

SUPPRESSOR T-CELL ACTIVITY IN RESPONDER ×
NONRESPONDER (C57BL/10 × DBA/1)F₁ SPLEEN CELLS
RESPONSIVE TO L-GLUTAMIC ACID⁶⁰-L-ALANINE³⁰-L-
TYROSINE¹⁰*

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Antibody responses of mice to the terpolymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT)¹ are controlled by an H-2-linked immune response (Ir) gene(s). The lack of response in nonresponder mice (H-2^{p^qs}) 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 ϕ) (2-4). Several observations indicate that the Ir gene defect is not expressed in M ϕ , at least in responses to GAT. First, responder and nonresponder M ϕ bind comparable amounts of GAT in vitro (5, 6). Second, allogeneic nonresponder and syngeneic responder GAT-M ϕ stimulate comparable primary IgG plaque-forming cell (PFC) responses by responder lymphoid cells in vitro (6). Third, nonresponder GAT-M ϕ stimulate GAT-specific helper T cells in responder mice in vivo that function in vitro only when stimulated with GAT on M ϕ syngeneic with the M ϕ used for initial immunization (6, 7).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 ϕ 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 ϕ -T cell interactions in other systems. (Responder × nonresponder)F₁ 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 ϕ (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 acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰; GAT-MBSA, GAT-complexed to methylated bovine serum albumin; GAT-M ϕ , GAT-pulsed macrophage(s); Ir gene, immune response gene(s); M ϕ , macrophages; PFC, plaque-forming cell(s).

² C. W. Pierce, and J. A. Kapp. Manuscript in preparation.

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

In the GAT-system, virgin (responder \times nonresponder)F₁ spleen cells develop comparable primary PFC responses to both parental and unrelated third party GAT-M ϕ . F₁ spleen cells from mice immunized with responder or nonresponder parental GAT-M ϕ develop secondary responses only when stimulated with the parental GAT-M ϕ used for immunization or F₁ GAT-M ϕ (14). These results are entirely consistent with the restrictions on M ϕ -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-M ϕ .

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

To analyze the paradox described above we have examined secondary PFC responses by (responder \times nonresponder)F₁ spleen cells from mice immunized with parental responder, nonresponder, and F₁ GAT-M ϕ and the effect of injecting soluble GAT simultaneously with GAT-M ϕ 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-2^b), DBA/2 (H-2^d), DBA/1 (H-2^a), C3H/He (H-2^k), (C57BL/10 \times DBA/2)F₁ (H-2^{b/d}), and (C57BL/10 \times DBA/1)F₁ (H-2^{b/a}) 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 \approx 45,000), was prepared as previously described for use as soluble GAT or GAT-MBSA in culture (15), preparing GAT-M ϕ (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-M ϕ as 1-ml cultures under modified Mishell-Dutton conditions (16). In some experiments, primed (C57BL/10 \times 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-M ϕ and Immunization of Mice. The preparation of GAT-M ϕ 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 \times 10⁶ of the appropriate GAT-M ϕ (bearing \approx 25 ng GAT/10⁶ cells); (b) 10 μ g 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
PFC Responses to GAT by Responder × Responder (C57BL/10 × DBA/2)F₁ and Responder × Nonresponder (C57BL/10 × DBA/1)F₁ Spleen Cells Stimulated by Parental and F₁ GAT-Mφ

Spleen cells	Day 5 IgG GAT-specific PFC/culture*				
	C57BL/10 Mφ	DBA/2 Mφ	DBA/1 Mφ	(C57BL/10 × DBA/2)F ₁ Mφ	(C57BL/10 × DBA/1)F ₁ Mφ
(C57BL/10 × DBA/2)F ₁					
Virgin	210	280	290	260	ND‡
GAT primed§	420	430	20	570	ND
Primed with (C57BL/10 × DBA/2)F ₁ GAT-Mφ	280	250	30	240	ND
(C57BL/10 × DBA/1)F ₁					
Virgin	240	240	260	ND	320
GAT primed§	240	<10	<10	ND	200
Primed with (C57BL/10 × DBA/1)F ₁ GAT-Mφ	610	<10	540	ND	670
GAT-MBSA primed¶	640	10	840	ND	700

