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Desensitization *in vitro*—The Specific Inhibition, by Antigen, of the Passive Transfer of Delayed Hypersensitivity by Peritoneal Exudate Cells

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Summary. A preliminary experiment showed that the injection of bovine γ-globulin into guinea-pigs with delayed hypersensitivity to bovine γ-globulin reduced the 24-hour skin reactions to bovine γ-globulin and (to a lesser extent) PPD. The peritoneal exudate cells from the desensitized donors had a reduced ability to transfer delayed hypersensitivity to bovine γ-globulin but a normal ability to transfer delayed hypersensitivity to PPD.

Likewise, it was possible to diminish the passive transfer of delayed hypersensitivity to bovine γ-globulin by peritoneal exudate cells, by exposure of the cells to bovine γ-globulin *in vitro*. The recipients were tested immediately after cell transfer. This *in vitro* desensitization was specific, in that the transfer of delayed hypersensitivity to PPD was unaffected.

Exposure of cells *in vitro* to hypotonic conditions and antibody to guinea-pig γ-globulin did not prevent the passive transfer of delayed hypersensitivity.

INTRODUCTION

Guinea-pigs develop delayed hypersensitivity after immunization with antigen in Freund's complete adjuvant. This delayed hypersensitivity can be reduced and sometimes abolished by the systemic injection of antigen about the time of performing the skin test. This reduction in delayed hypersensitivity is called desensitization. When delayed hypersensitivity is produced by immunization with egg albumin in Freund's complete adjuvant and testing is performed 9 days later as little as 18 μg of antigen causes partial desensitization (Uhr and Pappenheimer, 1958; see also Silverstein and Borek, 1966). However, in other situations it is reported that several milligrams of antigen injected several times are required for desensitization (Benacerraf and Kantor, 1963).

Uhr and Pappenheimer (1958) found that antigen would cause desensitization when given 5 hours after the skin test. This indicated that the cells which mediate delayed hypersensitivity could be desensitized following a short exposure to antigen and suggested that it should be possible to produce desensitization *in vitro*. It was found that passive transfer of delayed hypersensitivity to bovine γ-globulin by peritoneal exudate cells was specifically inhibited by exposure to antigen *in vitro*.

MATERIALS AND METHODS

Animals

Hartley strain, outbred guinea-pigs purchased from a dealer were used. The donors weighed 200–400 g and the recipients 200–300 g. Female animals were usually used.

Sensitization

The Hartley donors were sensitized with 50 μg bovine γ-globulin in 0·2 ml Freund's complete adjuvant containing 0·2 mg human tubercle bacilli. This was injected into the four footpads.

Passive transfer of delayed hypersensitivity

Good transfers of delayed hypersensitivity to bovine γ-globulin can be obtained if both peritoneal cells and immune serum are transferred to the recipient (Asherson and Loewi, 1966). Peritoneal exudate cells were harvested 3 weeks after immunization and 4 days after the intraperitoneal injection of liquid paraffin. The recipients were randomized and received 3 ml of serum intravenously. A few hours later the peritoneal exudate cells were injected and skin tests performed within an hour. Serum was active after freezing. All skin tests were read 'blindly' and the diameter of erythema recorded. Induration was measured with a Quicktest dial caliper gauge A 02 T (Schnelltaster, System Kroplin).

Desensitization in vivo

The donors were immunized 18 days before transfer and liquid paraffin was injected 4 days before transfer. The day before transfer 0·25 mg Anthisan (mepyramine maleate) was injected intramuscularly followed 10 minutes later by 1 mg bovine γ-globulin (BGG). Ten milligrams BGG was given subcutaneously at 45 minutes, 5 mg intramuscularly at 75 minutes and 5 mg intravenously at 3 hours. The skin tests were undertaken at 3½ hours. Five milligrams was given intravenously the following morning and the guinea-pigs used for transfer 1 hour later. The serum for the transfer was obtained from the control guinea-pigs only.

