Mφ lacking this product are unable to present antigen in an immunogenic form to stimulate T cells (13).

In the GAT-system, virgin (responder × nonresponder)F1 spleen cells develop comparable primary PFC responses to both parental and unrelated third party GAT-Mφ. F1 spleen cells from mice immunized with responder or nonresponder parental GAT-Mφ develop secondary responses only when stimulated with the parental GAT-Mφ used for immunization or F1 GAT-Mφ (14). These results are entirely consistent with the restrictions on Mφ-immune helper T-cell interactions previously observed (6). However, F1 mice immunized with soluble GAT develop secondary responses in vitro only when stimulated with responder parental or F1 GAT-Mφ.

Thus, a contradiction exists between (a) the ability of nonresponder GAT-Mφ to stimulate primary responses in both parental responder and (responder × nonresponder)F1 spleen cells, and to prime these mice such that development of secondary responses is restricted to the nonresponder GAT-Mφ, and (b) the failure of spleen cells from F1 mice primed with soluble GAT to develop secondary responses to nonresponder GAT-Mφ. This latter observation is comparable with the concept that Ir gene(s) function may be expressed in Mφ in the GAT system under some circumstances. Alternatively, the physical state of the GAT used for immunization, soluble GAT vs. GAT-Mφ, may be crucial in the priming process and the subsequent response patterns of immune F1 spleen cells to parental GAT-Mφ in vitro.

To analyze the paradox described above we have examined secondary PFC responses by (responder × nonresponder)F1 spleen cells from mice immunized with parental responder, nonresponder, and F1 GAT-Mφ and the effect of injecting soluble GAT simultaneously with GAT-Mbsa on these response patterns. The data demonstrate that the response patterns of immune F1 spleen cells are strictly dependent on the physical state of the GAT used for immunization and that suppressor T cells are stimulated in F1 mice immunized with soluble GAT.

Materials and Methods

Mice. C57BL/10 (H-2b), DBA/2 (H-2d), DBA/1 (H-2q), C3H/He (H-2k), (C57BL/10 × DBA/2)F1 (H-2b/c), and (C57BL/10 × DBA/1)F1 (H-2b/q) mice were bred in the animal facility in the Yalem Building 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, Culture System, and Hemolytic Plaque Assay. GAT (Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind.; lot 4, mol wt ≈45,000), was prepared as previously described for use as soluble GAT or GAT-MBSA in culture (15), preparing GAT-Mφ (5, 6), and coupling to sheep erythrocytes for use as indicator cells in the PFC assay (15). Spleen cells at 107 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., Kankakee, Ill.; lot M26302) were incubated with the indicated GAT-Mφ as 1-ml cultures under modified Mishell-Dutton conditions (16). In some experiments, primed (C57BL/10 × DBA/1)F1 spleen cells were treated with anti-Thy 1.2 serum plus guinea pig serum as a source of complement (C) to deplete T cells. IgG GAT-specific PFC responses were assayed using the slide modification of the Jerne hemolytic plaque assay (15).

Preparation of GAT-Mφ and Immunization of Mice. The preparation of GAT-Mφ using peptone-induced peritoneal exudate has been described in detail previously (5, 6). F1 mice were immunized by i.p. injection of: (a) 3–4 × 106 of the appropriate GAT-Mφ (bearing ≈25 ng GAT/106 cells); (b) 10 μg GAT in a mixture of magnesium aluminum hydroxide gel (Maalox; William H. Rorer, Inc., Ft. Washington, Pa.) and pertussis vaccine (Eli Lilly and Co.,
Table I

| Spleen cells                  | (C57BL/10 × DBA/2)F1 | (C57BL/10 × DBA/2)F1 × DBA/2 F1 | (C57BL/10 × DBA/2)F1 × DBA/1 F1 |
|------------------------------|----------------------|-------------------------------|-------------------------------|
| Virgin Mφ                    | 210                  | 280                           | 260                           |
| GAT primed§                  | 420                  | 430                           | 20                            |
| Primed with (C57BL/10 × DBA/2)F1 Mφ | 280                  | 250                           | 30                            |

Days 5 IgG GAT-specific PFC/culture

Virgin or immune (C57BL/10 × DBA/2)F1 or (C57BL/10 × DBA/2)F1 spleen cells at 10⁷ cells/culture were incubated with 5 × 10⁴ of the indicated GAT-Mφ bearing 1 ng GAT/10⁴ cells.
§ ND, not done.