* Virgin or immune (C57BL/10 × DBA/2)F₁ or (C57BL/10 × DBA/1)F₁ spleen cells at 10⁷ cells/culture were incubated with 5 × 10⁴ of the indicated GAT-Mφ bearing ≈ 1 ng GAT/10⁵ 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/10⁵ 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 × 10⁵ cells/ml, and added to cultures in 0.1 ml volumes. GAT bound per 5 × 10⁴ 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 syngenicity 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-M ϕ . Third, as observed previously (14), (responder \times nonresponder) F_1 spleen cells from mice immunized with soluble GAT responded to F_1 or responder parental, but not nonresponder parental or unrelated third party, GAT-M ϕ . However, if these F_1 mice were immunized with F_1 GAT-M ϕ or GAT-MBSA, their spleen cells responded to F_1 and both parental GAT-M ϕ , but not third party DBA/2 GAT-M ϕ . Thus, (responder \times nonresponder) F_1 spleen cells exhibit restrictions in secondary responses with regard to unrelated third party GAT-M ϕ regardless of how the spleen cells were primed. However, immunization with F_1 GAT-M ϕ 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 F_1 spleen cells respond to nonresponder parental GAT-M ϕ .

Effects of Soluble GAT on Response Patterns to Parental and F_1 GAT-M ϕ and GAT-MBSA in (Responder \times Nonresponder) F_1 Mice. The hypothesis tested with the experiments in Table II was that simultaneous administration of soluble GAT and parental or F_1 GAT-M ϕ or GAT-MBSA to (responder \times nonresponder) F_1 mice will convert the response pattern normally induced by that immunizing agent to the response pattern normally induced by soluble GAT. Virgin F_1 spleen cells and spleen cells from F_1 mice primed with soluble GAT responded as observed previously. It is possible that these results reflect an artifact induced by pulsing M ϕ with GAT in vitro at 4°C in serum-free medium at \geq pH 9. Therefore, C57BL/10 and DBA/1 peritoneal exudate M ϕ were pulsed under physiological conditions by injecting 10 μ g GAT into the peritoneal cavity and harvesting the cells 1 h later. These M ϕ bound quantities of GAT comparable to M ϕ pulsed in vitro and stimulated responses in virgin and GAT-primed F_1 spleen cells comparable to those stimulated by in vitro pulsed parental M ϕ (Table II, exp. 1 and 2). Thus, the ability of nonresponder parental GAT-M ϕ to stimulate primary responses and their failure to stimulate secondary responses in GAT-primed F_1 spleen cells was not due to an artifact, such as denaturation of GAT, induced by the conditions employed for in vitro pulsing of M ϕ . As expected from previous experiments (14), spleen cells from F_1 mice primed with responder (C57BL/10) or nonresponder (DBA/1) parental GAT-M ϕ responded to F_1 and the immunizing parental GAT-M ϕ , but failed to respond to the opposite parental or unrelated third party GAT-M ϕ (Table II, exp. 1). This response pattern was not altered by addition of soluble GAT to the immunizing C57BL/10 GAT-M ϕ , but soluble GAT induces the same response pattern as C57BL/10 GAT-M ϕ in these F_1 mice and no alteration was expected. By contrast, soluble GAT completely reversed the response pattern in F_1 mice immunized with DBA/1 GAT-M ϕ . Instead of being restricted for and responding to the immunizing DBA/1 GAT-M ϕ , these F_1 mice responded identically to those immunized with soluble GAT alone and developed PFC responses only when stimulated with responder parental C57BL/10 or F_1 GAT-M ϕ (Table II, exp. 1). Further, the response patterns of F_1 mice primed with F_1 GAT-M ϕ (Exp. 2) and GAT-MBSA (Exp. 3), which developed secondary responses when stimulated with either parental GAT-M ϕ , were altered in a similar manner by inclusion of soluble GAT in the immunizing regimen. These F_1 spleen cells responded to responder parental C57BL/10 and F_1 GAT-M ϕ , but no longer responded to the nonresponder parental DBA/1 GAT-M ϕ .

