IMMUNE SUPPRESSION IN VIVO WITH ANTIGEN-MODIFIED SYNGENEIC CELLS

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

By NAI-KONG CHEUNG, DAVID H. SHERR, KRIKOR M. HEGHINIAN, BARUJ BENACERRAF, AND MARTIN E. DORF

From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02116

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).[1] 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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[1] *Abbreviations used in this paper:* C, complement; GL, poly-(L-Glu^{60}-L-Lys^{40})$_n$; GLϕ, poly-(L-Glu^{56}-L-Lys^{35}-L-Phe^{9})$_n$; FγG, fowl gamma globulin; GLϕ-FγG, covalent conjugate of GLϕ and FγG; GL-FγG, covalent conjugate of GL and FγG; 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, pGLϕ-coupled spleen cells.

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-(L-Glu^{56}-L-Lys^{35}-L-Phe^{9})$_n$, GLϕ, lot GLPl, average mol wt 38,000, and poly-(L-Glu^{60}-L-Lys^{40})$_n$, GL, lot L641, average mol wt 40,000 were prepared by Miles Yeda, Ltd., Rehovot, Israel. Fowl gamma globulin (FγG) was purchased from Miles Laboratories, Inc., Elkhart, Ind. GLϕ-FγG and GL-FγG were synthesized with the Schiff base method (10). The GLϕ-FγG or GL-FγG conjugates along with unreacted FγG 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 FγG. 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-GLT^{5}-I^{125} 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 10^6 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 6×10^7 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-FγG in Maalox and pertussis adjuvant i.p., and the GLϕ and FγG-specific spleen plaque-forming cells (PFC) assayed 8 days afterwards.

Anti-Thy-1 Treatment. 10^8 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, 3×10^7 p-GLϕ-coupled spleen cells (p-GLϕ-spl cells) were injected i.v. to induce tolerance.

PFC Assay. The antibody responses to GLϕ-FγG and GL-FγG 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). FγG-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 inhibitable with 25–50 μg per slide of free ligand were reported as antigen-specific plaques. Generally, 95–100% PFCs are specifically inhibitable. 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

TABLE I

GLϕ-Specific Tolerance Induction with p-GLϕ in C57BL/6 Mice

Experiment number	Pretreatment	Number of mice	GLϕ-specific PFC/spleen ± SE*	
			IgM	IgG
1	PBS‡	7	8,471 ± 882	8,014 ± 917
	10 μg p-GLϕ	6	3,150 ± 495‖	3,200 ± 650‖
	1 μg p-GLϕ	3	6,600 ± 435	8,200 ± 251
2	10^7 Normal spl cells§	3	26,133 ± 634	36,467 ± 1,471
	10^7 GLϕ-spl cells	4	7,400 ± 1,779‖	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-FγG 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, 10^7 GLϕ-spl cells were injected into C57BL/6 mice. Control mice received 10^7 normal spleen cells. 3 days later both groups were immunized with 20 μg GLϕ-FγG in M/P. On day 8 their spleens were assayed.

‖ Indicates $P < 0.01$.

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-FγG in M/P (day 0). On day 8 their spleens were assayed for GLϕ-specific and FγG-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 FγG 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 10^7 GLϕ-coupled syngeneic spleen cells (p-GLϕ-spl cells) or with 10^7 control spleen cells. 3 days later they were immunized with 20 μg GLϕ-FγG 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 10^7 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-FγG (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. 1 A, 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-FγG PFC response was not affected (Fig. 1 B). Furthermore, the PFC response to trinitrophenyl-keyhole