§§ Mice were immunized 28 days previously by intraperitoneal injection of 10 µg GAT in Maalox-pertussis.
¶ Mice were immunized 28 days previously by intraperitoneal injection of 4 × 10⁶ of the indicated Mφ bearing 30 ng GAT/10⁶ cells.
¶¶ Mice were immunized 28 days previously by intraperitoneal injection of 10 µg GAT as GAT-MBSA. At culture initiation, all these spleen cells had <30 IgG GAT-specific PFC/10⁷ cells.

Indianapolis, Ind.) (15); (c) 10 µg GAT as GAT-MBSA; or, (d) GAT-Mφ or GAT-MBSA plus 10 µg soluble GAT. PFC responses by immune spleen cells in vitro 21–35 days later were stimulated with 7 or 5 × 10⁴ Mφ bearing 1–2 ng GAT. In some experiments, Mφ were pulsed in vivo with GAT by injecting 10 µg GAT (containing 1% ¹²⁵I-GAT) into peritoneal cavities of mice 3 days after injection of 1.5 ml 10% sterile proteose peptone. After 1 h, peritoneal exudate cells were harvested, washed three times with Hanks’ balanced salt solution, adjusted to 5 x 10⁵ cells/ml, and added to cultures in 0.1 ml volumes. GAT bound per 5 x 10⁶ cells under these conditions ranged from 1.5 to 4.0 ng.

Results

PFC Responses to GAT by (Responder × Responder)F1 and (Responder × Nonresponder)F1 Spleen Cells Stimulated by Parental and F1 GAT-Mφ. Previous studies demonstrated that (responder × nonresponder)F1 spleen cells from mice immunized with soluble GAT in vivo responded to responder parental and F1 GAT-Mφ, but not to nonresponder parental GAT-Mφ in vitro (14). These studies were extended to spleen cells from (responder C57BL/10 × responder DBA/2)F1 mice immunized with soluble GAT and (responder × responder)F1 and (responder C57BL/10 × nonresponder DBA/1)F1 spleen cells from animals immunized with the syngeneic F1 GAT-Mφ (Table I). First, virgin F1 spleen cells developed comparable primary PFC responses to all GAT-Mφ tested. Second, (responder × responder)F1 spleen cells from mice immunized with soluble GAT or F1 GAT-Mφ responded to F1 and both parental GAT-Mφ, but failed to respond to the unrelated, third party DBA/1 GAT-Mφ. Thus, these immune F1 spleen cells exhibit genetic restrictions requiring syngenecity between the immunizing and stimulating GAT-Mφ, as observed previously (6) and, in contrast to soluble GAT-primed (responder × nonresponder)F1 spleen cells, developed second-
ary responses of comparable magnitude to either parental GAT-Mφ. Third, as observed previously (14), (responder × nonresponder)F₁ spleen cells from mice immunized with soluble GAT responded to F₁ or responder parental, but not nonresponder parental or unrelated third party, GAT-Mφ. However, if these F₁ mice were immunized with F₁ GAT-Mφ or GAT-MBSA, their spleen cells responded to F₁ and both parental GAT-Mφ, but not third party DBA/2 GAT-Mφ. Thus, (responder × nonresponder)F₁ spleen cells exhibit restrictions in secondary responses with regard to unrelated third party GAT-Mφ regardless of how the spleen cells were primed. However, immunization with F₁ GAT-Mφ or GAT-MBSA, in contrast to soluble GAT, produced significantly different response patterns suggesting that the physical state of the GAT used for immunization (soluble vs. insolubilized) is a major determining factor in whether the immune F₁ spleen cells respond to nonresponder parental GAT-Mφ.

