IN VIVO SUPPRESSION OF DELAYED HYPERSENSITIVITY: PROLONGATION OF DESENSITIZATION IN GUINEA PIGS*

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Effector mechanisms for delayed hypersensitivity (DH)[1] reactions have been extensively studied but the control of these mechanisms remains poorly understood (1). Several lines of evidence favor active regulation of the DH response (2). We have been particularly interested in the possibility that the anergic states seen in certain human diseases, such as sarcoidosis and Hodgkin's disease, may represent an exaggeration of a normal shutoff mechanism; i.e., an active rather than a passive process. In this report we present evidence that supports the concept that DH responses can be actively suppressed in vivo by exaggerated interaction between antigens and responding lymphocytes and that the anergy induced can be prolonged.

We used desensitized guinea pigs for these experiments. The systemic administration of a single large dose (milligram) of antigen to guinea pigs 7–8 days after immunization with that antigen resulted in loss of the normal DH skin response (1, 3). When desensitization was produced by a single injection of antigen the loss was transient, lasting 2–3 days (4). A most important detail of this phenomenon is that during the period of desensitization the animal did not express a DH response to heterologous antigens to which it had been successfully immunized, but not desensitized (1, 3). This non-specific desensitization was also transient. Previously, we reported that this generalized suppression of DH reactivity is not due to exhaustion of antigen or depletion of specifically reactive lymphocytes and/or their lymphokines (1–4). Desensitization appears to work via an active environmental factor(s); when we transferred competent lymphocytes into desensitized guinea pigs these cells lost their competence (4). Cells removed from desensitized animals regained their immunological reactivity (4). The experiments now described suggest that this active suppression can be maintained only while antigen is capable of reacting with sensitized, nontolerant cells.

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[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; CFA, complete Freund's adjuvant; DH, delayed hypersensitivity; FCS, fetal calf serum; FIF, feedback inhibition factor; HSA, human serum albumin; OA, ovalbumin; PE, peritoneal exudate; Pic-GPA, picrylated guinea pig albumin; PHA, phytohemagglutinin; PPD, purified protein derivative of tuberculin; SRF, skin reactive factor.

Materials and Methods

Animals. Hartley strain albino female guinea pigs weighing between 350 and 400 g or inbred strain 2 guinea pigs weighing between 400 and 550 g were immunized and used as donors of cells. Younger guinea pigs weighing approximately 250 g were used as recipients in cell transfer experiments.

Antigens. Picrylated guinea pig albumin (Pic-GPA), approximately 21 groups/mol was prepared by modification of the method described by Eisen et al. (5) and detailed in previous reports from this laboratory (6). Human serum albumin (HSA) was obtained from the American Red Cross. Bovine gamma globulin (BGG)-fraction II was obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; ovalbumin (OA) from Worthington Biochemical Corp., Freehold, N. J.; and a purified protein derivative of tuberculin (PPD) from Connaught Medical Research Labs, Willowdale, Canada.

Immunization. Guinea pigs were immunized by foot pad injection of 200 μg antigen in 0.05 ml of saline emulsified in an equal volume of complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.). When multiple antigens were administered, the emulsions were pooled, mixed, and an equal vol (0.1 ml) injected into each foot pad.

Desensitization. Animals to be desensitized were given a subcutaneous or intravenous injection of 2 mg of HSA and 2 mg of BGG commencing on the 7th or 8th day after immunization and this was continued daily for time periods given with the results of individual experiments.

Skin Tests. Separate intradermal injections (0.1 ml) of each antigen were given in the following amounts: Pic-GPA, 20 μg; HSA, 25 μg; OA, 25 μg; PPD, 25 μg; and BGG, 50 μg, except in one experiment detailed in the text where smaller doses of antigen were used. Skin reactions were read at 24 h and the mean diameter of erythema and induration measured. To assess the success of desensitization, intradermal challenge with all sensitizing antigens was performed only after at least two desensitizing injections of antigen and the reactions were compared with a control group immunized, but not desensitized. To detect any effect of locally administered serum from desensitized animals on DH responses in immunized animals the normal skin test dose of Pic-GPA, PPD, and HSA were injected into one flank and an injection of an equal volume of fluid containing the standard amount or half the standard amount of antigen in 50% desensitized serum was injected simultaneously into the other flank.