Incubation in vitro

The peritoneal cells were spun down, filtered to remove debris and resuspended in Krebs–Ringer–glucose–phosphate (Umbreit, Burris and Stauffer, 1957). They were then placed in incubation media containing 8–10 per cent normal guinea-pig serum which had been heated at 56° for 30 minutes, 100 units/ml penicillin and 100 μg/ml streptomycin. For desensitization, a 1 per cent solution of Armour bovine γ-globulin was added to give a final concentration of 1 mg/ml. In some experiments the bovine γ-globulin solution was filtered through a millipore membrane. In the main experiments (Tables 2 and 4) Krebs–Ringer–glucose–phosphate was used throughout and the cells were shaken on a Dubnoff metabolic shaker. All other experiments were conducted in Parker 199, or Hanks's balanced buffered saline, and without mechanical shaking. The peritoneal exudate cells from six donors were normally incubated in 50 ml of medium. After incubation the cells were washed in 25 ml Krebs–Ringer–glucose–phosphate containing 4, 2 and 1 per cent of heated guinea-pig serum. In experiments with incubation at 0° the cells were washed at 4°. The cells were finally suspended in 1 per cent heated guinea-pig serum. In each experiment of Table 2, twenty-four donors were used and there were twelve to fifteen recipients.

In most experiments control animals were included that received serum. These occasionally gave a small reaction to bovine γ-globulin at 24 hours, which was much smaller than the 24-hour reactions produced by un-desensitized cells and serum together.

RESULTS

DESENSITIZATION *in vivo*

Eight guinea-pigs were immunized with bovine γ-globulin (BGG) in Freund's complete adjuvant. Four were desensitized by the injection of 26 mg of BGG. Table 1 shows that

TABLE 1

DESENSITIZATION OF DELAYED HYPERSENSITIVITY TO BOVINE γ-GLOBULIN *in vivo*

	Time of reading (hours)	Diameter and induration of skin reactions to: BGG 125 μg Diam.*	BGG 125 μg Ind.†	BGG 25 μg Diam.*	BGG 25 μg Ind.†	BGG 5 μg Diam.*	BGG 5 μg Ind.†	PPD 20 μg Diam.*	PPD 20 μg Ind.†
Donors									
Control	3	18·1	40·8	16·1	34·5				
Desensitized	3	2·7	27	2·4	27·7				
Control	19	23·4	48	18·0	40·5	10·6	33	22·1	39·5
Desensitized	19	8·8	23·3	5	24	3·7	24	16·2	24·3
Recipients									
Control	24	12·1	31·0	11·0	25·2	6·0	23·2	13·4	23·2
Desensitized	24	6·5	27·7	5·2	21·7	1·5	20	15·7	24·5

Donors were immunized with bovine γ-globulin and desensitized by the injection of bovine γ-globulin. Peritoneal exudate cells ($1{\cdot}4 \times 10^8$) from desensitized (three) and control (four) guinea-pigs were used to transfer skin reactions to normal recipients in a one to one transfer.
The value before the skin test was about 22 (0·1 mm) units.

* Mean diameter of the skin reaction.
† Induration as measured by the mean skin fold thickness.

this reduced the 3- and 19-hour reaction to bovine γ-globulin. There was also some reduction in the reaction to purified protein derivate (PPD). This reduction was only slight as measured by the diameter of the 19-hour lesion but very considerable as measured by induration. These guinea-pigs were ill and this non-specific desensitization may have been due to prolonged anaphylaxis. Non-specific desensitization is also described by Uhr and Pappenheimer (1958) who attributed it to lymphopenia. Peritoneal exudate cells from the desensitized donors conveyed normal delayed hypersensitivity reactions to PPD but only poor reactions to bovine γ-globulin. It was concluded that desensitization *in vivo* reduced the ability of peritoneal exudate cell population to transfer delayed hypersensitivity to normal recipients.

DESENSITIZATION *in vitro*

The peritoneal exudate cells of guinea-pigs immunized with bovine γ-globulin in Freund's complete adjuvant will transfer delayed hypersensitivity to normal recipients. The effect of incubating the cells *in vitro* with bovine γ-globulin (BGG) and then washing to remove the free antigen was studied. In all experiments an aliquot of cells was incubated *in vitro* without antigen.

Table 2 shows that, in a series of five experiments, incubation of peritoneal exudate cells with bovine γ-globulin consistently diminished the passive transfer of delayed hyper-

TABLE 2

PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY BY PERITONEAL EXUDATE CELLS AFTER INCUBATION WITH BOVINE γ-GLOBULIN *in vitro*

