IMMUNE SUPPRESSION IN VIVO WITH ANTIGEN-MODIFIED SYNGENEIC CELLS

I. T-Cell-Mediated Suppression to the Terpolymer poly-(Glu, Lys, Phe)ₙ

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Intravenous injection of hapten-conjugated syngeneic cells results in specific immunological tolerance affecting both hapten-specific contact sensitivity and plaque-forming cell responses (1–4). Although the underlying basis for this nonresponsiveness is not fully understood, it appears that both the T- and B-cell compartments may be affected (3–5). In addition, several studies have reported the generation of specific suppressor T cells under these conditions (4–6).

The antigen that was used to induce specific nonresponsiveness (suppression) in these studies was the synthetic linear polypeptide of L-glutamic acid, L-lysine and L-phenylalanine (GLϕ). This antigen was chosen for several reasons. (a) The immune response to GLϕ is under histocompatibility-linked immune response gene control (7). (b) Although previous attempts to identify GLϕ specific suppressor T cells in nonresponder mice have been unsuccessful (8, 9), we questioned whether induction with antigen-modified syngeneic cells would be a more efficient means of inducing T suppressor cells. (c) In addition, we sought to provide evidence that nonresponder mice could generate functional T cells with specificity for GLϕ.

To date, all systems that analyzed nonresponsiveness induced with antigen-modified syngeneic cells employed small, chemically reactive molecules which were covalently conjugated to cell surface proteins. In the present study, we have used a different method of coupling antigen to spleen cells. This new coupling method uses the ability of palmitoyl-derivatized polypeptides to adhere to cell membranes. Thus, polypeptides can be coupled onto spleen cells without excessive chemical modification of the peptide and without the formation of covalent bonds between the polypeptide antigens and any cell surface molecules. We now demonstrate that the intravenous administration of syngeneic spleen cells modified with a palmitoyl-derivatized polypeptide results in antigen-specific nonresponsiveness and the induction of specific suppressor T cells.

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Abbreviations used in this paper: C, complement; GL, poly-(L-Gluₙ, L-Lysₙ); GLϕ, poly-(L-Gluₙ, L-Lysₙ, L-Pheₙ); FYG, fowl gamma globulin; GLϕ-FYG, covalent conjugate of GLϕ and FYG; GL-FYG, covalent conjugate of GL and FYG; M/P, suspension of Maalox and pertussis vaccine in PBS; MEM, minimal essential medium; NMS, normal mouse serum; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SRBC, sheep erythrocytes; p-GLϕ, the palmitoyl derivative of GLϕ; p-GLϕ-spl cells, p-GLϕ-coupled spleen cells.

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Materials and Methods

Mice. C57BL/6 male mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. All experimental animals were between 8 and 12 wk of age at the beginning of immunization. Animals were age matched in each experiment.

Antigen. The linear terpolymers poly-(t-Glu56-l-Lys35-l-Phe)₉₉, GLΦ, lot GLPI, average mol wt 38,000, and poly-(t-Glu60-l-Lys60)₉₉, GL, lot L641, average mol wt 40,000 were prepared by Miles Yeda, Ltd., Rehovot, Israel. Fowl gamma globulin (FyG) was purchased from Miles Laboratories, Inc., Elkhart, Ind. GLΦ-FyG and GL-FyG were synthesized with the Schiff base method (10). The GLΦ-FyG or GL-FyG conjugates along with unreacted FyG were separated from the unreacted GLΦ or GL with 45 or 100% saturated ammonium sulfate respectively, and then dialyzed extensively in phosphate-buffered saline (PBS).

Immunization. Mice were immunized i.p. in a volume of 0.2 ml with 20 μg of GLΦ conjugated to FyG. The adjuvant was a mixture of 1% Maalox and 25% pertussis vaccine (M/P). Maalox was purchased from William H. Rorer, Inc., Fort Washington, Pa. and pertussis vaccine was purchased from Eli Lilly and Co., Indianapolis, Ind.

Palmitoyl-GLΦ. The palmitoyl derivative of GLΦ (p-GLΦ) was synthesized by the method of Cheung et al. (10). Trace amounts of palmitoyl-GLT²-I² were used to estimate the amount of antigen bound per cell (10).