Passive Transfer of DH. Viable peritoneal exudate (PE) or spleen cells (150–250 $\times$ 10^6) were obtained from immunized and desensitized guinea pigs as follows: 3 days after the injection of 30 ml of mineral oil into the peritoneal cavity, PE cells were harvested by the injection into and the subsequent drainage from the peritoneal cavity of 100 ml of Hanks' buffered salt solution. When splenectomy was performed, a single cell suspension was made by gently teasing the spleen through a stainless steel sieve (80 gauge mesh) into a Petri dish containing modified Eagle's media (MEM) and 10% fetal calf serum (FCS). Both the peritoneal and spleen cells were centrifuged at 350 *g* for 10 min and the pellets washed twice. Cells from two or three animals were pooled to provide 150–250 $\times$ 10^6 viable cells. After culture for 16 h in a 37°C CO_2 incubator or after immediate resuspension in a final vol of 2 ml of MEM containing 10% FCS, cells were drawn through a 25 gauge needle into a syringe and injected intravenously into the recipient guinea pigs. Recipients were skin tested immediately after the cell transfer.

Hemagglutinating Antibody. Antibody levels to HSA and OA were measured by their ability to agglutinate human red blood cells coated with antigen. Human type "O" blood was defibrinated and washed. The red cells were removed, packed, and mixed at room temperature for 4 min with 3 ml of 0.1% of $CrCl_3$ (Mallinckrodt Chemical Works, St. Louis, Mo.) and 3 ml of HSA or OA at a concentration of 10 mg/ml. The treated cells were washed five times and resuspended at 5% concentration in saline. Finally, the red cells were diluted 1:5 in phosphate-buffered saline (PBS). The serum to be examined for antibody was tested at a dilution of 1:10 with PBS containing 1% rabbit serum previously absorbed with human red blood cells. Hyperimmune antisera to OA and HSA made in rabbits by Dr. Philip W. Askenase was used for control purposes. IgG antibody was measured by treating serum with mercaptoethanol (Pierce Chemical Co., Rockford, Ill.). To the 0.1 ml of serum to be tested was added 0.3 ml of 0.133 M mercaptoethanol; the mixture was held at room temperature for 2 h. Serial dilutions of serum were added to autotiter hemagglutination plates containing treated red cells and the agglutination end point read. For simplicity, antibody results are expressed as the well number where agglutination ended on a scale of 1–15 where 1 is

the well representing a titer of 1/10, and 15 a titer of 1/160,000.

Serum from Desensitized Animals. This serum for either in vitro or in vivo experiments was obtained by immunizing guinea pigs as above, and selecting those animals who exhibited the best generalized reduction in DH reactions. These animals were exsanguinated by cardiac puncture and the serum recovered, pooled, and stored at −4°C after passage through a Millipore filter.

Cell Cultures. Experiments were performed in which either guinea pig or human cells were stimulated in vitro with phytohemagglutinin (PHA-P) (Difco Laboratories), or PPD in a variety of test sera. Blood was drawn into a heparinized syringe and spun for 45 min through a Ficoll-Hypaque gradient (Pharmacia Laboratories, Piscataway, N. J.), and the lymphocyte layer recovered and washed three times in MEM containing penicillin and streptomycin. The cells were brought to a final concentration of 2–5 × 10^6/ml in MEM containing L-glutamine (Microbiological Associates, Inc., Bethesda, Md.), penicillin and streptomycin, and 30% serum. The type of serum is identified in each experiment. To 1 ml of cells in media was added 0.5 ml of media containing 1/400, 1/800, or 1/1,600 of the stock solution of PHA-P. PPD stimulation was achieved using 25 μg of PPD for guinea pig cells and 100 μg PPD for human cells. PHA cultures were terminated at 72 h, PPD cultures after 6 days, and in both cases cultures were pulsed with 1.0 μCi of tritiated thymidine (New England Nuclear, Boston, Mass.), for the 24 h before termination. All cultures were performed in triplicate. Our laboratory's method for termination, harvesting, and scintillation counting have previously been described in detail (7). The results are reported as stimulation indices with the mean value and standard deviation provided. The counts per minute from unstimulated lymphocytes are also provided so that absolute increases in counts per minute can be calculated.

