Immunology 1976 **30** 593

Central and peripheral action of suppressor cells in contact sensitivity in the guinea-pig

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Received 24 *September* 1975; *accepted for publication* 23 *October* 1975

Summary. Suppressor cells were demonstrated in the spleen of guinea-pigs made specifically unresponsive to dinitrofluorobenzene (DNFB) with dinitrobenzene sulphonic acid ($DNBSO_3$). Transfusion of these cells at the same time as sensitization with DNFB, produced a significant reduction in the immunoblasts proliferating in the draining lymph node 4 days later. Transfusion on the day of skin testing produced no greater suppression of skin reactivity than cells taken from animals made hyporeactive to DNFB by contact with dinitrothiocyanate benzene (DNTB). It is concluded that there are at least two sites that suppressor cells can act. In the case of total unresponsiveness induced by $DNBSO_3$, action is both central and in the periphery. In the case of hyporeactivity induced by DNTB, in which there is no defect in proliferation of T cells in response to antigen, the action of these cells is confined to the periphery. Results of spleen weight studies suggest that suppressor cells homing in the spleen respond by proliferation to epicutaneously applied DNFB.

INTRODUCTION

Immunological unresponsiveness to dinitrophenyl compounds induced by intravenous injection of dinitrobenzene sulphonic acid, sodium salt ($DNBSO_3$) or feeding of dinitrochlorobenzene is normally associated with an inhibition of proliferation of large pyroninophilic cells in the paracortical area of the lymph node draining the site of local application of sensitizer (Turk and Stone, 1963; Polak, Geleick and Turk, 1975). In two situations, however, peripheral hyporeactivity may be produced without such inhibition; these are when $DNBSO_3$ is injected intravenously at the same time as contact sensitization (Polak, Frey and Turk, 1973) and when the animals are made hyporeactive by skin contact with the cross-reacting dinitrothiocyanate benzene (DNTB) (Sommer, Parker and Turk, 1975).

The present study aims to demonstrate that spleen cells from animals made unresponsive by the intravenous injection of $DNBSO_3$ have a central effect on T-cell proliferation in recipient animals. In addition it was found that these cells have a peripheral action, similar to that previously demonstrated with cells from animals made hyporeactive by contact with DNTB (Sommer *et al.*, 1975).

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MATERIALS AND METHODS

Animals

Outbred Hartley strain guinea-pigs of either sex weighing 350–500 g were used. The animals were from stocks bred either at the Royal College of Surgeons or purchased from Messrs A. Tuck &

Son, Ltd, Rayleigh, Essex. They were fed on pelleted diet RPG (E. Dixon & Sons, Ware, Herts) liberally supplemented with cabbage.

Reagents

DNFB. 1-Fluoro-2, 4-dinitrobenzene was obtained from Hopkin & Williams Ltd, Chadwell Heath, Essex.

$DNBSO_3$. 2,4-Dinitrobenzene sulphonic acid, sodium salt was obtained from Eastman, Kodak Ltd, Liverpool.

DNTB. 2,4-Dinitrothiocyanatebenzene was purchased from Eastman Kodak Ltd, Liverpool.

Oxazolone. 4-Ethoxymethylene-2-phenyl-oxazolone was purchased from B.D.H. Chemicals, Poole, Dorset.

Cy. Cyclophosphamide monohydrate was purchased from Koch-Light Laboratories Ltd, Colnbrook, Bucks.

Induction of tolerance with $DNBSO_3$

Guinea-pigs received two intravenous injections of 500 mg/kg body weight of $DNBSO_3$ in distilled water. Injections were performed 28 and 14 days before animals were killed and spleen cells taken for transfer.

Induction of hyporesponsiveness with DNTB

0·1 ml of a 2 per cent solution of DNTB in acetone (w/v) was applied to the dorsum of the left ear, 14 and 7 days before the animals were killed and spleen cells taken for transfer.

Sensitization

DNFB. 0·05 ml of a 10 per cent (w/v) solution of DNFB in acetone: olive oil (1:1) was applied epicutaneously to the dorsum of the ear.

Oxazolone. 0·2 ml of a 10 per cent solution of oxazolone in ethanol was applied to the dorsum of the ear.

Skin tests. Skin tests were performed by dropping 0·02 ml of the test solution onto the shaved flanks of the guinea-pigs. The following solutions were used: (a) DNFB, 0·05 per cent, 0·1 per cent, 0·25 per cent and 0·5 per cent (w/v) in acetone; olive oil (4:1); (b) oxazolone, 0·06 per cent, 0·125 per cent and 0·25 per cent (w/v) in olive oil. Skin reactions were read as described previously (Parker and Turk, 1970) and the mean intensity in groups of at least five animals recorded for each concentration.