Induction of Suppression. Twice-washed single spleen cell suspensions were pelleted and resuspended in 1 mg/ml p-GLΦ in PBS. 2 μl of the p-GLΦ solution was used for every 10⁶ spleen cells. Control cells were incubated in PBS instead of p-GLΦ. After incubation at 37°C for 30 min, the cells were twice washed in minimal essential medium (MEM) containing 0.3% heparin. After they were washed, the cells were resuspended to 6 × 10⁷ cells/ml, and 0.5-ml quantities were injected per mouse, i.v.

Adoptive Transfer. 5 days after being primed with p-GLΦ-coupled spleen cells, mice were sacrificed, the recipient spleens were teased in sterile MEM with 0.3% heparin, and the cells were washed twice and resuspended in heparinized MEM for i.v. injection into normal recipients. These recipient mice were then immediately challenged with 20 μg GLΦ-FyG in Maalox and pertussis adjuvant i.p., and the GLΦ and FyG-specific spleen plaque-forming cells (PFC) assayed 8 days afterwards.

Anti-Thy-1 Treatment. 10⁶ suppressor spleen cells from p-GLΦ-cell primed mice were pelleted and incubated with 0.5 ml of a 1:2 dilution of AKR anti-C3H thymocyte serum or normal mouse serum as control. After 30 min at 20°C the serum was removed and 0.5 ml rabbit complement diluted 1:5 was added in L-15 medium with 1% DNase for a 30-min incubation at 37°C and the cells were resuspended in the same media for i.v. injection.

Cyclophosphamide Treatment. Cyclophosphamide (Cytoxan) was purchased from Mead Johnson & Co., Evansville, Ind. The drug was administered intraperitoneally in the dose of 5 mg/kg. 2 days after cytoxan treatment, 3 × 10⁶ p-GLΦ-coupled spleen cells (p-GLΦ-spl cells) were injected i.v. to induce tolerance.

PFC Assay. The antibody responses to GLΦ-FyG and GL-FyG were enumerated 7 or 8 days after immunization by a modification of the Jerne hemolytic plaque technique (10). GLΦ-sheep erythrocytes (SRBC) or GL-SRBC were prepared with respective palmitoyl conjugates (10). FyG-SRBC were prepared by incubating a chicken anti-SRBC antiserum with washed SRBC at 37°C for 30 min (9). A goat anti-μ antiserum provided by Dr. R. Asofsky, National Institutes of Health, Bethesda, Md. was used to inhibit IgM PFC, and a rabbit anti-mouse IgG was used to develop IgG PFC. Only PFC that were inhabitable with 25–50 μg per slide of free ligand were reported as antigen-specific plaques. Generally, 95–100% PFCs are specifically inhabitable. All results are expressed as PFC per spleen. The cell recovery per spleen did not vary significantly between groups.

Statistical Analysis. All data were analyzed for significance by using a two-tailed Student's t test, performed on a Wang 700 calculator (Wang Laboratories, Inc., Lowell, Mass.).

Results

GLΦ-Specific Nonresponsiveness. When appropriate quantities of p-GLΦ were injected i.v. into C57BL/6 mice, GLΦ-specific nonresponsiveness was induced (Table I). In the
first experiment mice were injected with 10 or 1 µg of p-GLΦ in PBS i.v. Three days later, they were immunized with 20 µg GLΦ-FyG in M/P (day 0). On day 8 their spleens were assayed for GLΦ-specific and FyG-specific PFC responses. When 10 µg p-GLΦ was injected, both the IgM and IgG anti-GLΦ responses were significantly reduced (Table I); however, there was no reduction of the FyG response (vide infra). In contrast, no significant tolerance was noted when 1 µg of p-GLΦ was used as pretreatment. In separate experiments 10 ng to 1 µg doses of soluble p-GLΦ also failed to affect the GLΦ-specific PFC response, whereas 10-µg doses were again tolerogenic (data not shown). It was likely that hydrophobic molecules such as p-GLΦ reacted with both serum proteins and cells. However, because the cell-bound antigen may be more effective in producing nonresponsiveness, the next experiment was carried out to elucidate the importance of cell surface-associated GLΦ molecules (Table I). Mice were injected with 10⁷ GLΦ-coupled syngeneic spleen cells (p-GLΦ-spl cells) or with 10⁷ control spleen cells. 3 days later they were immunized with 20 µg GLΦ-FyG in M/P (day 0). 8 days later their spleens were assayed for GLΦ PFC responses. In mice that received p-GLΦ-spl, both the IgM and IgG anti-GLΦ responses were decreased by 70–80%, compared with mice that received control cells. The efficiency of cell surface-associated GLΦ in the induction of tolerance was evident from the trace labeling studies. The data indicated that 0.1-µg quantities of GLΦ were carried by the 10⁷ p-GLΦ-spl cells able to induce tolerance, whereas, as demonstrated above, 0.01–1-µg quantities of p-GLΦ (when administered i.v. as the soluble form) were not able to decrease the GLΦ PFC response to GLΦ-FyG (Table I).