Results

Prolongation of Desensitization by Daily Injections of Antigens. 18 guinea pigs were immunized with HSA and BGG in CFA and 9 were desensitized with daily injections of 2 mg of HSA and BGG from the 6th to 30th day postimmunization. The antigens inducing desensitization (in this case, HSA and BGG), are hereafter referred to as the desensitizing antigens while the antigen whose DH effect they block (in this case, PPD) is referred to as the indifferent antigen. DH skin reactions to HSA and PPD were sought in both the control group and the desensitized animals seven times during the 30-day period and the intervals and results are shown in Fig. 1. Reduced responses to the indifferent antigen (PPD) could only be prolonged for 10 days by this regimen. Nonreactivity to the desensitizing antigen (HSA) continued throughout.

Humoral Immune Response to Desensitizing and Indifferent Antigens. To determine whether the repeated administration of antigen was inducing tolerance to that antigen, we measured antibody levels to both a desensitizing and an indifferent antigen during a 100 day period. 16 guinea pigs were immunized with HSA and OA in CFA. From the 7th to the 36th postimmunization day, eight were desensitized daily with 1 mg of HSA. On the 45th day after immunization all animals were reimmunized with HSA in CFA. Antibody to HSA and OA was measured each week by hemagglutination techniques and the results are shown in Table I. In both groups, antibody levels to OA (the indifferent antigen) rose steadily and remained high for the full 100-day period. Throughout the period of desensitization, even when DH responses to OA were markedly reduced, no significant suppression of either mercaptoethanol-sensitive or -resistant antibody to OA was observed. In comparison, while control animals developed and maintained high titers of anti-HSA antibody, the desensitized group exhibited an initial rise in HSA antibody and then a fall to very small titers and even reimmunization with HSA in CFA did not reverse this phenomenon. Onset

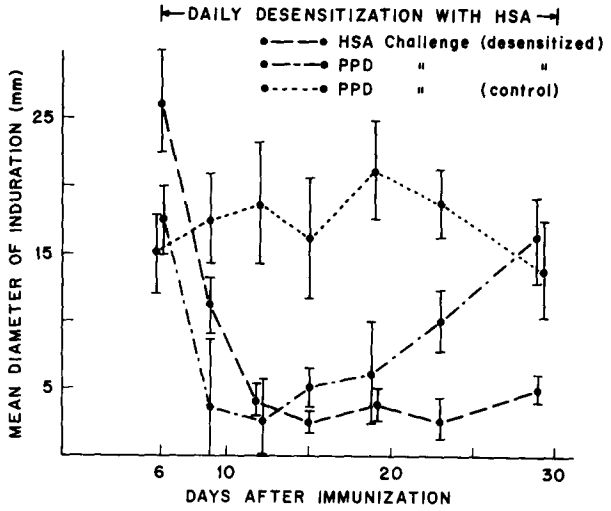

FIG. 1. 18 guinea pigs were immunized with PPD and HSA on day 0, and 9 were immunized with daily injections of 1 mg of HSA from day 6 to day 30. DH reactions to PPD in the control guinea pigs are compared with DH reactions to PPD and HSA in the desensitized guinea pigs. Note that both the HSA and PPD responses are affected by desensitization but that the PPD response returns to normal about 10 days after desensitization commences.

of the fall in HSA antibody levels in the desensitized group was associated with a return of DH to OA. These observations suggest that the establishment of immunological tolerance to an antigen terminates the ability of desensitizing amounts of that antigen to suppress DH responses to indifferent antigens.