Effects of Soluble GAT on Response Patterns to Parental and F₁ GAT-Mφ and GAT-MBSA in (Responder × Nonresponder)F₁ Mice. The hypothesis tested with the experiments in Table II was that simultaneous administration of soluble GAT and parental or F₁ GAT-Mφ or GAT-MBSA to (responder × nonresponder)F₁ mice will convert the response pattern normally induced by that immunizing agent to the response pattern normally induced by soluble GAT. Virgin F₁ spleen cells and spleen cells from F₁ mice primed with soluble GAT responded as observed previously. It is possible that these results reflect an artifact induced by pulsing Mφ with GAT in vitro at 4°C in serum-free medium at ≥pH 9. Therefore, C57BL/10 and DBA/1 peritoneal exudate Mφ were pulsed under physiological conditions by injecting 10 μg GAT into the peritoneal cavity and harvesting the cells 1 h later. These Mφ bound quantities of GAT comparable to Mφ pulsed in vitro and stimulated responses in virgin and GAT-primed F₁ spleen cells comparable to those stimulated by in vitro pulsed parental Mφ (Table II, exp. 1 and 2). Thus, the ability of nonresponder parental GAT-Mφ to stimulate primary responses and their failure to stimulate secondary responses in GAT-primed F₁ spleen cells was not due to an artifact, such as denaturation of GAT, induced by the conditions employed for in vitro pulsing of Mφ. As expected from previous experiments (14), spleen cells from F₁ mice primed with responder (C57BL/10) or nonresponder (DBA/1) parental GAT-Mφ responded to F₁ and the immunizing parental GAT-Mφ, but failed to respond to the opposite parental or unrelated third party GAT-Mφ (Table II, exp. 1). This response pattern was not altered by addition of soluble GAT to the immunizing C57BL/10 GAT-Mφ, but soluble GAT induces the same response pattern as C57BL/10 GAT-Mφ in these F₁ mice and no alteration was expected. By contrast, soluble GAT completely reversed the response pattern in F₁ mice immunized with DBA/1 GAT-Mφ. Instead of being restricted for and responding to the immunizing DBA/1 GAT-Mφ, these F₁ mice responded identically to those immunized with soluble GAT alone and developed PFC responses only when stimulated with responder parental C57BL/10 or F₁ GAT-Mφ (Table II, exp. 1). Further, the response patterns of F₁ mice primed with F₁ GAT-Mφ (Exp. 2) and GAT-MBSA (Exp. 3), which developed secondary responses when stimulated with either parental GAT-Mφ, were altered in a similar manner by inclusion of soluble GAT in the immunizing regimen. These F₁ spleen cells responded to responder parental C57BL/10 and F₁ GAT-Mφ, but no longer responded to the nonresponder parental DBA/1 GAT-Mφ.

These experiments clearly demonstrate that the physical state of the GAT used for
### Table II

**Effects of Soluble GAT on Priming Patterns of Parental Responders, Nonresponder and F1 GAT-Mφ, and GAT-MBSA in Responder × Nonresponder (C57BL/10 × DBA/1)F1 Mice**

| (C57BL/10 × DBA/1)F1 Spleen cells | Day 5 IgG GAT-specific PFC/culture* |
|------------------------------------|-------------------------------------|
|                                    | C57BL/10 Mφ | DBA/1 Mφ | (C57BL/10 × DBA/1)F1 Mφ | C3H/He Mφ |
| Experiment 1                       |            |          |                          |            |
| Virgin                             | 980        | 800      | 900                      | 1270       |
| GAT primed†                        | (980)*     | (750)    | ND§                      | ND         |
| C57BL/10 GAT-Mφ primed†            | 800        | 50       | 620                      | 30         |
| C57BL/10 GAT-Mφ + 10 µg soluble GAT primed** | 540 | <10 | 500 | <10 |
| DBA/1 GAT-Mφ primed¶               | 40         | 460      | 680                      | <10        |
| DBA/1 GAT-Mφ + 10 µg soluble GAT primed** | 920 | <10 | 870 | 40 |
| Experiment 2                       |            |          |                          |            |
| Virgin                             | 350        | 420      | 320                      | 430        |
| GAT primed†                        | (360)      | (570)    |                          |            |
| (C57BL/10 × DBA/1)F1 GAT-Mφ primed¶ | 620 | 780 | 630 | 80 |
| (C57BL/10 × DBA/1)F1 GAT-Mφ + 10 µg soluble GAT primed** | 330 | <10 | 370 | <10 |
| Experiment 3                       |            |          |                          |            |
| Virgin                             | 550        | 530      | 370                      | 390        |
| GAT primed¶                        | 310        | 40       | 310                      | 40         |
| GAT-MBSA primed¶                   | 330        | 320      | 350                      | <10        |
| GAT-MBSA + 10 µg soluble GAT primed§§ | 340 | 50 | 270 | <10 |

