REGULATION OF DELAYED HYPERSENSITIVITY

Failure to Transfer Delayed Hypersensitivity to Desensitized Guinea Pigs*

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Mechanisms controlling production of humoral antibodies include a well-characterized feedback inhibition system (1, 2). However, mechanisms regulating cellular immunity are not understood. Delayed hypersensitivity (DH)[1] reactions of varying intensities appear to diminish within a regular time sequence that suggests an active controlling process. 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 such a normal shutoff mechanism.

DH reactions appear to follow the specific recognition of an antigen by a thymus-derived lymphocyte (T cell). Accompanying the subsequent proliferation and differentiation of these cells is the secretion of humoral factors called lymphokines. These exert their action on cells (predominantly macrophages) producing the characteristic morphology, histology, and time-course of DH reactions.

Four possible explanations for termination of DH reactions have been examined. The first two, removal of the initiator of the reaction (exhaustion of antigen) and depletion of specifically reactive lymphocytes and/or their lymphokines, have been previously excluded (3). In this study we examined two further possibilities, namely that DH reactions cease when (*a*) the target cells (macrophages) become unresponsive to lymphokines, or (*b*) a feedback inhibition system becomes operative.

We used desensitized guinea pigs for these experiments. When guinea pigs, sensitized to an antigen, are given a large quantity of that antigen 7–8 days after immunization, ability to produce a DH response to the sensitizing antigen is lost (3–4). This loss is transient, lasting only a few days. During this period of desensitization, the animal cannot express a DH response to heterologous antigens to which it has been immunized but not desensitized (3, 5). This nonspecific desensitization is also transient and suggests a generalized suppression of DH reactions induced by the sensitizing dose of antigen.

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[1] *Abbreviations used in this paper*: BGG, bovine gamma globulin; CFA, Freund's complete adjuvant; DH, delayed hypersensitivity; FCS, fetal calf serum; FIF, feedback inhibition factor; HBSS, Hanks' buffered salt solution; HSA, human serum albumin; MEM, modified Eagle's medium; MIF, migration inhibition factor; OA, ovalbumin; PE, peritoneal exudate; Pic-GPA, picryl guinea pig albumin; PPD, purified protein derivative of tuberculin; SRF, skin-reactive factor; T cell, thymus-derived lymphocyte.

Is the period of DH nonreactivity in desensitized guinea pigs caused by unresponsiveness of target cells to lymphokines? We have examined this question by injection of skin-reactive factor (SRF) (6) into the skin of desensitized guinea pigs. SRF is a lymphokine produced by the interaction of antigen and sensitized cells and known to induce a DH-type response in normal skin. In the present work, it produced such a response in densensitized guinea pigs. At the same time, we found evidence for a generalized inhibition of DH reactivity in desensitized guinea pigs when attempting the passive cellular transfer of DH into such animals. We also removed cells from desensitized animals and transferred them into normal animals to assess their competency. The data that follow strongly suggest that densensitization is not due to macrophage unresponsiveness but to an active suppression of lymphocytes mediating DH, an observation compatible with the concept of a feedback inhibition system controlling cellular immunity.

Materials and Methods

Animals.—Hartley strain albino female guinea pigs weighing between 400 and 500 g were immunized and used as donors of cells. Younger guinea pigs of the same breeding stock weighing approximately 250 g were used as recipients. In one experiment inbred guinea pigs of strain 2, weighing 200–500 g, were used as donors or recipients.

Antigens.—Picryl guinea pig albumin (Pic-GPA, approximately 21 groups/mol) was prepared by modification of the method described by Eisen et al. (7) and detailed in previous reports from this laboratory (8). Human serum albumin (HSA) was obtained from the American Red Cross. Bovine gamma globulin-fraction II (BGG) was obtained from Schwarz/Mann Div., Becton, Dickinson and 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 footpad injection of 200 μg antigen in 0.05 ml of saline emulsified in an equal volume of Freund's complete adjuvant (CFA) (Difco Laboratories, Detroit, Mich.). When multiple antigens were administered, the emulsions were pooled, mixed, and an equal volume (0.1 ml) injected into each footpad.

Desensitization.—Animals to be desensitized were given a subcutaneous or intravenous injection of 2 mg of HSA and 2 mg BGG on the 7th and 8th days after immunization.

