Effects of Antigen on in vitro Cultures of Sensitized Peritoneal Cells

A great deal of research has been concerned with in vitro action of antigen on cells and tissues of animals with delayed hypersensitivity. Although the bibliography is extensive and sometimes contradictory, all evidence indicates that antigens do not exert any apparent action on in vitro cultures of parenchymal cells from animals with delayed hypersensitivity[1-3], but that antigens do have a specific action on cells and tissues of lymphoreticular origin[4-6].

MOEN and SWIFT[7] using cultures of pieces of animal spleen infected with tubercle bacilli or streptococci, demonstrated that cytotoxic action on cell migration shows specificity for illness, since cultures from tuberculin-sensitive animals were refractory to streptococcal protein, while animals which had been infected with streptococci were insensitive to tuberculin.

The dependence of cytotoxic action on a mechanism which involves delayed hypersensitivity was demonstrated by ARONSON[6], and by GEORGE and VAUGHAN[5]. On the other hand, they observed that immediate hypersensitivity with production of circulating antibodies was associated with a stimulating effect of specific antigen on cell migration and reproduction as found by several workers[8-10].

A consideration of the concentration of antigen used in culture both by the authors who describe a cytotoxic effect, and by those who find a stimulating action, indicates that the latter phenomenon is induced by low concentrations of antigen. Based upon this consideration, we have decided to study the effects of a low concentration of antigen in in vitro cultures of mononuclear peritoneal cells.

This work was divided into 2 parts. In the first part, we studied the influence of tuberculin added to in vitro cultures of peritoneal cells obtained from normal guinea-pigs and from guinea-pigs sensitized with 200 μg of PPD in water or in oil to induce immediate hypersensitivity, or with BCG (approximately 10^6 living bacillus) in complete Freund's adjuvant to induce delayed hypersensitivity; the antigens were injected s.c. in the neck of the guinea-pigs in a volume of 0.2 ml.

In the second part we studied the action of egg albumin added to cultures of peritoneal cells obtained from 3 groups of 6 guinea-pigs each. These were injected s.c. in the neck with 0.10 ml of antigen. The first group received saline, the second an emulsion of incomplete Freund's adjuvant with 50 μg of egg albumin to induce immediate hypersensitivity, and the third complete Freund's adjuvant with 50 μg of egg albumin to induce delayed hypersensitivity.

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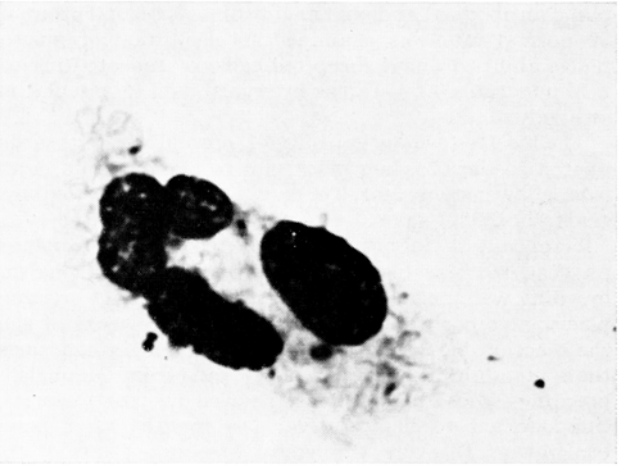

Fig. 2. 4 h culture of cells obtained from guinea-pigs with delayed hypersensitivity to egg albumin. Incubated in presence of 20 μg/ml egg albumin. × 2000.

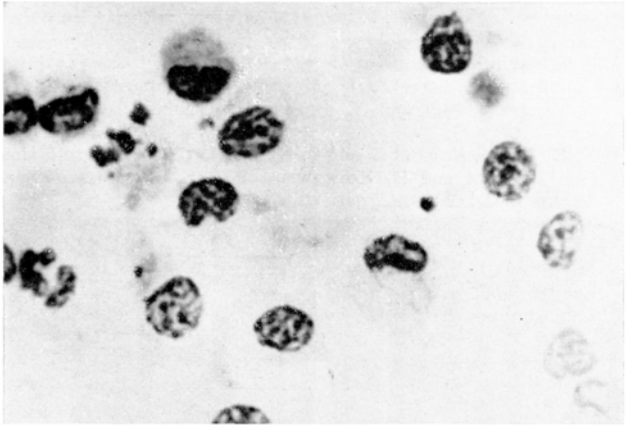

Fig. 1. 4 h culture of cells obtained from normal guinea-pigs. Incubated in the presence of 1/5000 tuberculin. × 800.

