Immunology, 1966, **10**, 179.

Selective and Specific Inhibition of 24-Hour Skin Reactions in the Guinea-Pig

II. THE MECHANISM OF IMMUNE DEVIATION

G. L. ASHERSON*

National Institute for Medical Research, Mill Hill, London

(*Received 27th July* 1965)

Summary. Guinea-pigs injected with antigen in Freund's complete adjuvant develop delayed hypersensitivity to the antigen. This is reduced by the prior injection of the same antigen precipitated with alum. This may be termed immune deviation and has been shown to differ from immune tolerance. Four possible mechanisms for this phenomenon were considered: (a) that there was a specific defect in the cell population responsible for the passive transfer of delayed hypersensitivity; (b) that there was an antibody blocking the detection of delayed hypersensitivity; (c) that there was an antibody blocking the induction of delayed hypersensitivity; and (d) that the cells responsible for delayed hypersensitivity were desensitized *in vivo*. Experiments on the passive transfer of delayed hypersensitivity from deviated and control guinea-pigs showed that there was a defect in the cell population involved in the passive transfer of delayed hypersensitivity. Serum transfer failed to reveal evidence of antibody blocking the induction or detection of delayed hypersensitivity. Transfer of cells into recipients which had been pretreated with alum-precipitated antigen gave no evidence of desensitization in the PPD system. The findings with bovine γ-globulin were equivocal. It was concluded that there was a defect in the cell population involved in the passive transfer of delayed hypersensitivity and, in the absence of evidence for other mechanisms, this was attributed to a direct effect of the first injection of antigen on the cells responsible for the state of delayed hypersensitivity after the injection of the same antigen in Freund's complete adjuvant.

INTRODUCTION

The response of the guinea-pig to antigen injected in Freund's complete adjuvant is altered by the prior injection of alum-precipitated antigen. There is a specific reduction in delayed hypersensitivity and circulating γ_2 antibody although the total antibody response is not reduced. This phenomenon may be called immune deviation (Asherson and Stone, 1965; Loewi, Holborow and Temple, 1966) and is distinct from immune tolerance and immune enhancement.

There are several possible ways in which the first (deviating) injection of antigen may affect the response to the injection of same antigen in Freund's complete adjuvant.

* Present address: Department of Bacteriology, London Hospital Medical College, London, E.1.

(i) It may cause a defect in the cell population responsible for delayed hypersensitivity. This can be demonstrated by the passive transfer of delayed hypersensitivity to normal recipients by immune cells from deviated and undeviated guinea-pigs immunized with antigen in Freund's complete adjuvant.

(ii) It may produce an antibody which blocks the induction of delayed hypersensitivity. This is analogous to the specific ability of antibody to prevent antibody synthesis (Uhr and Baumann, 1961) and can be excluded by serum transfer experiments.

(iii) It may produce an antibody which blocks the detection of delayed hypersensitivity. This is analogous to immune enhancement (Kaliss, 1958) and can be excluded by serum transfer.

(iv) It may desensitize the cells involved in delayed hypersensitivity. Desensitization occurs within 4 hours of exposure of immune cells to antigen *in vitro* (Asherson, unpublished observations). Its participation in immune deviation is made unlikely by experiments on the passive transfer of delayed hypersensitivity to PPD to recipients pretreated with alum-precipitated antigen.

The experiments described in this paper support the view that immune deviation is due to a defect in the cell population responsible for delayed hypersensitivity.

MATERIALS AND METHODS

Immune deviation

Immune deviation is demonstrated by randomly assigning guinea-pigs to an experimental (deviated) and control group. The guinea-pigs of the experimental group receive a first injection of alum-precipitated antigen (pretreatment). They are subsequently immunized with antigen in Freund's complete adjuvant (immunization) and finally skin tested with antigen. In the experimental group the same antigen was used for these three procedures. The guinea-pigs of the control group received the same immunization and skin tests as the experimental (deviated) group but were pretreated with an unrelated alum-precipitated antigen. This design elucidates the effect of pretreatment with the same (deviating) or unrelated (control) antigen on the delayed hypersensitivity which follows immunization with antigen in Freund's complete adjuvant. (See Asherson and Stone, 1965.)