Success of Desensitization Related to Degree of Immunization. In outbred desensitized guinea pigs, the degree of nonspecific anergy induced by antigen injection is variable. Comparison of the size of DH responses to immunogens before desensitization and the subsequent production of desensitization demonstrates that the better the DH responses before desensitization, the better the nonspecific anergy after desensitization. 64 guinea pigs were immunized with HSA and BGG in CFA. DH responses to PPD, BGG, and HSA were measured on the 6th day and after 2 days of desensitization with HSA and BGG. As is shown in Table II, grouping the 32 animals with the best DH responses before desensitization reveals that these animals develop statistically better ($P < 0.001$) desensitization to both the desensitizing and indifferent antigens than the 32 animals with inferior DH responses after immunization.

Prolongation of Tuberculin Anergy with Three Consecutive Desensitizing Regimens. If nonspecific anergy reverses when tolerance is induced (vide supra), then immunization and subsequent desensitization with new antigens should restore the nonspecific anergy. To test this hypothesis 24 guinea pigs were immunized with HSA in CFA and desensitized with daily injections of HSA from the 6th day to the 55th day after immunization. As shown in Fig. 2, the DH skin response to HSA diminished and remained minimal throughout. As previously observed, the DH skin response to PPD, the indifferent antigen, fell but returned after 10 days of desensitization with HSA. However, when animals

TABLE I

Antibody Level in Guinea Pigs Immunized with HSA and OA and Desensitized by Daily Injections of HSA for 30 days

Days since immunization	Desens status	Hemagglutinating antibody levels (mean ± SD)* to:							
		HSA				OA			
		Total		IgG		Total		IgG	
		Control‡	Desens‡	Control	Desens	Control	Desens	Control	Desens
6	Day before desens	2.7 ± 1.1	0	0	0	0	1.4 ± 0.7	0	0
8	Day after desens	7.7 ± 1.3	3.0 ± 1.2	4.2 ± 1.6	2.0 ± 0.8	6.9 ± 1.7	5.5 ± 2.5	3.5 ± 2.0	4.0 ± 1.0
15	Desens continuing	7.7 ± 1.3	3.2 ± 1.3	7.2 ± 1.4	2.5 ± 0.9	10.2 ± 1.4	9.1 ± 0.4	7.5 ± 2.6	9.2 ± 0.2
36	Final day of desens	12.4 ± 0.6	0.2 ± 1.0	11.4 ± 0.8	0	13.0 ± 0.4	13.0 ± 0.5	12.2 ± 0.7	12.6 ± 0.3
50	5 Days after reimmunization with HSA	13.2 ± 0.3	3.0 ± 2.6	11.4 ± 0.2	1.5 ± 1.0	13.4 ± 0.4	14.3 ± 0.6	13.0 ± 0.4	13.0 ± 0.5
100	No normal manipulations since day 45	13.2 ± 0.9	0.2 ± 0.1	11.2 ± 0.1	0	13.4 ± 0.6	14.5 ± 0.3	13.0 ± 0.3	13.6 ± 0.3

* Expressed as the mean ± SD of the well number at which the hemagglutinating end point was observed.

‡ Eight guinea pigs in each group.

TABLE II

Relationship of the Size of DH Skin Reactions Elicited after Immunization to the Subsequent Degree of Desensitization

Group	No. of animals	Mean diameter (mm) ± SD of DH skin responses					
		6 days after immunization*			After 2 days desensitization‡		
		PPD	BGG	HSA	PPD	BGG	HSA
"Good" DH	32	14 ± 3.2	21 ± 4.4	25 ± 6.1	5 ± 1.8	4 ± 1.2	3 ± 1.4
"Poorer" DH response after immunization§	32	11 ± 3.7	15 ± 2.4	11 ± 6.8	10 ± 3.4	9 ± 3.7	10 ± 4.1

* All animals immunized with BGG and HSA in CFA.