Experiment No.	Conditions of incubation	Incubation		No. of recipients	24-hour skin reaction mean and range		
		Time	Temperature		PPD 20 μg	BGG 50 μg	BGG 10 μg
I	Control	2 hr	37°	3	13·0 (13·0)	15·6 (13–16·5)	7·8 (7·5–8·5)
	Desensitized	2 hr	37°	3	12·3 (9–14·5)	6·5 (2·5–9)	0 (0)
	Control	2 hr	0°	3	13·3 (9·5–17·5)	15·5 (13–19)	10·1 (9–12)
	Desensitized	2 hr	0°	2	10·2 (10–10·5)	7 (5–9)	0 (0)
II	Control	2 hr	37°	3	12·0 (9·5–13·5)	16·6 (15–20)	7·3 (5–10)
	Desensitized	2 hr	37°	3	11·3 (6·5–14·5)	2·5 (0–5)	1·3 (0–2)
	Control	2 hr	0°	4	13·6 (11·5–14·5)	13·2 (4–17)	8·8 (3·5–12·5)
	Desensitized	2 hr	0°	4	12·5 (9·5–14·5)	10·2* (5·5–15)	5·7 (2–9·5)
III	Control with Ca, Mg	2 hr	37°	3	11·0 (10–14)	10·5 (8–12·5)	8·5 (6–11)
	Desensitized with Ca, Mg	2 hr	37°	3	13·8 (13·5–14)	2·8 (0–8·5)	0 (0)
	Control no Ca, Mg	2 hr	37°	4	12·9 (10–15)	13·7 (11–17·5)	9·4 (8·5–10)
	Desensitized no Ca, Mg	2 hr	37°	4	15·0 (13–17)	0·7 (0–3)	0 (0)
IV	Control	20 min	37°	3	11·8 (10–15)	14 (11–16·5)	6·8 (3–10·5)
	Desensitized	20 min	37°	3	11·5 (10–14)	4·2 (0–7·5)	2·2 (0–6·5)
	Control	1 hr	37°	3	11·0 (9·5–12)	13·3 (10·5–15)	8·2 (6–9·5)
	Desensitized	1 hr	37°	3	11·6 (11–13)	5·5 (0–9·5)	3·2 (0–7·5)
	Desensitized	2 hr	37°	3	9·8 (8–12·5)	0 (0)	0·6 (0–2)
V	Control	23 min	37°	3	12·3 (9·5–14·5)	16·8 (14·5–20)	6·6 (5–8·5)
	Desensitized	23 min	37°	3	11·2 (13–16)	11·1 (6·5–14·5)	6·8 (5–8)
	Control	15 min	0–15°	4	11·2 (10–14)	18·1 (12–22)	9·9 (6·5–12)
	Desensitized	15 min	0–15°	4	12·2 (5·5–18)	13·3† (10·5–17)	7·5 (5·5–9·5)

Skin reactions in recipients read 24 hours after transfer.

Cells were incubated in Krebs medium with or without added bovine γ-globulin. The range is shown in parentheses.

In Experiment III the cells were collected in the standard Krebs medium containing 2·58 mM Ca^{++} and 1·2 mM Mg^{++}. The cells from eight guinea-pigs were then placed in 50 ml of Krebs medium without added divalent ions. The added serum was dialysed before use against saline. The cells were washed in Krebs medium containing divalent ions.

* One guinea-pig showed diffuse erythema.

† One guinea-pig gave very faint reaction which was unmeasurable.

sensitivity to bovine γ-globulin. After 2 hours incubation at 37° without bovine γ-globulin the mean diameter of the 24-hour skin reactions to 50 μg bovine γ-globulin in groups of three to four recipients ranged from 10·5 to 16·6 mm; after incubation with bovine γ-globulin the mean figures ranged from 2·5 to 6·5 mm. These reductions were equivalent to a reduction in the test dose of antigen from 50 μg to less than 10 μg.

The reduction was specific, that is to say the mean diameter of the skin reaction to PPD showed little change. In some experiments there was a suggestion that the intensity of the reaction to PPD was slightly reduced.

TABLE 3

PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY BY PERITONEAL EXUDATE CELLS AFTER INCUBATION WITH BOVINE γ-GLOBULIN *in vitro*

Experiment No.	Conditions of incubation	Time (hours)	No. of recipients	24-hour skin reactions		
				PPD	BGG 125 μg	BGG 25 μg
IA	Control	1	3	14·3	18·3	8·3
	Desensitized	1	3	14·8	17·8	9·5
IIA	Control	4	5	13·9	13·3	9·5
	Desensitized	4	5	14·1	9·0	5·4

Skin reactions in recipients read 24 hours after transfer.

In these experiments the cells were taken under sterile conditions and incubated in 10 per cent guinea-pig serum in Parker 199. No mechanical shaking.