In the present experiments, unless otherwise stated, the donor guinea-pigs used for the passive transfer of delayed hypersensitivity were randomly assigned to two groups. One group received the deviating antigen (1 mg) precipitated with alum while the other group received the control antigen (1 mg) precipitated with alum, intravenously and subcutaneously. Fourteen days later the guinea-pigs were immunized with 50 μg of antigen in Freund's complete adjuvant and transfers were performed 3–5 weeks later. The same dosages were used in the experiments on the effect of antibody on the induction and detection of delayed hypersensitivity.

Passive transfer of delayed hypersensitivity

This was undertaken with peritoneal exudate and/or spleen cells which were pooled from a number of donors, washed once, and injected intravenously. The cell counts in the pools from the deviated and control guinea-pigs were comparable. Two to one transfers were commonly used, that is to say the cells derived from two donors were injected into one recipient. In the experiments on cell and serum transfer the donors

received 3–5 ml of heparinized plasma or serum from the donors which was given intravenously about an hour before the injection of cells. The recipients were tested within 1 hour of transfer by the intradermal injection of 0·1 ml of antigen. Unless otherwise stated, PPD, bovine γ-globulin and egg albumin were used at a concentration of 20, 100 and 100 μg/0·1 ml respectively. The mean diameter of erythema at 24 hours was recorded. Induration was measured with a Quicktest dial calliper and the original mean skin fold thickness of the group of guinea-pigs (usually 2·1 mm) was subtracted.

RESULTS

DEFECT IN THE IMMUNE CELL POPULATION IN IMMUNE DEVIATION

In the first two experiments, delayed hypersensitivity was transferred by spleen and peritoneal exudate cells from deviated and control donors to normal recipients. The donors were deviated with bovine γ-globulin, egg albumin or purified protein derivative derived from tuberculin (PPD) and were later immunized with bovine γ-globulin and egg albumin in Freund's complete adjuvant. The results are given in Table 1. The mean diameter of the 24-hour skin reaction to PPD in recipients that had received cells from guinea-pigs deviated with egg albumin or bovine γ-globulin was 14·4 mm. In contrast the reaction transferred by the cells of guinea-pigs deviated with PPD was only 7·2 mm.

TABLE 1

REDUCED ABILITY OF THE SPLEEN AND PERITONEAL EXUDATE CELLS OF GUINEA-PIGS SHOWING IMMUNE DEVIATION TO TRANSFER DELAYED HYPERSENSITIVITY TO NORMAL RECIPIENTS

Experiment	Donor deviated by:	Cells	No. of recipients	Mean and range of diameter (mm) of 24-hour skin reaction to			
				PPD		BGG*	
I	BGG*	Spleen	3	12·5	(7–15·5)	1·8	(1–3)
	PPD	Spleen	3	6·3	(4·5–8)	4·5	(3–6)
	BGG	Macrophages†	1	22		2	
	PPD	Macrophages	3	7·8	(7·5–8)	7·2	(4·5–9·5)
II	BGG	Spleen	3	14·3	(12–16·5)	2·3	(2–2·5)
	EA‡	Spleen	3	14·2	(8·5–18·5)	5·2	(2–8)
	PPD	Spleen	3	6·5	(6–7·5)	8	(5·5–10)
	BGG	Macrophages	3	13·5	(10–17·5)	5	(2–10·5)
	EA	Macrophages	1	16		9·5	
	PPD	Macrophages	3	8·3	(6·5–9·5)	9·3	(8·5–10·5)

Three to two transfers were undertaken 5 weeks after immunization.

* Bovine γ-globulin.
† Peritoneal exudate cells.
‡ Egg albumin.

A similar, specific reduction was found in the reaction to bovine γ-globulin. The delayed hypersensitivity reaction transferred by cells from control guinea-pigs deviated with PPD or egg albumin was 7·0 mm. In contrast, the mean reaction transferred by cells from guinea-pigs deviated with bovine γ-globulin was 2·9 mm.

These results showed that there was a specific defect in the cell population of guinea-pigs with immune deviation to PPD or bovine γ-globulin. The results with bovine γ-globulin were, however, somewhat difficult to interpret because the passively transferred lesions were diffuse and difficult to read.