Cyclophosphamide. 250 or 300 mg/kg body weight dissolved in 0·15 M NaCl was injected intraperitoneally into guinea-pigs.

Cell transfer studies

Two groups of recipient guinea-pigs were used. One group received Cy i.p. 3 days before sensitization with 10 per cent DNFB. These animals received an intravenous (i.v.) injection of cells 7 days after sensitization and 1 h before skin test. The other group received Cy i.p. and 3 days later received an i.v. injection of cells 1 h before sensitization with 10 per cent DNFB. Four days later, the draining lymph nodes and spleen were taken for further examination. Spleen cells from donor animals (described in the Results section) were washed three times in Eagle's medium and their viability determined by the trypan blue exclusion test. 3×10^8 viable spleen cells were injected intravenously into the recipient guinea-pigs.

As controls, cells from donor animals were transferred on the day of skin test into guinea-pigs treated with Cy 3 days before sensitization with oxazolone. Also 6 days after sensitization, the Cy–DNFB recipients were injected i.p. with 10 ml of serum from donor guinea-pigs. The recipients were skin tested 24 h later.

Histology

Lymph nodes were fixed in Carnoy's solution, sectioned at 5 μm and stained with pyronin–methyl green. Sections were examined for immunoblasts (large pyroninophilic cell proliferation) in the paracortical areas by counting the number of these cells in a microscopic field of 450 μm diameter. Lymph nodes were examined from at least five animals in each group.

Statistics

Statistical assessment of probability was by Student's *t*-test.

RESULTS

Action of spleen cells from unresponsive donors on cell proliferation in lymph nodes of sensitized recipients

3×10^8 Viable spleen cells from donors made unresponsive to DNFB by two injections of 500 mg/kg $DNBSO_3$ 28 and 14 days previously, were transfused into recipients on the day they were sensitized with 10 per cent DNFB. Animals were killed and examined

Table 1. Effect of transfusion of spleen cells from animals made tolerant by injections of $DNBSO_3$ on T-cell proliferation in draining auricular lymph nodes, 4 days after sensitization with DNFB

	Immunoblasts in paracortical area of auricular lymph node (field 450 μm diameter)	Auricular lymph node weights (mg)	Spleen weights (mg)
DNFB recipients			
(i) 'Tolerized' cells (7)*	109±47·5‡	87·8±10·1	1007±142
No cells (5)	217±77·3	71·5±25·7	690±218
(ii) Normal cells (8)	181±36·8	87·5±24·5	1062±356
No cells (6)	187±45·5	107·3±20·9	847±205
Cy–DNFB recipients			
(i) 'Tolerized' cells (6)	88±41·3†	33·7±15·0	962±480†
No cells (8)	177±57·7	92·1±14·2	419±79
(ii) Normal cells (7)	186±53·0	58·9±29·3	681±141
No cells (8)	178±36·7	61·5±13·1	578±135

* Number of animals in group.
† $P<0·01$.
‡ $P<0·02$.

4 days later. Table 1 gives the number of immunoblasts (large pyroninophilic cells) found in the paracortical area of the draining lymph node in a field 450 μm diameter selected as that in which these cells could be found in maximum concentration. Despite there being no change in lymph node weight, there was a significant difference in the number of immunoblasts; these were reduced by a half as compared with uninjected controls. Transfusion of the same number of normal spleen cells had no such effect. Similar findings were obtained with recipients that had been pretreated with Cy (250 mg/kg) 3 days before transfer and the results showed a greater degree of significance. An interesting observation,

Table 2. Comparison of 48-h skin reactions to DNFB and oxazolone after transfer of spleen cells or serum from $DNBSO_3$- or DNTB-treated donors

Cells transferred	Treatment of recipients	Number of recipients	Mean skin test reactions			
			DNFB (per cent)			
			0·5	0·25	0·125	0·05
0	Cy–DNFB	12	2·8	1·8	1·4	0·8
Viable $DNBSO_3$ cells	Cy–DNFB	7	1·9	1·3	0·6	0·1
Viable DNTB cells	Cy–DNFB	18	1·8	1·6	0·5	0·2
Killed $DNBSO_3$ cells	Cy–DNFB	5	2·9	2·5	1·9	0·9
Normal cells	Cy–DNFB	6	2·5	2·0	1·6	1·0
$DNBSO_3$ serum (10 ml)	Cy–DNFB	6	2·8	2·1	1·5	0·7
			Oxazolone (per cent)			
			0·25		0·125	0·06
0	Cy-Oxaz	5	1·7		1·5	1·0
Viable $DNBSO_3$ cells	Cy-Oxaz	5	1·6		1·5	1·3

made at the same time, was that Cy-pretreated animals receiving spleen cells from sensitized donors had significantly increased spleen weights, as compared with those from the same group, sensitized in the same way, but not receiving cells. Transfer of spleen cells from unsensitized donors had no such effect as compared with controls.