TABLE 4

PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY BY PERITONEAL EXUDATE CELLS AFTER INCUBATION UNDER HYPOTONIC CONDITIONS AND WITH RABBIT ANTIBODY TO GUINEA-PIG γ-GLOBULIN *in vitro*

Experiment No.	Conditions of incubation	24-hour skin reactions		
		PPD 20 μg	BGG 100 μg	BGG 20 μg
IB	Krebs medium 0·705 per cent saline	14	30·1	23
	Krebs medium 0·45 per cent saline	14·6	21·3	15·3
	Krebs medium 0·35 per cent saline	13·3	20	14·4
IIB	0°	11·5	15·5	14
	37°	11·3	18	15·8
	Added rabbit anti-guinea-pig γ-globulin at 37°	12·7	20·3	16·8
	Added rabbit anti-guinea-pig γ-globulin and guinea-pig serum	12·8	15·2	14·3

Skin reactions in recipients read 24 hours after transfer.

Two to one transfers, incubation for 1 hour at 37° with shaking. In Experiment IIB the cells were incubated in a volume of 25 ml. This contained 1·25 ml each of hyper-immune rabbit anti guinea-pig γ_1-globulin and γ_2-globulin. In a control 4 ml of guinea-pig serum was added.

Desensitization did not require prolonged *in vitro* incubation with antigen. Experiment IV (Table 2) shows that desensitization occurs within 20 minutes, and Experiment V illustrates that some desensitization occurs within 15–23 minutes.

Experiment I suggests that desensitization can occur when the cells are incubated at 0°. However, the cells incubated at 0° were unshaken in Experiments I and II and this

requires further investigation. Experiment II shows that desensitization occurs when the incubation with antigen is conducted in a medium free of added calcium and magnesium ions.

In earlier experiments the cells were incubated in Parker 199 or in Hanks's buffered saline without mechanical shaking. In some of these experiments no desensitization was seen at 1 hour and even at 4 hours desensitization was limited (Table 3). This may indicate the importance of shaking for *in vitro* desensitization.

MISCELLANEOUS OBSERVATIONS

Lymphocytes are resistant to hypotonic saline (Trowell, 1963). This suggested that the passive transfer of delayed hypersensitivity would be unaffected by incubating in hypotonic media. Table 4 shows this is indeed the case.

It is possible that delayed hypersensitivity is mediated by cells possessing immunoglobulin receptors. However, incubation of peritoneal cells with mixed rabbit antisera to γ_1- and γ_2-globulins did not affect passive transfer.

DISCUSSION

These experiments show that it is possible to prevent the passive transfer of delayed hypersensitivity by peritoneal exudate cells by exposure to antigen *in vitro* and preliminary studies suggest that this is also true of lymph node cells. Prolonged *in vitro* exposure is not required and desensitization follows exposure to antigen for only 20 minutes. This suggests that the receptor concerned is readily accessible to bovine γ-globulin and this raises the question whether it is on the surface of the cell.

It is not clear whether the passive transfer of delayed hypersensitivity is due to donor cells which arrive at the skin test site which interact with antigen to cause the influx of further cells (Turk and Oort, 1963; McCluskey, Benacerraf and McCluskey, 1963), or whether they transfer a factor to recipient cells which then migrate to the skin test site. David (1966) has shown that sensitized lymphoid cells during incubation with antigen liberate a material which inhibits the migration of normal peritoneal cells. He raises the question whether this factor is an immunoglobulin. These views suggest that desensitization may prevent the passive transfer of delayed hypersensitivity either by blocking the arrival of the donor cells at the appropriate site or by altering their ability to liberate pharmacologically active agents or immunoglobulin.

It is likely that two separate phenomena are studied under the heading of desensitization. In some experiments (Uhr and Pappenheimer, 1958; Gell and Wolstencroft, 1967) desensitization only lasts a few days and may reflect the desensitization of cells involved in the mediation of delayed hypersensitivity. In other experiments (Leskowitz and Jones, 1965; Dvorak and Flax, 1966), desensitization lasts over 10 days and presumably affects cells whose progeny are the cells which mediate delayed hypersensitivity.

Gell and Wolstencroft (1967) injected sensitized lymph node cells into irradiated recipients and obtained delayed hypersensitivity two or more days later. Under these circumstances desensitization *in vitro* or *in vivo* had no effect on passive transfer.

The difference between their results and the present ones may be due to the fact that in their experiments the progeny of the injected cells were responsible for the passively transferred delayed hypersensitivity while in the present experiments the injected cells themselves were responsible.

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