Attempts to produce better passive transfer of delayed hypersensitivity to bovine γ-globulin led to the observation that well-defined 24-hour skin lesions could be transferred using cells *and* serum from the immunized donors (Asherson and Loewi, 1966). This method was used to study the ability of deviated guinea-pigs to transfer delayed hypersensitivity to bovine γ-globulin. One group of donor guinea-pigs was deviated by the prior injection of alum-precipitated bovine γ-globulin while the control group received alum-precipitated egg albumin. Both groups were immunized with bovine γ-globulin in Freund's complete adjuvant. The initial experiments in which the peritoneal exudate cells of two guinea-pigs and serum were transferred to one guinea-pig (two to one transfer) suggested that cells and serum from the deviated guinea-pigs transferred smaller lesions than cells and serum from the control guinea-pigs. The difference was however slight and clear-cut results were only obtained on reducing the

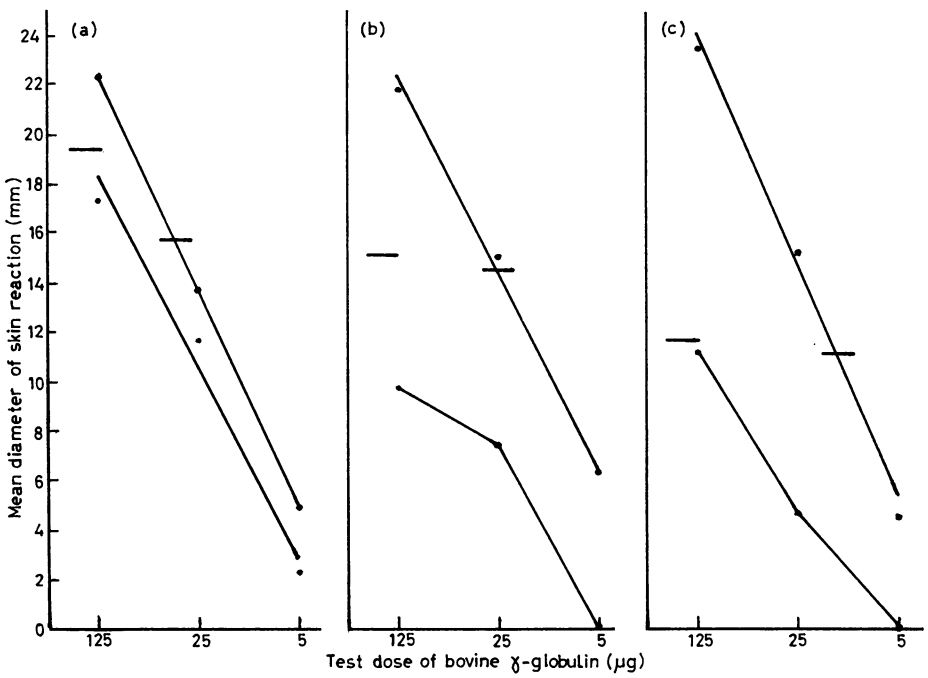

FIG. 1. The effect of the number of cells transferred on the mean diameter of delayed hypersensitivity reactions to varying test doses of bovine γ-globulin in guinea-pigs receiving peritoneal exudate cells from control and deviated guinea-pigs. (a) Two to one transfer, (b) one to one transfer, and (c) half to one transfer.

The guinea-pigs were given peritoneal exudate cells equivalent to two, one and half a donor. All the recipient guinea-pigs were also given serum from the deviated donors. Each point is based on the mean of three guinea-pigs. In each case the upper line indicates the reaction transferred by the control guinea-pigs and the lower line the reaction transferred by the deviated guinea-pigs. The mean reaction to 20 μg of PPD is shown by the horizontal lines.

number of cells transferred. Fig. 1 shows that the deviated cells had a slightly reduced ability to transfer delayed hypersensitivity in a two to one transfer. However, the deviated guinea-pigs had poor ability to transfer delayed hypersensitivity in a one to one and a half to one transfer. Both groups of guinea-pigs had similar ability to transfer

delayed hypersensitivity to PPD. It was concluded that there was a specific defect in the cell population responsible for the passive transfer of delayed hypersensitivity in the deviated guinea-pigs.

It was also possible to demonstrate a defect in the peritoneal exudate cells of guinea-pigs which had been pretreated with bovine γ-globulin in a soluble form. Table 2 shows that 6 weeks after transfer there was no demonstrable defect in the cells of animals deviated by the injection of alum-precipitated antigen. However, the donor guinea-pigs that had been pretreated with soluble bovine γ-globulin transferred very weak delayed hypersensitivity reactions. As these donor guinea-pigs had reduced antibody titres to bovine γ-globulin they were considered to be in a state of immune paralysis.