* Virgin or immune spleen cells at 10⁶ cells/culture were incubated with 5 × 10⁴ of the indicated GAT-Mφ bearing 4 ng GAT/10⁶ cells.

† Numbers in parentheses are PFC responses stimulated by 5 × 10⁶ C57BL/10 or DBA/1 Mφ pulsed in vivo by injection of 10 µg GAT containing 1% [³H]-GAT into peritoneal cavities of mice 3 days after injection of 1.5 ml 10% proteose peptone. After 1 h, peritoneal exudate cells were harvested, washed three times with Hanks’ balanced salt solution, and adjusted to 5 × 10⁵ cells/ml for addition to cultures in 0.1 ml vol. GAT/5 × 10⁴ cells was: experiment 1 — C57BL/10 — 2.9 ng, DBA/1 — 1.9 ng; experiment 2 — C57BL/10 — 3.5 ng, DBA/1 — 3.0 ng.

‖ Virgin or immune spleen cells were immunized 28 days previously by intraperitoneal injection of 10 µg GAT in Maalox-pertrussis.

¶ Virgin or immune spleen cells were immunized 28 days previously by intraperitoneal injection of 4 × 10⁶ C57BL/10, DBA/1, or F1 GAT-Mφ bearing 30 ng GAT/10⁶ cells.

** Virgin or immune spleen cells were immunized 28 days previously by intraperitoneal injection of 4 × 10⁶ C57BL/10, DBA/1, or F1 GAT-Mφ bearing 30 ng GAT/10⁶ cells plus 10 µg soluble GAT.

†† Virgin or immune spleen cells were immunized 28 days previously by intraperitoneal injection of 10 µg GAT as GAT-MBSA.

§§ Virgin or immune spleen cells were immunized 28 days previously by intraperitoneal injection of 10 µg GAT as GAT-MBSA plus 10 µg soluble GAT.

§ ND, not done.

Immunization is critical in determining the subsequent response pattern of the immune F1 spleen cells. Further, soluble GAT can alter response patterns induced by parental or F1 GAT-Mφ such that these immune spleen cells respond as if they were immunized with soluble GAT alone. Because these F1 mice can develop secondary responses to nonresponder parental GAT-Mφ after immunization with these Mφ, but
Table III
Suppressor Cells in GAT and F1 GAT-Mφ Primed Responder × Nonresponder (C57BL/10 × DBA/1)F1 Spleen Cells

| F1 spleen cells (×10⁶)/culture | Day 5 IgG GAT-specific PFC/culture* |
|-------------------------------|-----------------------------------|
| Virgin GAT-primed             | C57BL/10/Mφ | DBA/1/Mφ | (C57BL/10 × DBA/1)F1/Mφ | C3H/He/Mφ |
| GAT-Mφ primed†                | 480         | 570      | 420                      | 400       |
| 10                            | 330         | 350      | 290                      | 420       |
| 14                            | 540         | 30       | 620                      | 40        |
| 10                            | 610         | 520      | 530                      | <10       |
| 10                            | 400         | 30       | 490                      | <10       |
| 10                            | 480         | 460      | 440                      | <10       |

* Virgin or immune (C57BL/10 × DBA/1)F1 spleen cells were incubated with 5 × 10⁶ of the indicated GAT-Mφ bearing 2 ng GAT/10⁶ cells.
† (C57BL/10 × DBA/1)F1 mice were immunized 35 days previously by intraperitoneal injection of 10 μg GAT in Maalox-pertussis (GAT primed) or 3 × 10⁶ F1 GAT-Mφ bearing 20 ng GAT/10⁶ cells (F1 GAT-Mφ primed).