To demonstrate that the decrease in the GLΦ PFC response on day 8 after immunization was not the result of a shift in the appearance of the peak of the antibody response, the PFC responses of the tolerized mice were analyzed at various days after immunization. As shown in Fig. 1A, day 8 after immunization was the peak of the PFC response for both control and tolerant mice. Moreover, the immune response of the tolerant mice remained significantly diminished throughout the period of day 6 to day 12. This tolerance is GLΦ specific, because the anti-FyG PFC response was not affected (Fig. 1B). Furthermore, the PFC response to trinitrophenyl-keyhole

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### Table I

| Experiment number | Pretreatment | Number of mice | GLΦ-specific PFC/spleen ± SE* |
|-------------------|-------------|----------------|-------------------------------|
| 1                 | PBS‡ 10 g | 7              | IgM 8,471 ± 882, IgG 8,014 ± 917 |
|                   | 10 µg p-GLΦ | 6             | IgM 3,150 ± 495, IgG 3,200 ± 650 |
|                   | 1 µg p-GLΦ | 3             | IgM 6,600 ± 435, IgG 8,200 ± 251 |
| 2                 | 10⁷ Normal spl cells§ | 3 | IgM 26,133 ± 634, IgG 36,467 ± 1,471 |
|                   | 10⁷ GLΦ-spl cells | 4 | IgM 7,400 ± 1,779, IgG 8,900 ± 2,399 |

* Arithmetic mean of PFC response ± SE.
‡ C57BL/6 mice were injected with 10 or 1 µg p-GLΦ i.v. Control mice received PBS. 3 days later they were immunized with 20 µg GLΦ-FyG in M/P (day 0) and their spleens were assayed 8 days later (day 8).
§ p-GLΦ was coupled onto spleen cells. To induce tolerance, 10⁷ GLΦ-spl cells were injected into C57BL/6 mice. Control mice received 10⁷ normal spleen cells. 3 days later both groups were immunized with 20 µg GLΦ-FyG in M/P. On day 8 their spleens were assayed.
|| Indicates P < 0.01.
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Fig. 1. (A) Kinetics of GLΦ-specific PFC response in control (-----) and tolerant (-----) C57BL/6 mice. 3 days after the administration of $3 \times 10^8$ p-GLΦ-spl cells or normal cells, mice were immunized with 20 μg GLΦ-FyG in M/P (day 0). Their spleens were assayed at various days after immunization. GLΦ-specific IgM response is depicted as open circles (○) and IgG as solid circles (●). Each point represents the arithmetic mean of the PFC response of three to five mice ± SE. (B) Kinetics of FyG-specific PFC response in control (-----) and tolerant (-----) C57BL/6 mice. Only IgG PFC responses were assayed.

limpet hemocyanin or horse erythrocytes was not decreased in mice that were given an i.v. injection of GLΦ spleen cells, confirming the specificity of the p-GLΦ-spl-induced nonresponsiveness (data not shown).