TABLE 2

PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY BY PERITONEAL EXUDATE CELLS AND SERUM OF GUINEA-PIGS PRETREATED WITH BOVINE γ-GLOBULIN AND IMMUNIZED WITH BOVINE γ-GLOBULIN IN FREUND'S COMPLETE ADJUVANT

Donor prior treatment	Mean and range of 48-hour skin reaction to:		
	BGG (diameter)	PPD (diameter)	BGG (induration)
No pretreatment	10·3 (9–12)	11·5 (10·5–12·5)	1·7 (1·05–2·4)
Pretreated with soluble BGG (paralysis)	2·5 (0–7·5)	11·3 (10·5–13)	0·9 (0·8–1·0)
Pretreated with alum-precipitated BGG	12·2 (11–13)	14·2 (13–16·5)	1·7 (1·0–2·15)

Two to one transfers were undertaken 6 weeks after immunization. The donors were treated with 10 mg of soluble or alum-precipitated antigen intravenously and subcutaneously and immunized with bovine γ-globulin in Freund's complete adjuvant 14 days later.

Each figure is based on the mean of three guinea-pigs. All the recipient guinea-pigs received 5 ml of pooled serum from deviated guinea-pigs taken 3 weeks after immunization.

TABLE 3

THE EFFECT OF THE PASSIVE TRANSFER OF SERUM FROM DEVIATED DONORS ON THE INDUCTION OF DELAYED HYPERSENSITIVITY TO PPD AND BOVINE γ-GLOBULIN

Prior treatment	Mean diameter and range of 24-hour skin reaction to:			
	PPD (tested Day 8)	BGG (tested Day 8)	PPD (tested Day 15)	BGG (tested Day 15)
Serum from PPD donor	11·6 (4·5–13·5)	23 (22–25)	11·5 (10·5–13·5)	10·1 (9–12)
Serum from BGG donor	12·0 (11–13)	25·5 (23–28)	12·6 (9·5–15·5)	10·1 (7·5–16)
Serum from normal donor	11·6 (11–13·5)	25·0 (21·5–26)	13·0 (9·5–16·5)	11·4 (9–12·5)
Nil	11·1 (9–11·5)	20·1 (18–22·5)	11·0 (10–11·5)	11·25 (11–12·5)

The serum donors were prepared by injecting 1 mg of alum-precipitated bovine γ-globulin or PPD. Twelve and 14 days later 8 ml of serum was transferred to groups of four recipient guinea-pigs which were then immunized with 50 μg of bovine γ-globulin in Freund's complete adjuvant.

Test doses of antigen: PPD 6 μg/0·1 ml; bovine γ-globulin 10 μg/0·1 ml.

Serum transfer did not affect the induction of delayed hypersensitivity. Similar results were obtained in a second, separate, experiment.

INABILITY TO DEMONSTRATE ANTIBODY BLOCKING THE INDUCTION OF DELAYED HYPERSENSITIVITY

Donor guinea-pigs were deviated with bovine γ-globulin or PPD. Twelve and 14 days later they were bled and of 8 ml serum injected intravenously into recipient guinea-pigs on two occasions. Control guinea-pigs received normal guinea-pig serum or were left uninjected. The recipients were then immunized with bovine γ-globulin in Freund's complete adjuvant and skin tested with bovine γ-globulin and PPD 8 and 15 days later. Table 3 shows that in two separate experiments prior treatment with serum did not block the induction of delayed hypersensitivity to PPD or bovine γ-globulin.

INABILITY TO DEMONSTRATE ANTIBODY BLOCKING THE DETECTION OF DELAYED HYPERSENSITIVITY

Donor guinea-pigs were deviated with bovine γ-globulin or PPD or were left uninjected. Fourteen days later they were immunized with bovine γ-globulin in Freund's complete adjuvant. After another 14 days, 7 ml of their serum was injected intravenously on two occasions into guinea-pigs which had been immunized with bovine γ-globulin in Freund's complete adjuvant 21 days previously. The recipients were tested 2 days later with 10 μg/0·1 ml of bovine γ-globulin. The mean diameters of the 24-hour reactions in the two groups were 13·2 and 13·8 mm. It was concluded that under these conditions antibody did not prevent the detection of delayed hypersensitivity.