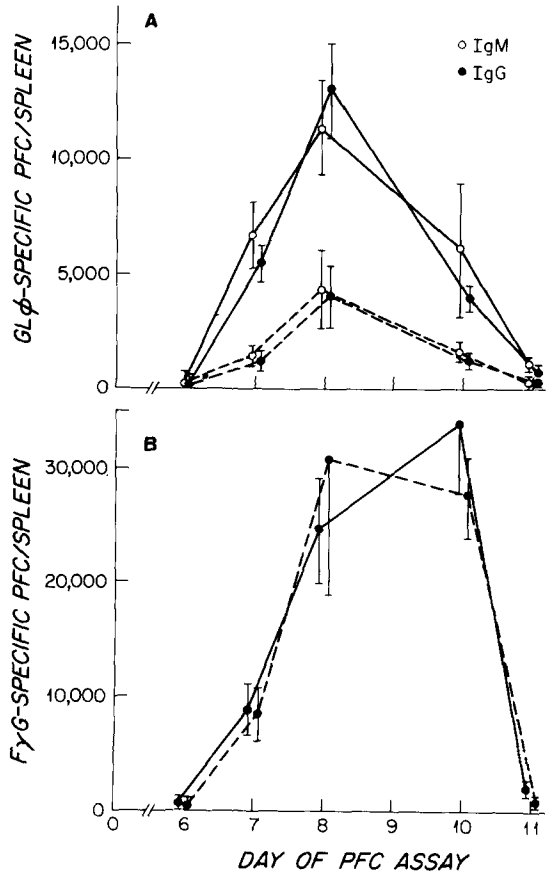

FIG. 1. (A) Kinetics of GLϕ-specific PFC response in control (——) and tolerant (-----) C57BL/6 mice. 3 days after the administration of 3×10^7 p-GLϕ-spl cells or normal cells, mice were immunized with 20 μg GLϕ-FγG in M/P (day 0). Their spleens were assayed at various days after immunization. GLϕ-specific 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 FγG-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-FγG or GL-FγG. As shown in Table II, the GLϕ- and GL-specific PFC responses were significantly reduced, while the FγG-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	Specific PFC/spleen ± SE‡		
		GLϕ or GL IgM	GLϕ or GL IgG	FγG IgG
Control cells	GLϕ-FγG	14,000 ± 5,630	6,450 ± 3,007	14,437 ± 4,732
GLϕ-spl	GLϕ-FγG	2,512 ± 1,239§	1,312 ± 1,214§	15,975 ± 2,667
Control cells	GL-FγG	12,727 ± 4,335	9,437 ± 3,559	13,050 ± 8,395
GLϕ-spl	GL-FγG	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.v., 3 days before challenge with 20 μg GLϕ-FγG or GL-FγG. 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.
§ Indicates $P < 0.02$.

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 3×10^7 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-FγG 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-FγG 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-FγG 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

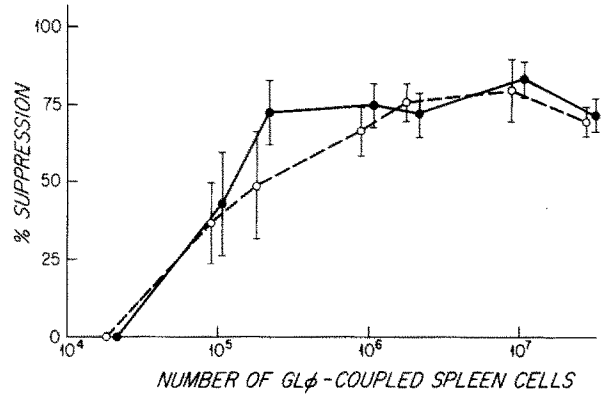

FIG. 2. Dose requirement for tolerance induction in C57BL/6 mice. Groups of mice (four to six mice/group) were injected i.v. with 2×10^4 to 3×10^7 p-GLϕ-spl cells while control groups received similar numbers of normal spleen cells. 3 days later they were challenged with 20 μg GLϕ-FγG in M/P (day 0) 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.

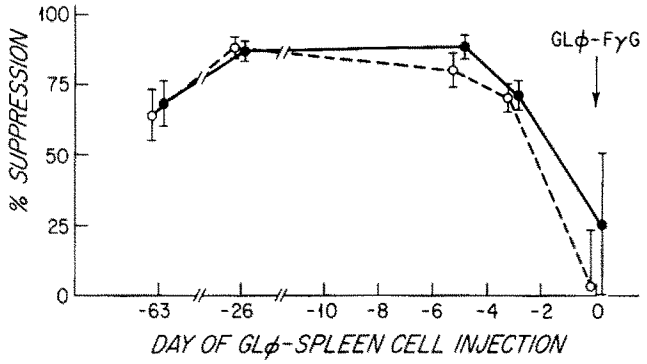

FIG. 3. Kinetics of tolerance induction in C57BL/6 mice. Mice were injected with 3×10^7 p-GLϕ-spl cells or control spleen cells and at various times (day 0 to day 63) afterward challenged with 20 μg GLϕ-FγG in M/P (day 0). 8 days later their spleens were assayed for GLϕ-specific IgM or IgG PFC responses. 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 3×10^7 p-GLϕ-spl cells. Control mice were given cyclophosphamide or saline 2 days before i.v. injection of 3×10^7 normal spleen cells. These mice were then challenged with 20 μg GLϕ-FγG 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.

TABLE III

T-Cell Dependency of GLϕ-Specific Suppression

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	8,980 ± 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-FγG in M/P. Spleen cells were assayed 8 days later for PFC.

‡ Arithmetic mean PFC response ± SE.

§ Indicates $P < 0.01$.

TABLE IV

GLϕ Suppression Is Cyclophosphamide Sensitive

Pretreatment*		Number of mice	GLϕ-specific PFC/spleen ± SE‡ GLϕ specific	
D-5	D-3		IgM	IgG
Saline	Control	4	9,750 ± 935	9,226 ± 1,029
Saline	p-GLϕ-spl	4	1,800 ± 367§	1,475 ± 315§
Cyclophosphamide	Control	3	10,933 ± 2,140	13,800 ± 4,539
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-FγG in M/P (day 0). Their spleens were assayed 8 days later.

‡ Arithmetic mean PFC/spleen ± SE.

§ 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 2×10^5 to 3×10^7 p-GLϕ-spl 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 FγG 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 subimmunogeneic 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 FγG-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 FγG-specific PFC responses. The failure to demonstrate linked FγG tolerance in the present experiments may be attributed to the presence of unconjugated FγG molecules in the GLϕ-FγG and GL-FγG preparations, which could activate FγG-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 × 10^5–3 × 10^7 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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