‡ 8 days after immunization with BGG and HSA in CFA and 2 days after desensitization.

§ Difference in skin test response between the two groups after desensitization are statistically significant.

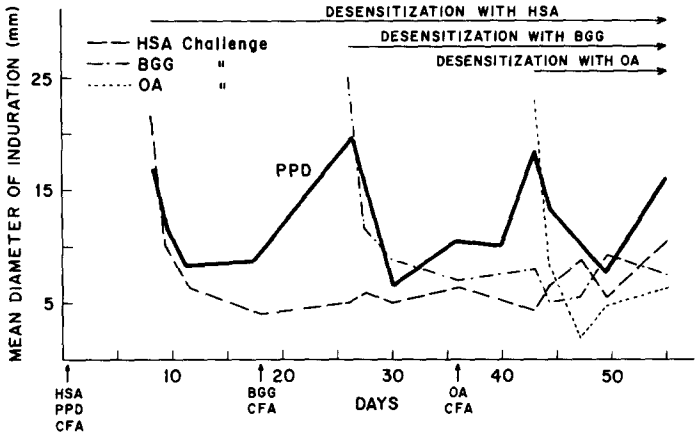

FIG. 2. Animals consecutively immunized and desensitized with HSA, BGG, and OA cannot produce a normal DH response to these antigens after desensitization. The mechanism involved clearly effects DH responses to PPD transiently, however, they remain susceptible to repeated re-establishment of the desensitized state.

were immunized with BGG in CFA on day 18 after the initial immunization and then desensitized with BGG from day 25, DH skin unresponsiveness to PPD was rapidly reinstated. Injections of BGG maintained this lack of DH to the indifferent antigen for 10 days; the cycle was repeated again by immunizing and subsequently desensitizing with OA. In Fig. 2, the mean values for the diameter of the induration induced by DH testing are given and the oscillations in PPD responses are clearly illustrated. This experiment demonstrates that (*a*) there is no exhaustion of the mechanism needed for preventing a DH response to the indifferent antigen; and (*b*) desensitization is again shown to depend upon sensitization.

Passive Transfer of DH using Lymphocytes from Short-Term or Long-Term Desensitized Guinea Pigs. Six guinea pigs immunized with HSA and BGG in

CFA were desensitized for 3 or 7 days before the transfer of peritoneal exudate cells to normal recipients. Two guinea pigs were required for sufficient cells for one transfer. Similarly, cells were transferred from 6 animals immunized 55 days before transfer and 6 similarly immunized animals who had been desensitized with HSA for 38 days before transfer, and with BGG for 29 days before transfer. In all, cells from 24 animals were transferred to 12 normal recipients. These animals were skin tested immediately after receiving the cells. Table III shows that cells from short-term desensitized animals and long-term immunized animals adequately transferred DH to normal animals. However, cells from long-term desensitized animals, while capable of transferring responsiveness to PPD (the indifferent antigen) which had escaped from the desensitizing influence, could not transfer DH to HSA and BGG (both desensitizing antigens) a finding that further strengthens the concept that true immunological tolerance to these antigens had been induced.

Effect of Transferring Spleen Cells from Long-Term Desensitized Animals to Immunized Recipients. The spleens from the long-term desensitized Hartley guinea pigs detailed in Table I were larger than spleens from long-term immunized animals used as controls (mean, 520 mg ± 80 SD for control animals and 830 mg ± 95 SD for desensitized animals). The spleen appears to be an excellent source of suppressor T cells (7) and we sought evidence for such activity in the large spleens. Eight inbred strain 2 guinea pigs were immunized with HSA and

TABLE III

Comparison of the Ability of PE Cells to Passively Transfer DH after Short- or Long-Term Desensitization

