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DERMAL EXUDATE MACROPHAGES

INDUCTION IN DERMAL CHAMBERS AND RESPONSE TO LYMPHOKINES

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SUMMARY

Chambers were implanted in the dorsum of guinea-pigs at the dermal-subcutaneous junction. Exudates were induced and harvested. Macrophages obtained were able to migrate *in vitro*. If procured from sensitized donors, macrophage migration was inhibited by the corresponding antigen. Dermal exudate macrophages are therefore subject to the effect of lymphokines. The chamber model may be useful for *in vivo* studies of cell to cell and cell–parasite interactions.

INTRODUCTION

Macrophages play an important role in cellular immunity. Skin macrophages, or those arriving to the skin after some appropriate stimulus (Ando, Dannenberg & Shima, 1972; Dannenberg, Ando & Shima, 1972) are involved in intracellular infections such as leprosy, cutaneous leishmaniasis and some deep mycoses. Lymphocyte–macrophage interaction is of great interest. Current concepts favour the view that macrophage activation by lymphokines is important, and that when it does not occur, stubborn, difficult-to-cure disease ensues. For instance, unrelenting proliferation of *Mycobacterium leprae* inside macrophages in lepromatous leprosy has been explained by absence of lymphocytes capable of becoming sensitized to *M. leprae*. Thus, macrophages would not become stimulated and could not digest the bacillus (Godal *et al.*, 1971). Conversely, it could be that deficiencies in initial processing of antigen by macrophages would explain lack of lymphocyte sensitization (Waldron, Horn & Rosenthal, 1973). Furthermore, actual lymphokine sensitivity of dermal macrophages has not been directly demonstrated, although local activation of lepromatous dermal macrophages, as determined by an *in vivo* test has been achieved (Convit *et al.*, 1974). It would be useful to have a source of such macrophages in significant quantities, enough to do cytological, biochemical and immunological studies. Cells should be obtained by a relatively easy procedure and one that would not impair the health of the donor. The ideal method should provide a way for repeated or continuous harvesting. We have used a chamber which introduced at the dermal-subcutaneous junction of the dorsal skin of guinea-pigs is

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tolerated for a sufficient time. Exudates may be induced and harvested. Macrophages from such source migrate *in vitro* and, when obtained from sensitized animals, this migration is inhibited by the corresponding antigen.

MATERIALS AND METHODS

Male guinea-pigs weighing in excess of 600 g were kept in individual, partially restraining cages. When appropriate, they were sensitized to *Mycobacterium tuberculosis*. One hundred milligrams of desiccated *M. tuberculosis* (Difco) were suspended in 10 ml of normal saline containing 0·5% phenol. Equal volumes of this suspension and of Freund's incomplete adjuvant (Difco) were emulsified. Each animal received 0·5 ml of this emulsion (about 2·5 mg of *M. tuberculosis*) distributed between the foot pads. Presence of sensitization was checked by intradermal injections of 0·1 ml of a 1:10 dilution of Lederle × 3 Old Tuberculin (OT).

The dermal chamber described was arrived at after extensive preliminary testing with other materials and designs. It follows some ideas outlined by Guyton (Guyton, Granger & Taylor, 1971). The chamber is of cilindroid shape and made of plexiglass. Its body and cap are pressure-adjusted. The former is perforated by numerous holes so as to allow entrance of connective-vascular axes and interchange between these and the lumen of the chamber. The lumen communicates with the exterior by two polyethylene catheters (Intramedic PE 205 Clay-Adams). Fig. 1 provides further data. Chambers were implanted as follows. Animals were

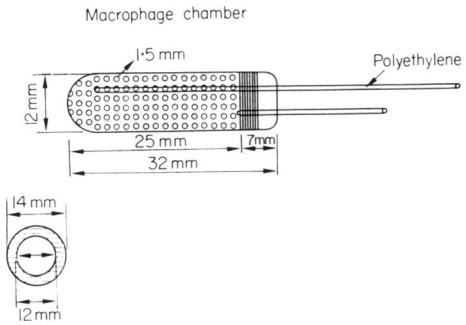

FIG. 1. Schematic drawing of the dermal chamber. Polyethylene catheters are anchored to the cap. The one nearly reaching the bottom of the chamber is the 'in' catheter. The cap is grooved to promote adherence of surrounding tissues.

clipped, and anaesthetized with intraperitoneal veterinary Nembutal (Abbott). All further procedures were done under sterile surgical conditions in an operating room provided with germicidal ultraviolet light. Chambers were sterilized with a solution of Urolocide (American Cystoscope Makers Incorporated) and extensively washed with sterile distilled water before use. Each was put in the dorsum of an animal at the dermal subcutaneous boundary, inside a cavity obtained by blunt dissection, through a transverse incision made 8 cm from the occipito-atloidal joint. The incision was closed with 4-0 Dermalon sutures and made watertight by a synthetic liquid plastic (Cabello-Díaz & Bernaola, 1973). Chambers were washed through the catheters with Hanks's balanced salt solution (HBSS) containing 200 μg/per ml of Gentamycin (Reagent Solution, Schering). Catheters were heat-sealed. The ensemble was protected and kept in place by elastic adhesive dressings (Tensoplast T. J. Smith & Nephew Ltd, England). Chambers were left undisturbed for an optimum of 7 days. By then, the outside is surrounded by a capsule, connective 'roots' penetrate into the lumen via the holes drilled in the body of the chamber. There is another membrane lining the inside of the chamber. The appearance resembles somewhat the one described by Arko (1972). In such conditions liquid put through the catheters will not diffuse into surrounding tissues or escape through the incision. Exudates were induced by injecting 1 ml of sterile liquid paraffin (Merck) through the 'in' catheter. Exudates should be collected after about 7 days. Harvesting is done by washing the chamber through the catheter with cold 'washing cocktail' (HBSS plus 10 μ/ml of heparin, 2% heat-inactivated normal guinea-pig serum

(NGPS) and antibiotic–antimycotic mixture (Gibco). The number of cells was estimated by haemocytometry, cell types by Wright-Giemsa staining of cover slip smears or cytocentrifuge (Shandon-Elliott) droplets. Viability was estimated by the method of Philips & Terryberry (1957). Contamination was checked by standard bacteriological techniques.