Comparison of peripheral suppressor activity of spleen cells from $DNBSO_3$-treated donors with those from DNTB-treated donors

It was possible to detect peripherally active suppressor cells using recipients treated with Cy (300 mg/kg) before sensitization (Table 2). In these experiments the activity of spleen cells from $DNBSO_3$-treated donors that were completely unresponsive was compared with that from DNTB-treated donors that were only partially unreactive (Sommer *et al.*, 1975). Spleen cells were transfused on the day of skin testing, 7 days after sensitization. Skin reactions in Cy-pretreated DNFB-sensitized controls reached their peak 48 h after skin testing and at this time maximum suppression could be observed with cells from both $DNBSO_3$- and DNTB-treated donors. It was of particular interest to find that the degree of suppression induced by cells from $DNBSO_3$-treated donors was no greater than that induced by cells from DNTB-treated donors. Cells from $DNBSO_3$-treated donors had a similar specificity in their suppressive effect as cells from DNTB-treated donors. Moreover, no suppression was produced with dead cells, live cells from normal donors or with 10 ml of fresh serum from $DNBSO_3$-treated donors (Table 2).

DISCUSSION

This communication continues the discussion raised in an earlier paper (Polak *et al.*, 1973) as to whether the factors operating in immunological unresponsiveness may act at two levels in the immune system, that is both centrally and in the periphery. Initially (Turk and Stone, 1963), it was noted that two i.v. injections of $DNBSO_3$ given at 14-day intervals before sensitization would inhibit what is now known to be T-cell proliferation in the lymph node draining the site of attempted sensitization as well as producing a state of peripheral unresponsiveness. It was later found (Polak *et al.*, 1973) that when one injection of $DNBSO_3$ was given at the same time as sensitization, peripheral unresponsiveness occurred in a situation in which central proliferation of T cells was not prevented. A similar state of unresponsiveness is produced by contact with DNTB before attempted sensitization with DNFB (Sommer *et al.*, 1975). In this form there is peripheral hyporeactivity associated with no defect in proliferation of T cells in response to antigen. However, suppressor cells can be demonstrated that are active peripherally in specifically damping down skin reactivity.

In the present study a return has been made to the initial model of unresponsiveness induced by two intravenous injections of $DNBSO_3$ at 14-day intervals before sensitization. Peripherally active suppressor cells can be demonstrated by the same technique used to demonstrate these cells in DNTB-treated animals. Despite the greater degree of skin unresponsiveness in the $DNBSO_3$ donors, suppressor cell activity of spleen suspensions was no greater than that found in animals treated by skin contact with 2 per cent DNTB. However, the same cell suspensions showed an activity capable of reducing T-cell proliferation in the lymph node by 50 per cent if injected i.v. into normal or Cy-pretreated donors at the time of sensitization. An additional feature which was observed was that where cells from tolerant animals were injected into Cy-pretreated recipients there was a significant increase in spleen size as compared with controls. However, when normal spleen cells were injected there was no significant increase in spleen size over that in parallel controls. This might indicate that some of the transfused cells 'homed' into the spleen and responded to soluble antigen that might be released from the sensitization site in the recipient. However, normal spleen cells, presumably 'homing' to the same extent, were not able to react to antigen in such a way as to cause a significant spleen weight increase.

As a result of these studies, it can be concluded that there are two separate sites of action of suppressor cells generated during the induction of immunological unresponsiveness in the contact sensitivity model of the guinea-pig. In all models investigated so far there are cells whose target is to suppress the reaction specifically at the periphery. However, in animals made tolerant by two injections of $DNBSO_3$ at 14-day intervals before sensitization there is, in addition, a population of cells, demonstrable in the spleen, that can act centrally to interfere with T-cell proliferation in the draining lymph node. These

cells are among those affected by Cy treatment in experiments in which this type of immunological unresponsiveness has been shown to be reversible (Polak and Turk, 1974).

ACKNOWLEDGMENTS

We wish to thank Mrs Susan Leach for her excellent technical assistance. We acknowledge the Cancer Research Campaign and the Arthritis and Rheumatism Council for financial support.

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