Table IV
Suppressor Cell Activity in GAT-Primed Responder × Nonresponder (C57BL/10 × DBA/1)F1 Spleen Cells is due to T Cells

| F1 Spleen Cells (×10⁶)/culture | Day 5 IgG GAT-specific PFC/culture* |
|-------------------------------|-----------------------------------|
| Virgin GAT-primed†            | C57BL/10/Mφ | DBA/1/Mφ | (C57BL/10 × DBA/1)F1/Mφ | C3H/He/Mφ |
| Anti-Thy 1 + C-Treated GAT-primed | 290 | 240      | 240                      | 260       |
| 10                            | 290         | 210      | 280                      | 240       |
| 10                            | 710§        | 10       | 630                      | 60        |
| 4                             | <10§        | ND       | ND                       | ND        |
| 10                            | 250         | 40       | 190                      | <10       |
| 10                            | 230         | 230      | 190                      | 220       |

* Virgin or immune (C57BL/10 × DBA/1)F1 spleen cells at the indicated cell densities were incubated with 5 × 10⁶ of the indicated GAT-Mφ bearing 1 ng GAT/10⁶ cells.
† (C57BL/10 × DBA/1)F1 mice were immunized 28 days previously by intraperitoneal injection of 10 μg GAT in Maalox-pertussis.
§ Primary IgM responses to SRBC by these spleen cells: GAT-primed, 1570; anti-Thy 1 + C treated GAT-primed, 140.
ND, not done.

soluble GAT reverses this response pattern, the possibility of suppressor cell activity induced by soluble GAT in these (responder × nonresponder)F1 mice was investigated.

Suppressor Cells in (Responder × Nonresponder)F1 Spleen Cells Primed With Soluble GAT. Because primary PFC responses to GAT are not governed by restrictions on Mφ-lymphocyte interactions (6, 14), primed F1 spleen cells can be added to virgin F1 spleen cells stimulated with various GAT-Mφ to test for suppressor cell activity (14). Small numbers of soluble GAT-primed F1 spleen cells, whose responses are restricted to F1 or responder parental GAT-Mφ, suppressed responses of virgin F1 spleen cells stimulated by nonresponder parental DBA/1, or third party C3H/He GAT-Mφ, but not responses stimulated by F1 or responder parental GAT-Mφ (Table III). However, F1 spleen cells from mice primed with F1 GAT-Mφ did not suppress responses of virgin...
SUPPRESSOR T CELLS IN F1 SPLEEN CELLS

F1 spleen cells stimulated with nonresponder parental GAT-Mφ, but had suppressor cells that were activated by unrelated third party C3H/He GAT-Mφ. Treatment of GAT-primed F1 spleen cells containing suppressor cells with anti-Thy 1.2 serum plus C abolished the suppression demonstrating that the suppression was T-cell mediated (Table IV).

Discussion

Three significant observations emerged from these experiments. First, (responder × nonresponder)F1 spleen cells from mice immunized with F1 GAT-Mφ or GAT-MBSA develop secondary PFC responses when stimulated with responder and nonresponder parental GAT-Mφ, but not when stimulated with unrelated third party C3H/He GAT-Mφ. These observations are in contrast to responses of soluble GAT-primed F1 spleen cells, which respond to F1 and responder parental GAT-Mφ but not to nonresponder parental GAT-Mφ, and emphasize the critical importance of the physical state of the GAT used for immunization in determining subsequent response patterns of F1 spleen cells. The observations with F1 spleen cells from mice primed with F1 GAT-Mφ and GAT-MBSA are consistent with responses of F1 spleen cells from mice primed with nonresponder parental GAT-Mφ and demonstrate further that F1 mice have T cells that can be primed by and subsequently respond to GAT presented on nonresponder parental Mφ. Collectively, these observations argue against functional expression of the Ir genes controlling the response to GAT in Mφ.