Skin Tests.—Separate intradermal injections (0.1 ml) of each antigen were given to each animal in the following amounts: Pic-GPA, 20 μg; HSA, 25 μg; OA, 25 μg; PPD, 25 μg; and BGG, 50 μg. 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 after the second desensitizing injection and the reactions compared with a control group of immunized but nondesensitized guinea pigs.

Production and Testing of SRF.—Following the method of Bennett and Bloom (6), lymph node lymphocytes from guinea pigs immunized to Pic-GPA 3 wk earlier were harvested; 24×10^6 cells/ml were placed in modified Eagle's medium (MEM) and incubated overnight in the presence of 100 μg/ml of Pic-GPA. A sample of the supernatant was assayed for the presence of macrophage migration inhibition factor (MIF) (9). If the supernatant caused a 40% or greater reduction in macrophage migration, the remainder of the supernatant was considered likely to contain SRF. The supernatant was then dialyzed against distilled water for 24 h, lyophilized and concentrated five times, and resuspended in saline. For testing, 0.1 ml of this preparation was injected intradermally. Histological examination of the reactions were made in a number of cases. A control preparation was made by the identical treatment of supernatant from sensitized cells cultured overnight without antigen.

Passive Transfer of DH.—Two types of experiments were performed. In one (*a*), cells sensitized to a heterologous antigen were transferred into desensitized recipients; in the other (*b*), cells from desensitized donors were transferred to normal animals.

(*a*) Guinea pigs were immunized with Pic-GPA or OA in CFA. 3 wk later 150–250 × 10^6 viable peritoneal exudate (PE) or spleen cells were obtained in the following manner. 3 days after the injection of 30 ml of mineral oil into the peritoneal cavity, PE cells were harvested by the injection into the peritoneal cavity and subsequent drainage of 100 ml of Hanks' buffered salt solution (HBSS). Splenectomy was then performed and a single-cell suspension made by gently teasing the spleen through a stainless steel sieve (80-gauge mesh) into a Petri dish containing 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 × 10^6 viable cells. After resuspension in a final volume of 2 ml of MEM and 10% FCS, cells were drawn through a fine gauge (no. 25) needle into a syringe and injected intravenously into the recipient guinea pigs. The recipients had been immunized with HSA and BGG in CFA 8 days before the transfer and desensitized to both antigens on the 7th and 8th days after immunization. Immediately after the injection into these animals of the cells from donor animals immunized only to OA, they were challenged with intradermal injection of HSA, BGG, PPD, and OA. Reactions were read at 24 h and compared with the reactions in control recipients which had not been desensitized, but had received the same donor cells. Identical experiments were performed with cells from donors immunized to a different antigen, Pic-GPA.

(*b*) To test the immunocompetency of cells removed from desensitized animals, half of a group of guinea pigs immunized with HSA, BGG, and either OA or Pic-GPA in CFA were desensitized to HSA and BGG on the 7th and 8th days after immunization. Skin testing of these animals on the 8th day revealed the loss of DH reactivity to both the desensitizing (HSA and BGG) and "indifferent" (PPD and OA or Pic-GPA) antigens. 150–250 × 10^6 PE or spleen cells from these animals, prepared as described above, were injected intravenously into normal guinea pigs. Skin testing with HSA, BGG, PPD, and either OA or Pic-GPA followed immediately. Reactions were read at 24 h and compared with those in normal guinea pigs that had received cells from immunized but not desensitized control guinea pigs.

RESULTS

Response of Desensitized Guinea Pigs to Skin-Reactive Factor (SRF).—16 guinea pigs sensitized to HSA in CFA, when challenged intradermally with HSA and PPD, exhibited normal DH responses (see first line in Table I). Intradermal injection of these antigens into the skin of normal animals did not produce a visible reaction (not shown in Table I). The response to 0.1 ml of SRF, administered intradermally, was not significantly different from the response to antigen, either in the mean diameter of the resulting erythema or the intensity of the reaction. A minimal reaction was obtained when the control supernatant was injected. 16 guinea pigs were similarly sensitized to HSA and then desensitized to HSA. As shown in the lower portion of Table I, intradermal challenge with both HSA and PPD elicited much smaller and less intense reactions than those produced in the nondesensitized controls. This result confirms previous results showing that after desensitization to one antigen (HSA), an indirect effect on responsiveness to an indifferent antigen (PPD) also occurs (3–5). However, as noted in the last column of Table I, the response to SRF in these animals did not significantly differ ($P > 0.05$) from that seen in the sensitized