Fine Specificity of GLΦ Nonresponsiveness. We have previously demonstrated that the antibody responses to GLΦ and GL are highly cross-reactive (7) and that these antibodies share idiotypic determinants (11). Therefore, we tested the fine specificity of GLΦ nonresponsiveness with the cross-reacting polymer GL. Nonresponsiveness was induced in C57BL/6 mice with GLΦ-palmitoyl-coupled syngeneic cells. 3 days after induction, mice were challenged with either GLΦ-FyG or GL-FyG. As shown in Table II, the GLΦ- and GL-specific PFC responses were significantly reduced, while the FyG-PFC responses were not affected. Thus, the effector mechanism(s) which mediate this tolerance can not distinguish between the closely related cross-reactive polypeptides GL and GLΦ.
TABLE II
Fine Specificity of GLφ Tolerance

| Pretreatment* | Challenge   | GLφ or GL | IgM       | GLφ or GL | IgG       | FyG | IgG   |
|---------------|-------------|-----------|-----------|-----------|-----------|-----|-------|
| Control cells | GLφ-FyG     | 14,000 ± 5,630 | 6,450 ± 3,007 | 14,437 ± 4,732 |
| GLφ-spl       | GLφ-FyG     | 2,512 ± 1,239§ | 1,312 ± 1,214§ | 15,975 ± 2,667 |
| Control cells | GL-FyG      | 12,727 ± 4,335 | 9,437 ± 3,559 | 13,050 ± 8,395 |
| GLφ-spl       | GL-FyG      | 4,387 ± 1,144§ | 1,987 ± 1,245§ | 11,287 ± 4,421 |

* C57BL/6 mice were given 3 × 10^7 GLφ coupled spleen cells, i.e., 3 days before challenge with 20 μg GLφ-FyG or GL-FyG. Animals were sacrificed 8 days after challenge for PFC assay.

§ Arithmetic mean of PFC response ± SE; three to four mice are included in each group.

Dose Requirements for Induction of Nonresponsiveness. To establish the optimal dose of p-GLφ-spl cells used for tolerance induction, C57BL/6 mice were injected with 2 × 10^4 to 3 × 10^5 p-GLφ-spl cells, while control groups received equivalent numbers of normal spleen cells. 3 days later, the mice were immunized with 20 μg GLφ-FyG in M/P (day 0) and their spleens were assayed on day 8. The degree of tolerance in the experimental mice was expressed as percent reduction of the control PFC response. As shown in Fig. 2, there was a dose dependence for tolerance induction by p-GLφ-spl. At least 2 × 10^5 cells were required for optimal reduction of both IgM and IgG responses. Trace labeling studies revealed that 2 ng GLφ was carried on 2 × 10^5 p-GLφ-spl cells.

Kinetics of Tolerance Induction. The time required for the development of nonresponsiveness and the tolerant state was next investigated. C57BL/6 mice were treated with 3 × 10^7 p-GLφ-spl cells i.v., while control groups were given 3 × 10^7 normal spleen cells. As shown in Fig. 3, groups of mice were immunized with 20 μg GLφ-FyG in M/P at various times after receiving control or GLφ-coupled spleen cells, and their spleens were assayed 8 days later. Under optimal conditions GLφ-specific IgM and IgG responses were decreased by 80-90% of control responses. The induction of nonresponsiveness required a latent period (1-2 days before challenge) and the state of tolerance lasted for at least 63 days after injection.

p-GLφ-spl Cells Induce GLφ-Specific Suppressor Cells. Since these antigen modified cells induced such a strong state of nonresponsiveness lasting at least 2 mo, an active suppressor mechanism was considered to be involved. Two transfer experiments were performed in which similar results were obtained in each experiment. Donor animals were sacrificed 5 days after tolerance induction with p-GLφ-spl and their spleen cells were transferred into normal syngeneic recipients. Control mice received an equal number of normal spleen cells. All mice were immediately challenged with 20 μg GLφ-FyG in M/P and 8 days later their spleens were assayed. 2 × 10^7 viable spleen cells from p-GLφ-spl-treated donors led to significant (60-70%, P < 0.01) suppression in the recipient animals (Table III). The treatment of such suppressor spleen cells with anti-Thy 1.2 serum plus complement (C) but not with normal mouse serum (NMS) plus C before transfer removed the suppressive activity (Table III). Thus, the suppression of GLφ responses is a T-cell-dependent process.