Status of guinea pig donors*	Pooled group no.	Mean diameter of DH skin reactions in:					
		Donors			Recipients		
		HSA	BGG	PPD	HSA	BGG	PPD
		mm	*mm*	*mm*	*mm*	*mm*	*mm*
Desensitized for 3 days before transfer with HSA and BGG	1	5.5	7.0	6.5	7.5	7.5	5.0
	2	4.0	7.0	7.5	8.5	8.5	7.0
	3	3.0	6.5	8.5	5.5	7.5	5.5
Desensitized for 7 days before transfer with HSA and BGG	4	5.0	10.0	7.5	9.5	0	8.5
	5	3.0	8.5	6.0	7.5	9.0	8.0
	6	3.0	7.5	8.0	8.5	8.5	7.5
Immunized 55 days earlier	7	30.0	28.0	14.0	9.0	13.5	8.5
	8	27.0	26.0	11.5	14.0	8.0	8.0
	9	25.0	18.0	15.5	12.5	9.5	8.0
Desensitized with HSA for 38 days and BGG for 29 days before transfer	10	4.0	2.0	17.0	0	0	11.5
	11	3.0	5.0	11.5	0	0	7.5
	12	5.0	4.0	9.5	0	0	7.0

* All guinea pigs were immunized with HSA in CFA. Animals in groups 1–6 were immunized with BGG and HSA simultaneously; for animals in groups 7–12, BGG immunization followed HSA by 17 days. Desensitization commenced 7 days after immunization.

BGG in CFA and were desensitized for at least 25 days with HSA and BGG. 15 days before cell transfer, these animals were immunized with OA in CFA and desensitized with OA for the 7 days before transfer. DH to PPD was still suppressed by this regimen when spleen cells were harvested. Eight strain 2 recipients were prepared for cell transfer by immunization with Pic-GPA in CFA 3 wk before transfer. Four of these animals received pooled cells from the spleen of two of the desensitized donors. No suppressive effect of spleen cells from desensitized donors was observed in this system as DH responses to Pic-GPA were equivalent in the control and recipient groups. The mean diameter (millimeters) ± SD of DH responses to PPD and Pic-GPA were, respectively, 13.5 ± 3.7 and 24.5 ± 6.4 for the control group, and 14.0 ± 4.5 and 22.5 ± 7.4 for the group given cells from desensitized animals.

Passive Transfer of DH after Exposure of Sensitized Cells to Serum from Desensitized Animals. A series of experiments were designed to look for a humoral suppressant of DH in the serum from desensitized guinea pigs. In each, the desensitized serum was collected from animals known to have good generalized suppression of DH reactivity. Passive transfer of DH to normal animals was attempted with sensitized lymphocytes cultured in serum from desensitized animals. In another approach the effect on DH of an intravenous injection of this serum into immunized animals was examined. 18 guinea pigs were immunized with HSA and BGG in CFA and skin tested 3–4 wk later. A similar number of animals were immunized with HSA and BGG in CFA but were desensitized on the 7th and 8th days after immunization; i.e., the 2 days before cell harvesting. Peritoneal exudate cells were harvested from both groups and put into tissue culture for 16 h. For half of the cell samples, the culture media was constituted with 30% serum from desensitized animals. These short-term cultured cells were then transferred into four groups of four normal guinea pigs. One group received PE cells from immunized animals; a second, PE cells from immunized animals cultured in the serum from desensitized animals; a third, cells from desensitized animals; and a fourth, cells from desensitized animals cultured in serum from desensitized animals. Exposure to the serum from desensitized guinea pigs could not be shown to interfere with DH transfer. In addition, eight guinea pigs immunized with HSA and BGG in CFA 3 wk earlier each received a 3 ml intravenous injection of the serum from desensitized animals and were then tested for DH to HSA, BGG, and PPD. No suppression of DH occurred.

Effect on DH of Injecting Serum from Desensitized Animals Directly into Skin Challenge Sites. 12 guinea pigs were immunized with Pic-GPA and HSA in CFA. 3 wk later all were skin tested with Pic-GPA, HSA, and CFA. Four animals were skin tested with a standard dose of antigen but into one flank the antigens were administered with serum from desensitized animals. Four animals were similarly tested except that half the normal skin test dose was used. A final group of four guinea pigs had desensitized serum injected into three skin sites on one flank 3 h before antigen was given into the same sites. In each case, DH responsiveness on both flanks were comparable, the serum from desensitized animals having no apparent effect.