Exudates were used to study macrophage migration and its inhibition by corresponding antigen. We used a modification of the method of Ferraresi *et al.* (1969). Exudates were washed twice with the cocktail and cells put inside capillary tubes (Clay Adams, Yankee microhematocrit tube plain 1021). These were affixed with silicone grease (Merck) to the bottom of 60×15 mm Falcon Tissue culture plastic Petri dishes. Culture medium (McCoy 5-A (Gibco), 15% NGPS and 100 μg/ml of gentamycin, was employed. In appropriate instances PPD (Parke-Davis preservative free) 80 μg/ml was added. Dishes were put on the platform of a Five-0-88 Portable Projector (3M) placed inside a Napco tissue culture incubator with a 5% CO_2, 95% air atmosphere. After 24 hr, areas of migration were evaluated by projecting them, drawing, transferring drawings to constant weight paper, cutting out and weighing. Results were calculated by considering migration in the absence of antigen as 100%. After projection, dishes were washed and adhering exudate cells fixed and stained to obtain permanent preparations. In most cases, after harvest, chambers were taken out to study gross and microscopic features. In three sensitized animals they were left *in situ* and further liquid paraffin added. After 8 days, new exudates were harvested and processed.

For control purposes, peritoneal exudate macrophage migrations were also done. Exudates were induced by liquid paraffin, either in sensitized but otherwise untreated animals, or in animals with chambers but after dermal exudate was already collected. Harvest took place 5 days after induction and exudates were processed and evaluated essentially as above.

RESULTS

Table 1 illustrates magnitude of harvests. In representative instances we obtained up to about 30×10^6 cells of which more than half were macrophages, with a high proportion of viable cells.

As shown in Tables 2 and 3, migration of dermal exudate macrophages from normal animals is not inhibited *in vitro* by PPD. Dermal exudate macrophages from sensitized animals migrate also, but this migration is inhibited by PPD. The difference between the two groups (normal *vs* sensitized) is statistically significant ($0{\cdot}001 < P < 0{\cdot}01$ by the *t*-test for small samples). There is an overall good correlation between PPD inhibition of migration of dermal and peritoneal macrophages. Reharvesting experiments were limited in number, but in two out of the three (not shown in tables) areas of migration in the presence of PPD were less than 75% of the areas covered without PPD.

TABLE 1. Harvest from Dermal chambers. Data are from three representative experiments

Exudate inducer (days)	Number of cells harvested	Macrophages (%)	Lymphocytes (%)	Polymorphonuclear leucocytes (%)	Viable mononuclear cells (%)
Liquid paraffin (5)	26×10^6	65	5	30	73
Liquid paraffin (7)	$31{\cdot}5 \times 10^6$	60	28	12	68
Liquid paraffin (7)	26×10^6	51	5	44	90

TABLE 2. Exudate macrophage migration from sensitized animals

Animal	OT 1/10 (intradermally)	Chamber exudate (percentage migration with PPD*) (without PPD = 100%)	Peritoneal exudate (percentage migration with PPD) (without PPD = 100%)
1	—	43·4	—
2	—	—	28·7
4	20 mm†	48·4	34·4
9	18 mm	95·1‡	—
10	13 mm	26·2	41·6
11	21 mm	107·0§	46·5
12	17 mm	80·6	24·3
13	—	—	58·7
15	16 mm	69·9	57·2
16	—	—	34·3
Mean	17·5 mm	67·2	40·7

— = Not done.

* Eighty μg/ml.
† Millimetres induration at 48 hr.
‡ Animal was sick, died soon afterwards.
§ Dermal exudate acid, rich in polymorphonuclear leucocytes.

TABLE 3. Dermal exudate macrophage migration from non-sensitized animals

Animal	Chamber exudate (percentage migration with PPD*) (without PPD = 100%)
II	90·6
III	91·7
IV	88·7
VIII	130·2
IX	112·8
X	116·1
XI	85·4
XII	82·5
XVI	102·7
XVII	105·4
XVIII	108·6
Mean	101·4

* Eighty μg/ml.

DISCUSSION

We may conclude as follows. The method is operative, and permits sufficient and even repeated, harvest of exudate cells. More than half of such cells are macrophages. The latter are viable and able to migrate *in vitro*. In sensitized animals, macrophage migration is significantly inhibited by the corresponding antigen. This means that macrophages from dermal exudates (coming from the dermis or from the blood stream) are susceptible to the effect of lymphokines. This in its turn, lends credence to current thoughts of macrophage activation by lymphocytes in certain dermal intracellular infections. Findings were not self-evident, because such susceptibility of macrophages to lymphokines has been reported not to happen with alveolar macrophages (Leu *et al.*, 1972). The range of potential use of dermal chambers is wide. It may not be far fetched to foresee the use of similar ones, when *in vivo* sequential studies of cell to cell or cell–parasite interactions are planned.

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