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

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

(C57BL/10 × DBA/1)F ₁ Spleen cells	Day 5 IgG GAT-specific PFC/culture*			
	C57BL/10 Mφ	DBA/1 Mφ	(C57BL/10 × DBA/1)F ₁ Mφ	C3H/He Mφ
Experiment 1				
Virgin	980 (980)‡	800 (750)	900 ND§	1270 ND
GAT primed	610 (790)	50 (<10)	760 ND	40 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 (360)	420 (570)	320	430
GAT primed	650 (560)	<10 (<10)	680	<10
(C57BL/10 × DBA/1)F ₁ GAT-Mφ primed¶	620	780	630	80
(C57BL/10 × DBA/1)F ₁ 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 10⁷ 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 × 10⁴ C57BL/10 or DBA/1 Mφ pulsed *in vivo* by injection of 10 μg GAT containing 1% ¹²⁵I-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.

|| (C57BL/10 × DBA/1)F₁ mice were immunized 28 days previously by intraperitoneal injection of 10 μg GAT in Maalox-pertussis.

¶ (C57BL/10 × DBA/1)F₁ mice 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.

** (C57BL/10 × DBA/1)F₁ mice 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.

‡‡ (C57BL/10 × DBA/1)F₁ mice were immunized 28 days previously by intraperitoneal injection of 10 μg GAT as GAT-MBSA.

§§ (C57BL/10 × DBA/1)F₁ mice 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

F ₁ spleen cells ($\times 10^{-6}$)/culture			Day 5 IgG GAT-specific PFC/culture*			
Virgin	GAT primed‡	F ₁ GAT-Mφ primed‡	C57BL/10 Mφ	DBA/1 Mφ	(C57BL/10 × DBA/1)F ₁ Mφ	C3H/He Mφ
10	—	—	480	570	420	400
14	—	—	330	350	290	420
—	10	—	540	30	620	40
—	—	10	610	520	530	<10
10	4	—	400	30	490	<10
10	—	4	480	460	440	<10

* Virgin or immune (C57BL/10 × DBA/1)F₁ spleen cells were incubated with 5×10^4 of the indicated GAT-Mφ bearing ≈ 2 ng GAT/10⁵ 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 3×10^6 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

F ₁ Spleen cells ($\times 10^{-6}$)/culture			Day 5 IgG GAT-specific PFC/culture*			
Virgin	GAT-Primed‡	Anti-Thy 1 + C Treated GAT-Primed	C57BL/10 Mφ	DBA/1 Mφ	(C57BL/10 × DBA/1)F ₁ Mφ	C3H Mφ
10	—	—	290	240	240	260
14	—	—	290	210	280	240
—	10	—	710§	10	630	60
—	—	10	<10§	ND	ND	ND
10	4	—	250	40	190	<10
10	—	4	230	250	190	220

* Virgin or immune (C57BL/10 × DBA/1)F₁ spleen cells at the indicated cell densities were incubated with 5×10^4 of the indicated GAT-Mφ bearing ≈ 1 ng GAT/10⁵ 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

F₁ spleen cells stimulated with nonresponder parental GAT-M ϕ , but had suppressor cells that were activated by unrelated third party C3H/He GAT-M ϕ . 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 \times nonresponder)F₁ spleen cells from mice immunized with F₁ GAT-M ϕ or GAT-MBSA develop secondary PFC responses when stimulated with responder and nonresponder parental GAT-M ϕ , but not when stimulated with unrelated third party C3H/He GAT-M ϕ . These observations are in contrast to responses of soluble GAT-primed F₁ spleen cells, which respond to F₁ and responder parental GAT-M ϕ but not to nonresponder parental GAT-M ϕ , 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-M ϕ and GAT-MBSA are consistent with responses of F₁ spleen cells from mice primed with nonresponder parental GAT-M ϕ and demonstrate further that F₁ mice have T cells that can be primed by and subsequently respond to GAT presented on nonresponder parental M ϕ . Collectively, these observations argue against functional expression of the Ir genes controlling the response to GAT in M ϕ .