Effect of Serum from Desensitized Guinea Pigs on In Vitro Responses of Cultured Cells to PHA. Dose response curves of the response of lymphocytes to

PHA stimulation in either commercial guinea pig sera or sera from desensitized animals were constructed. PHA stimulation of lymphocytes from normal, immunized, and desensitized guinea pigs were compared in both types of sera; no effect of the desensitized serum was observed nor were the cells from desensitized animals inferior in their response to PHA. Control values for unstimulated cultures ranged between 279 and 602 cpm.

Effect of Serum from Desensitized Animals on the In Vitro Response of Lymphocytes to PPD. Can sera from guinea pigs in whom the expression of DH to PPD has been markedly suppressed by injection of HSA and BGG similarly effect the in vitro response of cells to PPD? 14 guinea pigs were immunized with HSA, BGG, and OA in CFA; 16 were similarly immunized but desensitized for the 2 days before cell culture, and 4 normal human subjects known to have strongly positive DH to tuberculin were bled and their peripheral blood lymphocytes stimulated with PPD in media containing serum from either normal, immunized, or desensitized guinea pigs. To be sure the lymphocytes were capable of response, PHA stimulation was performed simultaneously. As can be seen in Table IV, all cells responded well to PHA in any serum. However, both the sera from immunized and desensitized animals markedly reduced the cellular response to PPD. Certainly no suppression related to the desensitized state could be claimed. Guinea pig control counts from unstimulated controls ranged from 280 cpm to 497 cpm for PHA and from 480 cpm to 720 cpm for PPD. Unstimulated human cells had background counts of 376–674 cpm for PHA cultures, and 590–870 cpm for PPD cultures.

TABLE IV

Effect of Serum from Desensitized Guinea Pigs on In Vitro Response of Lymphocytes to PPD

Source of cultured cells	No. of donors studied	DNA synthesis in stimulated lymphocytes expressed as a mean stimulation index*					
		Normal serum‡		Serum from immunized guinea pigs		Serum from desensitized guinea pigs	
		PHA	PPD	PHA	PPD	PHA	PPD
Guinea pigs immunized with PPD§	14	21	18	18	3.4	20	3.5
Guinea pigs immunized with PPD but desensitized ‖	16	18	15	11	4	17	6
Humans with positive tuberculin sensitivity	4	83	54	42	13	47	6

* Actual counts per minutes and range are included in test.

‡ Normal guinea pig serum used for guinea pig cells, normal pooled human serum for human cells.

§ Immunized with HSA, BGG, and OA in CFA 10 days before culture.

‖ Immunized to HSA, BGG, OA, and PPD and desensitized with HSA and BGG 2 days before cell culture.

Discussion

Since its discovery in 1958 (3), desensitization has remained a poorly understood phenomenon. Administration of moderate amounts of an antigen to an immunized animal leads to a transient (3 days with one injection) loss of DH reactivity (2). Desensitization can be induced in species other than guinea pigs (8) and has been considered to affect cellular rather than humoral immunity (9). It is not associated with a lack of available macrophages, the target cells in a DH response (1), nor is it due to exhaustion of antigen. Lymphocytes capable of specifically reacting to antigen with lymphokine release are present in the desensitized state (1). We have previously suggested that DH reactions may be terminated by a feedback inhibition factor (FIF) which would act as a nonspecific terminator of DH (4). Desensitization may represent an exaggeration of this normal mechanism induced by an intensive reaction between sensitized cells and large amounts of antigen resulting in a systemic effect. The fact that immunocompetent cells transferred into desensitized animals lose that competence and that immunocompetent cells regain their competence once removed from desensitized animals is consistent with an active suppressive state (3).