Second, spleen cells from (responder × nonresponder)F1 mice immunized with GAT-Mφ or GAT-MBSA plus soluble GAT develop secondary PFC responses when stimulated with F1 or responder parental GAT-Mφ, but not nonresponder parental GAT-Mφ, regardless of the response pattern of the F1 spleen cells immunized with GAT-Mφ or GAT-MBSA alone. Thus, soluble GAT does not alter the response pattern of spleen cells from mice immunized with responder parental GAT-Mφ which normally respond only to those GAT-Mφ. However, soluble GAT clearly modulated the secondary response patterns of F1 spleen cells immunized simultaneously with nonresponder parental or F1 GAT-Mφ or GAT-MBSA such that these F1 spleen cells no longer respond to nonresponder parental GAT-Mφ. These observations again demonstrate the critical importance of the physical state of the GAT used for immunization and further demonstrate that soluble GAT can block priming of T cells which are normally primed and respond to subsequent stimulation with nonresponder parental GAT-Mφ.

Third, soluble GAT-primed (responder × nonresponder)F1 spleen cells have a population of suppressor T cells which inhibit primary PFC responses of virgin F1 spleen cells stimulated with nonresponder parental GAT-Mφ. Thus, soluble GAT stimulates at least two populations of T cells in F1 mice; one functions as helper T cells for responses stimulated by responder parental GAT-Mφ, the other functions as suppressor T cells in responses of virgin F1 spleen cells to nonresponder parental and unrelated GAT-Mφ. By contrast, spleen cells from F1 mice immunized with F1 GAT-Mφ have helper T cells which can be stimulated by both responder and nonresponder parental GAT-Mφ and suppressor T cells which are active in suppressing responses of virgin F1 spleen cells stimulated with unrelated third party C3H/He GAT-Mφ. This last observation is consistent with results where suppressor T-cell activity is elicited in spleen cells from responder mice immunized with syngeneic or allogeneic GAT-Mφ by any GAT-Mφ allogeneic to the Mφ used for immunization (8). The behavior of
these suppressor T cells suggests they recognize GAT alone and not in the context of a Mφ membrane complex.

These apparently paradoxical observations can be reconciled by considering the immunobiology of Ir gene control of antibody responses to GAT and the concept that subsets of T cells exist in F1 animals, each of which is capable of interacting with one, but not the other, parental Mφ (17–19). Responder strains of mice, including (responder × nonresponder)F1 mice, develop GAT-specific, radioreistant helper T cells after immunization with soluble GAT, GAT-Mψ, and GAT-MBSA that function only when stimulated by GAT presented on Mψ syngeneic to the Mψ which presented the GAT during the in vivo immunization process (2–4, 6, 8). Radiosensitive, GAT-specific suppressor T cells are present in these populations of immune T cells which suppress primary PFC responses stimulated by GAT on Mψ allogeneic to the Mψ which presented GAT during the immunization process (8). Thus, responder mice can develop both GAT-specific helper and suppressor T cells after immunization with GAT. In nonresponder mice, soluble GAT preferentially stimulates suppressor T cells which appear to block or inhibit the development of helper T cells (1–4). However, because nonresponder mice can develop radioreistant, GAT-specific helper T cells after immunization with GAT-Mψ and GAT-MBSA (2–4), the defect in nonresponder mice is not a failure of T cells to recognize GAT or a lack of the capacity to develop helper cells. However, simultaneous administration of soluble GAT and GAT-MBSA to nonresponder mice abrogates development of helper T cells and only suppressor T cells are demonstrated (1–3). Thus, as in responder mice, nonresponder mice can develop both helper and suppressor T cells specific for GAT, but the physical state of the GAT (soluble vs. insoluble as GAT-Mψ or GAT-MBSA) appears to be the major factor determining whether helper or suppressor T cells predominate. This suggests that the Ir gene product does not encode a recognition unit for GAT, but functions as a regulatory gene after recognition of GAT by the T cells. Further, GAT-specific suppressor T cells in both responder and nonresponder mice are unable to inhibit the function of immune helper cells, but readily inhibit stimulation of virgin helper T cells and therefore the development of primary PFC responses (1–3, 8). This may account for the failure of nonresponder mice immunized with soluble GAT to develop either primary PFC responses or helper T cells.