TABLE I

Skin Reactions of Desensitized Guinea Pigs to Skin-Reactive Factor

Guinea pig status (no. animals)	Mean skin diameter ± SEM* of reactions to skin challenge			
	HSA	PPD	SRF‡	SRF control§
	mm	*mm*	*mm*	*mm*
Immunized (16)	15.5 ± 0.5	9.2 ± 0.5	13.5 ± 0.5	6.0 ± 0.5
Immunized and desensitized‖ (16)	6.2 ± 0.7	5.0 ± 0.7	11.6 ± 0.3	3.8 ± 0.5

* SEM, standard error of the mean.

‡ SRF = skin-reactive factor.

§ Control = supernatant from cells cultured without antigen.

‖ Desensitized to HSA alone.

animals. Histological examination revealed a cellular response with macrophages predominating.

Passive Transfer of DH from Heterologously Sensitized to Desensitized Guinea Pigs.—15 guinea pigs were immunized with HSA and BGG in CFA. An equal number of animals were similarly sensitized but desensitized to HSA and BGG. As shown in Table II, skin responses to both HSA and BGG, the desensitizing antigens, and PPD, the indifferent antigen, were markedly less than the responses in the immunized animals. Both the immunized and desensitized groups received at least 150×10^6 peritoneal exudate cells from guinea pigs sensitized only to OA, and in separate but identical experiments, cells from animals sensitized only to Pic-GPA. In both experiments, the transfer of marked responsiveness to OA or Pic-GPA was achieved in the control guinea pigs sensitized to HSA, BGG, and PPD. However, the transfer of responsiveness into desensitized guinea pigs was significantly reduced (Table III).

Passive Transfer of DH to Normal Animals with Cells from Desensitized Animals.—12 normal guinea pigs received $150–250 \times 10^6$ PE cells and 14 normal guinea pigs, $150–250 \times 10^6$ spleen cells, from animals which had been sensitized to HSA, BGG, PPD, and OA 8 days earlier. An equal number received cells from similarly sensitized animals which, in addition, had been desensitized to HSA and BGG on the 2 days preceding the transfer. As shown in Table IV, the transfer of responsiveness was equally well obtained with PE cells from desensitized animals as with cells from control animals. Frequently skin reaction elicited in recipients of cells from desensitized donors exceeded the skin reactions noted in these same donors before the transfer (not shown in Table IV). Parallel experiments were performed with spleen cells with similar results as shown in Table V. Passive transfer of DH into normal guinea pigs was possible with cells from animals sensitized for only 8 days although the size and intensity of the reactions were less than those of the previous transfer experiments in which donors had been immunized for 3 wk. To facilitate the transfer

TABLE II

Skin Reaction of Immunized Guinea Pigs Prepared to Receive Cells Transferred from Heterologously Immunized Donors

Guinea pig status* (no. animals)	Mean diameter ± SEM‡ of skin reactions to antigen		
	HSA	BGG	PPD
	mm	*mm*	*mm*
Immunized (18)	24.6 ± 0.8	17.0 ± 2.8	14.6 ± 1.9
Immunized and desensitized§ (18)	5.4 ± 2.4	6.5 ± 1.1	6.1 ± 0.5

* All immunized to HSA, BGG, and PPD.
‡ SEM, standard error of the mean.
§ Desensitized to HSA and BGG.

TABLE III

Passive Transfer of Delayed Hypersensitivity into Desensitized Guinea Pigs Using Either Peritoneal Exudate or Spleen Cells from Heterologously Immunized Donors

Recipient status	Mean diameter ± SEM* of recipient skin reactions to antigens used to immunize the donors			
	Peritoneal exudate cell transfer		Spleen cell transfer	
	OA	Pic-GPA	OA	Pic-GPA
	mm	*mm*	*mm*	*mm*
Immunized	13.6 ± 1.6	13.5 ± 0.9	8.6 ± 1.3	14.5 ± 0.2
Immunized and desensitized‡	7.0 ± 1.3	7.6 ± 0.9	4.0 ± 1.0	5.6 ± 2.1

* SEM, standard error of the mean.
‡ Immunized to HSA, BGG, and PPD, desensitized to HSA and BGG (see Table II).