The evidence presented herein supports the concept that the onset of desensitization is associated with the triggering of an inhibitory regulator for DH rather than the exhausting of a required cellular mediator. Once immunological tolerance is established to the desensitizing antigen, generalized anergy disappears. It is clear that cells sensitized to the indifferent antigens do not lose their responsiveness to the desensitizing mechanism as they respond repeatedly to new desensitizing regimens.

An hypothesis to explain desensitization would have antigen interacting both with sensitized effector cells and a subpopulation of suppressor T cells susceptible to exaggerated triggering by the desensitizing injections. Such suppressor cells would reduce ordinary reactivity of effector T cells either by cell to cell interaction, or by the release of a humoral factor (FIF) that in turn would interfere with the development of a DH response. The spleen of the mouse has been shown to contain much of the suppressor T-cell population (7). It seemed likely that cells from this organ in long-term desensitized animals would have some suppressive effect on DH in immunized animals receiving these cells. In our experiment, this was not so despite the enlargement of the spleen in desensitized animals. This may have been due to the fact that nonspecific anergy, although present in the donor animals, was beginning to wane. If nonspecific desensitization in guinea pigs could be prolonged, it would make an attractive model for the nonspecific anergic state seen with human diseases such as leprosy (10) and sarcoidosis (11, 12). It should be possible to reduce the dose of desensitizing antigen to a level that prevents tolerance induction, but preliminary experiments have pointed out the difficulty of doing this due to the development of immediate hypersensitivity reactions with the lower doses.

A humoral factor which can inhibit lymphocyte responsiveness has been reported in syphilis (13), sarcoidosis (14), mucocutaneous candidiasis (15), and leprosy (10). Thus, we were anxious to see if serum from adequately desensitized animals was immunosuppressive. We found no supportive evidence for such a factor, and although none of our experiments eliminated this possibility, it is

clear that if such a factor is present it is difficult to demonstrate. Our in vitro studies established that cells from desensitized animals can respond normally to PHA and antigens in culture and can transfer DH to normal animals after a night in culture. The ability of these cells and cells from immunized but not desensitized hosts to transfer DH is not reduced by a 16 h exposure to 30% serum from desensitized animals. An injection of 3 ml of this serum into immunized animals does not reduce their capacity for DH responsiveness. Perhaps this is a suboptimal dose but it is the maximum that can be given intravenously in a single injection to guinea pigs. The fact that serum from animals immunized as well as desensitized to PPD reduces the ability of cells sensitized to PPD to respond to that antigen in culture suggests that a blocking antibody mechanism may be responsible. This blocking factor makes it impossible to dissect the immunized from the desensitized state in our current system. A more definitive experiment to search for a desensitization factor that can nonspecifically block antigen stimulation of cells in tissue culture would use serum from animals immunized and desensitized with antigens that had not been administered to the donors of the cells used for stimulation with antigen. We are pursuing this approach. Our experiments demonstrate desensitization to be an actively produced phenomenon and leave intact the hypotheses that a suppressor cell mechanism is responsible.

Summary

Administering moderate (milligram) amounts of antigen to a guinea pig immunized with that antigen leads to a transient loss of all delayed hypersensitivity (DH) responses in that animal. In this study, we demonstrate that this "desensitization" can be prolonged for 10 days by repeated injection of antigen. At this time, tolerance to the desensitizing antigen develops in both the humoral and cellular systems of the immune response and DH responsiveness to other antigens returns. Repeated cycles of sensitization and desensitization produce repeated episodes of generalized anergy. Neither cells nor serum from desensitized animals could be shown to exert a suppressor effect when transferred to immunized animals and the cells responded normally to antigen and mitogen in tissue culture. The best generalized depression of DH was seen in those animals producing the best DH before desensitization. The inability of antigen to react with tolerant cells to produce desensitization suggests that this phenomenon is an active rather than a passive one and may represent an exaggeration of a normal regulatory mechanism for DH triggered by a regimen of antigen administration that activates suppressor cells to produce a systemic effect.

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