Distinct subsets of T cells, each capable of interacting with antigen presented on one but not the other parental Mψ have been demonstrated in F1 guinea pigs (17) and mice (18, 19). Because both responder and nonresponder parental mice can develop helper and suppressor T cells after exposure to the appropriate form of GAT, extension of the F1 T-cell subset concept leads to the hypothesis that in (responder × nonresponder)F1 mice, subsets of T cells exist which, after immunization with soluble GAT, behave functionally as responder or nonresponder parental T cells and that a phenotypic or functional allelic exclusion of the Ir gene function occurs in these subsets. Many of the predictions of this hypothesis have been borne out in the present experiments. First, immunization of F1 mice with F1 GAT-Mψ stimulates helper T cells capable of being stimulated by either parental GAT-Mψ, whereas immunization with either parental GAT-Mψ alone stimulates helper cells capable of responding subsequently only to the immunizing parental GAT-Mψ. Thus, the two subsets of T cells which function as helper T cells with the appropriate parental GAT-Mψ have been demonstrated. Moreover, suppressor T cells which inhibit responses to Mψ allogeneic to those used for immunization have been demonstrated in F1 spleen cells
of mice immunized by F1 GAT-Mφ as predicted. After immunization with soluble GAT, F1 spleen cells contain helper cells which function only when stimulated with responder parental GAT-Mφ and, most significantly, contain suppressor T cells which function with nonresponder parental GAT-Mφ as well as unrelated GAT-Mφ. Thus, subsets of T cells are stimulated in F1 mice by soluble GAT which behave phenotypically as responder parental (helper cells) and nonresponder parental (suppressor cells). Definitive proof of the hypothesis awaits experiments selecting the four respective subsets of T cells in these F1 mice.

Summary

The ability of spleen cells from (responder × nonresponder)F1 mice immunized with various GAT-Mφ, GAT-MBSA, and soluble GAT to develop IgG GAT-specific PFC responses in vitro after stimulation with responder and nonresponder parental and F1 GAT-Mφ was investigated. F1 spleen cells from mice immunized with F1 GAT-Mφ or GAT-MBSA developed secondary responses to responder and nonresponder parental and F1 GAT-Mφ, but not to unrelated third party GAT-Mφ. Spleen cells from F1 mice immunized with either parental GAT-Mφ developed secondary responses to F1 GAT-Mφ and only the parental GAT-Mφ used for immunization in vivo. Soluble GAT-primed F1 spleen cells responded to F1 and responder parental, but not nonresponder parental, GAT-Mφ. Simultaneous immunization in vivo with the various GAT-Mφ or GAT-MBSA plus soluble GAT modulated the response pattern of these F1 spleen cells such that they developed secondary responses only to F1 and parental responder GAT-Mφ regardless of the response pattern observed after immunization with the various GAT-Mφ or GAT-MBSA alone. These observations demonstrate the critical importance of the physical state of the GAT used for immunization in determining the subsequent response pattern of immune F1 spleen cells to the parental and F1 GAT-Mφ. Further, suppressor T cells, capable of inhibiting primary responses to GAT by virgin F1 spleen cells stimulated by nonresponder parental GAT-Mφ, were demonstrated in spleens of F1 mice immunized with soluble GAT, but not those primed with F1 GAT-Mφ. Because responder parental mice develop both helper and suppressor T cells after immunization with GAT-Mφ, and soluble GAT preferentially stimulates suppressor T cells whereas GAT-Mφ stimulate helper T cells in nonresponder parental mice, these observations suggest that distinct subsets of T cells exist in F1 mice which behave phenotypically as responder and nonresponder parental T cells after immunization with soluble GAT and GAT-Mφ.

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