TABLE IV

Passive Transfer of Delayed Hypersensitivity from Immunized and Desensitized Guinea Pigs into Normal Guinea Pigs Using Peritoneal Cells

Donor status (no. animals)	Recipient skin reactions (mean ± SEM*) to antigenic challenge			
	HSA	BGG	PPD	OA
	mm	*mm*	*mm*	*mm*
Immunized‡ (12)	5.6 ± 0.9	5.1 ± 0.4	9.5 ± 1.0	9.9 ± 1.3
Immunized and desensitized§ (12)	4.7 ± 0.6	4.9 ± 0.3	9.6 ± 1.2	11.0 ± 2.2

* SEM, standard error of the mean.
‡ Immunized to HSA, BGG, PPD, and OA.
§ Desensitized to HSA and BGG.

of DH with cells sensitized for only 8 days, PE cells from sensitized or desensitized inbred strain 2 guinea pigs prepared as described previously were transferred into normal guinea pigs of the same strain. The recipients were then challenged with all four antigens. The results of skin tests in donors as well as recipients are shown in Table VI. Clearly cells from the desensitized animals did

TABLE V

Passive Transfer of Delayed Hypersensitivity from Immunized and Desensitized Guinea Pigs into Normal Guinea Pigs Using Spleen Cells

Donor status (no. animals)	Recipient skin reactions (mean ± SEM*) to antigenic challenge			
	HSA	BGG	PPD	OA
	mm	*mm*	*mm*	*mm*
Immunized‡ (12)	4.9 ± 0.3	5.0 ± 0.3	6.8 ± 0.5	4.8 ± 0.3
Immunized and desensitized§ (12)	5.0 ± 0.3	4.7 ± 0.2	8.2 ± 0.7	5.2 ± 0.4

* SEM, standard error of the mean.
‡ Immunized to HSA, BGG, PPD, and OA.
§ Desensitized to HSA and BGG.

TABLE VI

Skin Reactions of Donor Guinea Pigs and Recipients of PE Cells from These Donors

Group tested	Status	Source of donor cells	Skin reactions (mean diameter + SEM*)			
			HSA	BGG	PPD	OA
			mm	*mm*	*mm*	*mm*
Donors‡	Immunized	—	25.5 ± 3.2	30.5 ± 4.5	18.5 ± 1.5	23.5 ± 4.0
	Immunized and desensitized	—	3.1 ± 0.4	5.6 ± 0.5	4.8 ± 0.5	5.5 ± 0.6
Recipients§	Not immunized	Immunized	9.0 ± 1.0	8.5 ± 0.5	14.0 ± 1.4	19.5 ± 1.3
	Not immunized	Immunized and desensitized	7.5 ± 0.9	9.2 ± 1.6	6.7 ± 0.6	10 ± 0.7

* SEM, standard error of the mean.
‡ Eight inbred strain 2 guinea pigs, all immunized to HSA, BGG, OA, and PPD, and four desensitized with HSA and BGG.
§ Six normal inbred strain 2 guinea pigs.

passively transfer DH responsiveness to normal animals, although for the antigens PPD and OA the responsiveness was less than that acquired by the transfer of cells from the nondesensitized donors. However, although these reactivities were less marked than those of the control group, they exceeded the reactions elicited by the same antigens in the desensitized donors, thus demonstrating a considerable recovery of the competency of these cells.

DISCUSSION

Desensitization remains a poorly understood phenomenon. Its essential features include the transiency of the loss of DH responsiveness to the antigen inducing this state, and its nonspecificity as demonstrated by the decrease in response to indifferent antigens (3–5). It occurs in species other than the guinea pig (10) and has been considered to affect cellular rather than humoral immunity (11). It has been postulated that desensitization may result from an induced lack of available macrophages, the target cells in a DH response (12). There is a well-demonstrated temporary disappearance of peripheral monocytes and macrophages from peritoneal exudate occurring within hours of an injection of large doses of antigen into sensitized animals (13–15). SRF seemed an ideal agent with which to test this hypothesis. Exposure of lymphocytes from immunized guinea pigs to antigen in vitro results in the release of soluble substances, which have been named SRF, which are able to induce an inflammatory reaction in the skin of normal guinea pigs. The inflammation is characterized by erythema and induration which reaches a peak in 6–8 h after injection; and histological examination reveals a mixed polymorph-mononuclear infiltrate in the dermis (6, 16). The data reported here clearly demonstrate that SRF is capable of inducing a predominantly macrophage-mediated response in the skin of desensitized guinea pigs of equal intensity to that response seen in normal animals. This finding suggests that neither the specific nor nonspecific weakening of DH that follows desensitization can be explained by a lack of available macrophages. Optimal suppression of DH is seen 24–48 h after desensitization (3), a time-course compatible with normal DH reactions. It has been postulated that desensitization provides an exaggerated model of normal DH (3). If this is so, it would follow that normal DH responses could terminate while target cells remained fully responsive to lymphokines.