Second, spleen cells from (responder \times nonresponder)F₁ mice immunized with GAT-M ϕ or GAT-MBSA plus soluble GAT develop secondary PFC responses when stimulated with F₁ or responder parental GAT-M ϕ , but not nonresponder parental GAT-M ϕ , regardless of the response pattern of the F₁ spleen cells immunized with GAT-M ϕ or GAT-MBSA alone. Thus, soluble GAT does not alter the response pattern of spleen cells from mice immunized with responder parental GAT-M ϕ which normally respond only to those GAT-M ϕ . However, soluble GAT clearly modulated the secondary response patterns of F₁ spleen cells immunized simultaneously with nonresponder parental or F₁ GAT-M ϕ or GAT-MBSA such that these F₁ spleen cells no longer respond to nonresponder parental GAT-M ϕ . 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-M ϕ .

Third, soluble GAT-primed (responder \times 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-M ϕ . 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-M ϕ , the other functions as suppressor T cells in responses of virgin F₁ spleen cells to nonresponder parental and unrelated GAT-M ϕ . By contrast, spleen cells from F₁ mice immunized with F₁ GAT-M ϕ have helper T cells which can be stimulated by both responder and nonresponder parental GAT-M ϕ and suppressor T cells which are active in suppressing responses of virgin F₁ spleen cells stimulated with unrelated third party C3H/He GAT-M ϕ . 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-M ϕ by any GAT-M ϕ allogeneic to the M ϕ used for immunization (8). The behavior of

these suppressor T cells suggests they recognize GAT alone and not in the context of a M ϕ 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 M ϕ (17-19). Responder strains of mice, including (responder \times nonresponder)F₁ mice, develop GAT-specific, radioresistant helper T cells after immunization with soluble GAT, GAT-M ϕ , and GAT-MBSA that function only when stimulated by GAT presented on M ϕ syngeneic to the M ϕ 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 M ϕ allogeneic to the M ϕ 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 radioresistant, GAT-specific helper T cells after immunization with GAT-M ϕ 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-M ϕ 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 M ϕ 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 \times 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-M ϕ stimulates helper T cells capable of being stimulated by either parental GAT-M ϕ , whereas immunization with either parental GAT-M ϕ alone stimulates helper cells capable of responding subsequently only to the immunizing parental GAT-M ϕ . Thus, the two subsets of T cells which function as helper T cells with the appropriate parental GAT-M ϕ have been demonstrated. Moreover, suppressor T cells which inhibit responses to M ϕ allogeneic to those used for immunization have been demonstrated in F₁ spleen cells

of mice immunized by F₁ GAT-M ϕ as predicted. After immunization with soluble GAT, F₁ spleen cells contain helper cells which function only when stimulated with responder parental GAT-M ϕ and, most significantly, contain suppressor T cells which function with nonresponder parental GAT-M ϕ as well as unrelated GAT-M ϕ . 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 \times nonresponder)F₁ mice immunized with various GAT-M ϕ , GAT-MBSA, and soluble GAT to develop IgG GAT-specific PFC responses in vitro after stimulation with responder and nonresponder parental and F₁ GAT-M ϕ was investigated. F₁ spleen cells from mice immunized with F₁ GAT-M ϕ or GAT-MBSA developed secondary responses to responder and nonresponder parental and F₁ GAT-M ϕ , but not to unrelated third party GAT-M ϕ . Spleen cells from F₁ mice immunized with either parental GAT-M ϕ developed secondary responses to F₁ GAT-M ϕ and only the parental GAT-M ϕ used for immunization in vivo. Soluble GAT-primed F₁ spleen cells responded to F₁ and responder parental, but not nonresponder parental, GAT-M ϕ . Simultaneous immunization in vivo with the various GAT-M ϕ 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-M ϕ regardless of the response pattern observed after immunization with the various GAT-M ϕ 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-M ϕ . Further, suppressor T cells, capable of inhibiting primary responses to GAT by virgin F₁ spleen cells stimulated by nonresponder parental GAT-M ϕ , were demonstrated in spleens of F₁ mice immunized with soluble GAT, but not those primed with F₁ GAT-M ϕ . Because responder parental mice develop both helper and suppressor T cells after immunization with GAT-M ϕ , and soluble GAT preferentially stimulates suppressor T cells whereas GAT-M ϕ 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-M ϕ .

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