Induction is Cyclophosphamide Sensitive. Since suppressor T cells have been shown to
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Fig. 2. Dose requirement for tolerance induction in C57BL/6 mice. Groups of mice (four to six mice/group) were injected i.v. with $2 \times 10^6$ to $3 \times 10^7$ p-GLΦ-spl cells while control groups received similar numbers of normal spleen cells. 3 days later they were challenged with 20 μg GLΦ-FyG in M/P (day 0) and their spleens were assayed for GLΦ-specific PFC response on day 8. Results are expressed as percent suppression of the control group responses. Control IgM and IgG responses averaged 10,000–20,000 PFC/spleen. Each point represents the arithmetic mean of percent suppression of control PFC responses ± SE. Open circles (○) denote percent suppression of IgM while solid circles (●) represent that of IgG PFC response.

Fig. 3. Kinetics of tolerance induction in C57BL/6 mice. Mice were injected with $3 \times 10^7$ p-GLΦ-spl cells or control spleen cells and at various times (day 0 to day 63) afterward challenged with 20 μg GLΦ-FyG in M/P (day 0). 8 days later their spleens were assayed for GLΦ-specific IgM or IgG PFC responses. Each point (four-eight mice/group) represents the arithmetic mean of the percent suppression of the control group response ± SE. Control IgM and IgG responses averaged 10,000–20,000 PFC/spleen. Open circles (○) represent the percent suppression of IgM and solid circles (●) that of IgG PFC responses.

be cyclophosphamide sensitive in other systems (12, 13) we have analyzed the cyclophosphamide sensitivity of the GLΦ-specific suppression. C57BL/6 mice were given 5 mg/kg cyclophosphamide or saline i.p. 2 days before suppression induction with $3 \times 10^7$ p-GLΦ-spl cells. Control mice were given cyclophosphamide or saline 2 days before i.v. injection of $3 \times 10^7$ normal spleen cells. These mice were then challenged with 20 μg GLΦ-FyG in M/P and their spleens were assayed on day 8. As shown in Table IV, suppression could not be demonstrated in the cyclophosphamide-pretreated group given p-GLΦ-spl. Thus, the generation of nonresponsiveness in this system is sensitive to small doses of cyclophosphamide.
'5 days after suppressor cell induction, 2 × 10^7 spleen cells from tolerant or control groups were transferred into normal recipients. Tolerant cells were also treated with either NMS plus C or anti-Thy 1.2 serum plus C before transfer into normal recipients. All mice were then challenged with 20 μg GL-γG in M/P. Spleen cells were assayed 8 days later for PFC.

TABLE III

| Cells transferred* | Number of mice | GLφ-specific PFC/spleen ± SE‡ |
|--------------------|----------------|-------------------------------|
|                    |                | IgM                           | IgG                           |
| Control cells      | 3              | 10,333 ± 328                  | 12,466 ± 754                  |
| GLφ-tolerant cells | 4              | 3,575 ± 620§                  | 4,635 ± 1,421§                |
| GLφ-tolerant NMS + C treated | 5  | 3,920 ± 1,232§ | 3,980 ± 1,415§ |
| GLφ-tolerant anti-Thy 1.2 + C | 5  | 9,300 ± 3,134 | 9,800 ± 2,199 |

* 5 days after suppressor cell induction, 2 × 10^7 spleen cells from tolerant or control groups were transferred into normal recipients. Tolerant cells were also treated with either NMS plus C or anti-Thy 1.2 serum plus C before transfer into normal recipients. All mice were then challenged with 20 μg GLφ-FyG in M/P. Spleen cells were assayed 8 days later for PFC.

§ Indicates P < 0.01.

TABLE IV

GLφ Suppression Is Cyclophosphamide Sensitive

| Pretreatment* | Number of mice | GLφ-specific PFC/spleen ± SE‡ |
|---------------|----------------|-------------------------------|
|                |                | IgM                           | IgG                           |
| D-5 Saline    | 4              | 9,750 ± 935                   | 9,226 ± 1,029                 |
| Saline p-GLφ-spl | 4         | 1,800 ± 367§                  | 1,475 ± 315§                  |
| D-3 Saline    | 3              | 10,933 ± 2,140                | 13,800 ± 4,539                |
| Cyclophosphamide Saline | 4 | 10,000 ± 867                 | 9,550 ± 1,270                 |
| Cyclophosphamide p-GLφ-spl | 4 | 10,000 ± 867                 | 9,550 ± 1,270                 |

* Mice were treated with 5 mg/kg cytoxan in 0.5 ml saline i.p. 2 days later, suppression was induced with 3 × 10^7 p-GLφ-spleen cells. Control cytoxan group was induced with 3 × 10^7 normal spleen cells. 3 days after induction mice were immunized with 20 μg GLφ-FyG in M/P (day 0). Their spleens were assayed 8 days later.