TABLE 4

EFFECT OF THE PRIOR INJECTION OF ALUM-PRECIPITATED ANTIGEN INTO RECIPIENTS ON THE PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY

Prior treatment	Mean diameter and range of 24-hour skin reaction to:		
	PPD*	BGG†	EA‡
Experiment I			
Alum-precipitated PPD 7 days before transfer	15·7 (15–16)	6·1 (3–13)	3·0 (0–4·5)
Alum-precipitated BGG 7 days before transfer	15·5 (13–17·5)	2·5 (0–5·5)	3·5 (0–5·5)
Nil	16·5 (14·5–19)	8·7 (6·5–11)	3·9 (3·5–4·5)
Experiment II			
Alum-precipitated PPD 12 days before transfer	14·6 (11·5–17)	5·3 (3–7·5)	
Alum-precipitated BGG 12 days before transfer	14·9 (13·5–17)	1·2 (0–4)	
Nil	15·8 (15–17·5)	4·7 (2·5–7)	

One to one transfer with mixed peritoneal exudate and spleen cells.
Each figure is based on the mean of five guinea-pigs.

* Purified protein derivative.
† Bovine γ-globulin.
‡ Egg albumin.

THE EFFECT OF INJECTION OF ANTIGEN INTO RECIPIENTS ON PASSIVELY TRANSFERRED DELAYED HYPERSENSITIVITY (DESENSITIZATION)

Guinea-pigs were injected intravenously and into the footpads with either alum-precipitated bovine γ-globulin, alum-precipitated PPD or were left uninjected. Seven days later they received cells from donors immunized with bovine γ-globulin and egg albumin in Freund's complete adjuvant. Twelve days later, other pretreated guinea-pigs received cells from donors immunized with bovine γ-globulin in Freund's complete adjuvant. Table 4 shows that the prior injection of alum-precipitated PPD into the recipients did not affect the passive transfer of delayed hypersensitivity. There was some suggestion that the reaction to bovine γ-globulin was inhibited by the prior injection of alum-precipitated bovine γ-globulin. However, as often happens in transfers made without immune serum, the bovine γ-globulin lesions were diffuse and difficult to read. It was concluded that immune deviation in the PPD system could not be attributed to desensitization.

DISCUSSION

Guinea-pigs pretreated (deviated) with alum-precipitated antigen develop less delayed hypersensitivity after injection of the same antigen in Freund's complete adjuvant than appropriate control guinea-pigs. The present results show that these deviated guinea-pigs also have a reduced ability to transfer delayed hypersensitivity to normal recipients. This reduction is specific; that is to say, guinea-pigs which have been pretreated (deviated) with bovine γ-globulin show reduced ability to transfer delayed hypersensitivity to bovine γ-globulin but normal ability to transfer delayed hypersensitivity to PPD. Analogous specificity is shown by guinea-pigs deviated by PPD.

The experiment involving serum transfer showed that the reduced ability of the peritoneal exudate and spleen cells of deviated guinea-pigs to transfer delayed hypersensitivity was unlikely to be due to the production of an antibody blocking the detection or induction of delayed hypersensitivity. However, the difficulty in demonstrating the defect in the cell population of the deviated guinea-pigs when large numbers of cells were used for passive transfer suggested that this point deserved further attention. The evidence was also against the view that desensitization played an important role and it was concluded that there was a direct effect of alum-precipitated antigen on the induction of delayed hypersensitivity by the same antigen in Freund's complete adjuvant. The question arises whether contact of antigen with certain peripheral white cells before they enter the lymph node is important in immune deviation.

These considerations show that immune deviation is a separate phenomenon from immune tolerance and no evidence was found that it resembled immune enhancement, in which antibody is responsible for the depression of immune responses.

ACKNOWLEDGMENTS

I wish to thank Dr J. H. Humphrey, F.R.S., for helpful discussions and Mr G. Kenney for excellent technical assistance.

REFERENCES

ASHERSON, G. L. and STONE, S. H. (1965). 'Selective and specific inhibition of 24-hour skin reactions in the guinea-pig. I. Immune deviations: description of the phenomenon and the effect of splenectomy.' *Immunology*, **9**, 205.

LOEWI, G., HOLBOROW, E. J. and TEMPLE, A. (1966). 'Inhibition of delayed hypersensitivity by pre-immunization without complete adjuvant.' *Immunology*, **10**, 339.

KALISS, N. (1958). 'Immunological enhancement of tumour homografts in mice; a review.' *Cancer Res.*, **18**, 992.

UHR, J. W. and BAUMANN, J. B. (1961). 'Antibody formation. I. The suppression of antibody formation by passively administered antibody.' *J. exp. Med.*, **113**, 935.