The most striking finding of these studies is the inability to transfer DH responses with immunologically competent lymphocytes into desensitized recipients. Clearly donor cells from heterologously immunized animals had the ability to impart DH responsiveness to control recipients, while this ability was suppressed in the recipients that were desensitized. The presence of a circulating humoral factor capable of interfering with the response of these cells to antigen seems a probable explanation. This hypothesis received support from experiments in which attempts were made to transfer DH responses with cells from desensitized animals. Our results suggest that such cells, once removed from the desensitized environment, rapidly recover their responsiveness to antigens. Two previous attempts to transfer DH with desensitized cells produced conflicting results. Gell and Wolstencroft reported (17) that cells from animals which had been desensitized in vitro transferred DH as effectively as cells from fully sensitive animals. However, Asherson and Stone (4) claimed that PE cells from desensitized donors had a reduced ability to transfer DH. They used a small number of animals, and the reduced ability was marginal and only demonstrable

when transferring sensitivity to the desensitizing antigens. All transfer experiments using donor immunization for only 8 days are difficult, since cells from short-term immunized animals are far less efficient at transferring DH than cells from animals immunized for longer periods. Desensitization, however, must be completed before antibodies develop to the sensitizing antigen which might induce an anaphylactic response to the desensitizing dose of antigen. It is significant, however, that in our experiments and in those of Asherson and Stone, 150×10^6 cells from desensitized donors frequently produced a DH response in a normal recipient of greater magnitude than the DH response to the same antigen elicited in the donor before the cells were taken for transfer. This is never seen in a normal transfer wherein the donor's skin reactivity is always greater than that of the recipient. Thus, a large degree of recovery from the desensitized state has occurred. These suggestions are confirmed by our experiments with inbred guinea pigs where similar histocompatibility allows for better transfer with short-term immunized cells.

Schlossman et al. (12) have suggested that desensitization affects peritoneal exudate lymphocytes but not lymph node lymphocytes, the former cells being unable to incorporate tritiated thymidine while the latter cells do so readily. While an anatomical compartment may protect lymphocytes from desensitizing influences, our data suggest that such protection is not due to qualitative differences in lymphocytes since cells from both spleen and peritoneal cavity were equally susceptible to the suppressive environment produced by desensitization, and were equally capable of mediating DH when removed from desensitized donors and injected into normal recipients.

We have previously speculated on the existence of a feedback inhibition factor (FIF) that might terminate DH responses (3). The evidence presented here of an environmental factor inhibiting DH in desensitized states is still indirect, but compatible with such a concept. Direct evidence for this factor is now being sought. It would provide better understanding of normal DH reactions and possibly those states of generalized energy seen in human granulomatous disease. Such diseases may represent not a deficiency but rather an active inhibition of cellular immunity induced by a normal product of antigen-lymphocyte interaction produced at an exaggerated level. A factor which inhibits lymphocyte responses has been reported in leprosy (18), sarcoidosis (19, 20), mucocutaneous candidiasis (21), and syphilis (22). It is too early to equate these states with desensitization but a relationship and the isolation of a desensitizing factor is being vigorously pursued.

SUMMARY

Two potential mechanisms for terminating delayed hypersensitivity (DH) reactions have been examined in desensitized guinea pigs. Lack of macrophage responsiveness to lymphokines was sought as an explanation for the reduced ability of these animals to express delayed hypersensitivity. Skin-reactive factor

was injected into the skin of desensitized guinea pigs and a control group of similarly immunized animals. The resulting inflammatory reactions were similar in size and intensity in both groups indicating normal macrophage responsiveness in the desensitized state. Passive cellular transfer of DH responses to desensitized animals was markedly less successful than transfer to normal animals. However, cells from desensitized guinea pigs did transfer DH responsiveness to normal animals. These data support the concept of a humoral suppressant of cellular immunity, perhaps acting as a feedback inhibitor, produced when guinea pigs are desensitized.

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