§ Indicates P < 0.01.

Discussion

The present experiments demonstrate the striking efficiency of cell surface-associated GLφ in inducing a long-lasting state of nonresponsiveness. The intravenous administration of 2 × 10^8 to 3 × 10^7 p-GLφ-spleen cells carrying 1–100 ng inhibits the primary GLφ-specific IgM and IgG PFC responses. This state of nonresponsiveness lasts at least 63 days. Although the response to the closely related, serologically cross-reactive polymer GL is also inhibited, the nonresponsiveness is antigen specific, because the PFC response to the FyG carrier is not affected. Suppressor cells appear to be responsible for initiating and (or) maintaining this state of tolerance, because spleen cells from tolerant mice can transfer tolerance to normal recipients. Moreover, the induction of nonresponsiveness is cyclophosphamide sensitive and the cells which can transfer suppression are Thy 1.2 positive.

The phenomenon we have described contrasts with the state of “low zone” tolerance described by Dresser and Mitchison (14) and Rajewsky and Brenig (15). The induction of low zone tolerance required repeated injection of subimmunogenic concentrations of antigen over a prolonged period of time. In contrast, GLφ-specific tolerance was rapidly induced (<3 days) after a single intravenous administration of tolerogen.
Furthermore, in the present studies, nanogram quantities of soluble p-GLφ failed to induce suppression unless the p-GLφ was associated with cells.

The adoptive transfer experiments clearly demonstrated that Thy 1.2-positive cells from tolerant mice were required to specifically suppress the GLφ antibody response in normal recipients. Antigen carryover cannot account for the ability to adoptively transfer suppression, because p-GLφ-spl cells administered on the day of immunization with GLφ-FγG could not decrease the day 8 GLφ PFC response. In addition, cyclophosphamide treatment, which has been shown to interfere with the process of suppressor cell induction (12, 13), abrogates the induction of GLφ tolerance. Thus, from these various experiments it appears that suppressor T cells are required for the generation of GLφ-specific nonresponsiveness induced with p-GLφ-spl cells.

It is well established that haptenated syngeneic cells can be used to specifically suppress a hapten-specific PFC response (3, 4) or contact sensitivity reaction (1, 2). The most commonly used haptens for these studies have been trinitrophenyl and dinitrophenyl. The induction of hapten-specific T-cell tolerance by the i.v. administration of nitrophenylated syngeneic cells is at least in part mediated by Thy 1-positive suppressor cells (4–6). Furthermore, the induction of trinitrophenyl-specific suppressor T cells was demonstrated to be cyclophosphamide sensitive (16). The results presented in this report closely parallel those noted in the hapten systems, in that small numbers of antigen-coupled cells are sufficient to induce a potent, long-lasting T-cell-dependent suppression. However, unlike the chemical conjugation methods that covalently couple hapten to cell surface proteins, the palmitoyl coupling method does not covalently associate GLφ with any cell surface molecules. We believe that the palmitoyl-derivatized peptides are anchored into the lipid portion of the membrane and may be presented to the immune system in the same form as a foreign minor histocompatibility determinant. The palmitoyl coupling method should prove generally applicable to other lysine-containing peptides and proteins. Indeed, initial experiments have indicated that proteins can be coupled to syngeneic cells to induce specific suppression.

The route of administration of antigen-modified syngeneic cells may be crucial in the induction of suppression. In other systems, the i.v. administration of modified syngeneic cells appears to be the most efficient route for the induction of suppressor cells (1–3, 17). Experiments are in progress to define (a) the importance of the route of antigen administration, (b) the cell type(s) that are most efficient in the induction of suppression, and (c) the nature of the cell surface molecules that provide the induction signals.

C57BL/6 mice are genetic nonresponders to GLφ by virtue of the fact that they lack an H-linked Ir gene which is required for GLφ responsiveness (7). The genetic defect in GLφ nonresponder mice appears to function at the level of the induction of GLφ-specific helper T cells. Thus, C57BL/6 nonresponder mice produce anti-GLφ antibody, after immunization with GLφ that has been conjugated to an immunogenic carrier, such as FγG (10), indicating that nonresponder mice do not have a defect in their B-cell repertoire. Hence, the GLφ polypeptide may function as a “hapten” in C57BL/6 nonresponder mice, and as a result the many similarities between the suppression induced with antigen-modified cells in the hapten and GLφ systems may not be coincidental.

The mechanism(s) of GLφ-specific suppression are under investigation. Several
possible mechanisms can be considered at this time. First, the GLΦ suppressor cells may act directly upon GLΦ or GL helper T cells. In opposition to this possibility are the data from various systems, which indicate C57BL/6 nonresponder mice do not produce helper cells to these polypeptides (18, 19). A second possibility, which has been suggested previously (3) and which may apply here, is that the GLΦ suppressor cells act directly on the GLΦ- or GL-specific PFC precursor cells. This mechanism could also explain the lack of fine specificity with respect to GLΦ and GL, since Kipps et al. (11) have demonstrated that the anti-GLΦ and anti-GL responses of C57BL mice share idiotypic determinants. Finally, an unprecedented mechanism may be invoked whereby the GLΦ-specific suppressor cells may inactivate a subpopulation of FyG-specific helper T cells, which preferentially cooperate with GLΦ- or GL-specific B cells. Ward et al. (20) have presented evidence supporting the existence of hapten-restricted T-cell clones. One expectation from such a model would be suppression of the FyG-specific PFC responses. The failure to demonstrate linked FyG tolerance in the present experiments may be attributed to the presence of unconjugated FyG molecules in the GLΦ-FyG and GL-FyG preparations, which could activate FyG-specific PFC responses independently of GLΦ responses.

This report represents the first indication that GLΦ nonresponder mice possess functional T cells with specificity for GLΦ, and suggests that GLΦ nonresponder animals can generate a T-cell receptor with specificity for this polymer. Heretofore, the data have suggested that nonresponder mice lacked helper and suppressor T cells (8, 18, 19). Transferable T-cell-mediated suppression was not previously demonstrated in GLΦ nonresponder mice (9). The present experiments demonstrate that GLΦ-specific suppressor cells can be induced in at least one nonresponder strain, and indicate that the intravenous administration of antigen-modified syngeneic cells is a very potent means of inducing such cells.

Summary

The palmitoyl derivative of the linear polypeptide of poly-(L-Glu-L-Lys-L-Phe)ₙ (GLΦ) can be coupled to spleen cells directly. The intravenous administration of 2 × 10⁶–3 × 10⁷ GLΦ-coupled syngeneic spleen cells induces GLΦ-specific suppressor T cells in C57BL/6 nonresponder mice. The suppression is antigen specific and can be detected by the inhibition of the primary GLΦ plaque-forming cell response to challenge with GLΦ-fowl gamma globulin. The number of inducer cells required for suppression carry less than 0.1 μg of antigen. Spleen cells from tolerized mice can transfer suppression to normal syngeneic recipients. The suppression is cyclophosphamide sensitive and the suppressor cells bear the Thy 1.2 marker. This method of inducing antigen-specific suppressor cells may be generally applicable to other antigen systems.

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References

1. Asherson, G. L., and M. Zembala. 1974. Suppression of contact sensitivity by T cells in the
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mouse. I. Demonstration that suppressor cells act on the effector phase of contact sensitivity; and their induction following in vitro exposure to antigen. *Proc. R. Soc. Lond. B Biol. Sci.* **187**:329.

2. Claman, H. N., S. D. Miller, and S. W. Moorhead. 1976. Tolerance: Two pathways of negative immunoregulation in contact sensitivity to DNFB. *Cold Spring Harbor Symp. Quant. Biol.* **41**:105.

3. Miller, S. D., and H. N. Clamen. 1976. The induction of hapten-specific T cell tolerance by using hapten-modified lymphoid cells. I. Characteristics of tolerance induction. *J. Immunol.* **117**:1519.

4. Moody, C. E., J. B. Innes, G. W. Sikind, and M. E. Weksler. 1978. Tolerance induced by TNP-derivatized syngeneic erythrocytes: Evidence for cooperation between hapten-specific T and hapten-specific B lymphocytes in the immune response. *J. Immunol.* **120**:844.

5. Miller, S. D., M. Sy, and H. N. Clamen. 1977. H-2 restriction of suppressor T-cell induction by hapten-modified lymphoid cells in tolerance to 1-fluoro-2,4-dinitrobenzene contact sensitization. *J. Exp. Med.* **145**:1071.

6. Scott, D. W. 1978. Role of self carriers in the immune response and tolerance. III. B cell tolerance induced by hapten-modified self involves both active T cell mediated suppression and direct blockade. *Cell. Immunol.* **37**:327.

7. Dorf, M. E., and B. Benacerraf. 1975. Complementation of H-2 linked Ir genes in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3671.

8. Kipps, T. J., B. Benacerraf, and M. E. Dorf. 1978. Regulation of antibody heterogeneity by suppressor T cells. I. Diminishing suppressor T cell activity increases the number of DNP clones in mice immunized with DNP-GL\textsubscript{O} or DNP-GLA. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2914.

9. Cheung, N. K. V., K. M. Heghinian, M. E. Dorf, and B. Benacerraf. 1978. H-2 control of tolerance induction to L-glutamic acid, L-lysine containing polymer. *J. Immunol.* **121**:1370.

10. Cheung, N. K. V., M. E. Dorf, and B. Benacerraf. 1977. Development of a hemolytic plaque assay for glutamic acid, lysine-containing polypeptides. Demonstration that non-responder mice produce antibodies to these peptides when conjugated to an immunogenic carrier. *J. Immunol.* **119**:901.

11. Kipps, T. J., B. Benacerraf, and M. E. Dorf. 1977. Presence of common idiotypes on antibodies induced by glutamic acid-lysine-containing terpolymers in responder and non-responder mice with the IgG\textsubscript{1b} heavy chain allotype. *Eur. J. Immunol.* **7**:865.

12. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T cell sets. II. Physiological role of feedback inhibition in vivo: Absence in NZB mice. *J. Exp. Med.* **147**:1116.

13. Debrec, P., C. Waltenbaugh, M. E. Dorf, and B. Benacerraf. 1976. Genetic control of specific immune suppression. IV. Responsiveness to the random copolymer l-glutamic acid\textsubscript{50}-l-tyrosine\textsubscript{50} induced in BALB/mice by cyclophosphamide. *J. Exp. Med.* **144**:277.

14. Dresser, D. W., and N. A. Mitchison. 1968. The mechanism of immunological paralysis. *Adv. Immunol.* **8**:121.

15. Rajewsky, K., and C. Brenig. 1974. Tolerance to serum albumins in T and B lymphocytes in mice. Dose dependence, specificity, and kinetics of escape. *Eur. J. Immunol.* **4**:120.

16. Miller, S. P., M-S. Sy, and H. N. Clamen. 1977. The induction of hapten-specific T cell tolerance using hapten-modified lymphoid cells. II. Relative roles of suppressor T cells and clone inhibition in the tolerant state. *Eur. J. Immunol.* **7**:165.

17. Greene, M. I., M. Sugimoto, and B. Benacerraf. 1978. Mechanisms of regulation of cell-mediated immune responses. I. Effect of the route of immunization with TNP-coupled syngeneic cells on the induction and suppression of contact sensitivity to picryl chloride. *J. Immunol.* **120**:1604.

18. Katz, D. H., M. E. Dorf, and B. Benacerraf. 1976. Control of T-lymphocyte and B-
lymphocyte activation by two complementing Ir-GLφ immune response genes. *J. Exp. Med.* 143:906.

19. Baltz, M., P. H. Maurer, C. F. Merryman, and M. Feldman. 1978. Complementation of H-2 linked Ir genes: Use of helper factor to analyze responses to GLphe. *Immunogenetics.* 6:471.

20. Ward, K., H. Canto, and E. A. Boyse. 1977. In Immune System: Genetics and Regulation. E. Sercarz, L. A. Herzenberg, and C. F. Fox, editors. Academic Press, Inc., New York. 397.