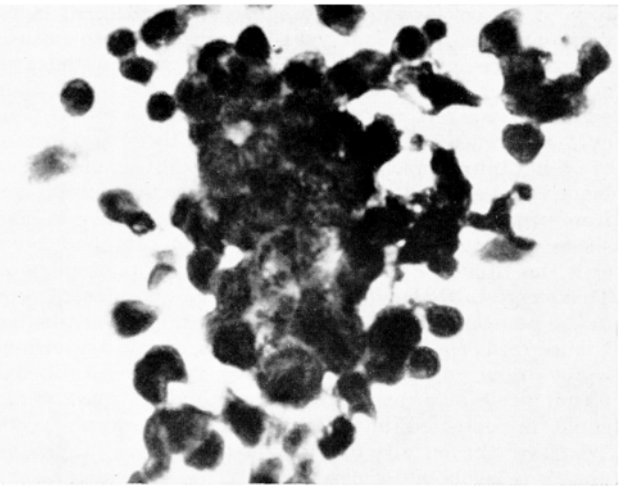

Fig. 3. 24 h culture of cells obtained from guinea-pigs with delayed hypersensitivity to tuberculin. Incubated in the presence of 1/5000 tuberculin. × 500.

All 3 groups were skin tested 21 days after immunization with 10 μg of egg albumin in 0.1 volume of saline solution, and reactions were read at 4, 24 and 48 h. The group of guinea-pigs sensitized with the incomplete Freund's adjuvant showed at 4 h a reaction averaging 17 mm of diameter, disappearing at 24–48 h.

The group of guinea-pigs sensitized with the antigen plus complete Freund's adjuvant showed similar 4 h reaction, but increased to an average 21 mm at 24 h, and was still noticeable at 48 h.

For the in vitro cultures the cells were removed from the peritoneal cavity without prior irritation by a single washing with Hanks' solution. The cell concentration used was 10^6 cells/ml; and the cultured medium consisted of 20% fetal calf serum in Hanks' solution. 1.5 ml of cells were added to Leighton tubes with glass slides and incubated at 37 °C. The slides were removed at intervals and stained by May-Grunwald-Giemsa technique.

The addition of the specific antigen to cultures of cells obtained from guinea-pigs with delayed hypersensitivity to tuberculin or to egg albumin produced a high frequency of multinucleated cells. We evaluated this phenomenon by determining the number of multinucleated cells/1000 cells on each slide. This data was statistically analyzed by the Student index *t*.

The *t* values were highly significant (P: 0.001) when the 4, 7 and 24 h delayed hypersensitive cultures with antigen were compared to the same cultures without antigen; in cultures of cells from untreated animals and from those with immediate hypersensitivity there was no statistically significant difference between the groups with and without antigen (Tables I and II).

In the 24 h cultures from animals with delayed hypersensitivity to tuberculin or to egg albumin the antigen produced a lower number of multinucleated cells than in the 7 h cultures; this number was still significantly higher than the controls. This reduction was accompanied by the appearance of larger numbers of cell aggregates than in the control groups (Figure 3).

Multinucleated cells with an average diameter of 20–40 μ (2–5 nuclei/cell) were observed consistently when the antigen was added to cultures of peritoneal cells obtained from delayed hypersensitive animals. This suggests a close relationship between the delayed hypersensitive state and the formation of multinucleated cells[11].

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Table I. No. of multinucleated cells in cultures of peritoneal cells from normal and tuberculin sensitized animals

Groups of 3 guinea-pigs immunized with	h of culture	Multinucleated cells No. ‰[a]		*t* index
		Cultures with antigen added[b]	Cultures without antigen added	
None	4	13 ± 2.2	10 ± 1.4	3.6
	7	8 ± 2.2	9 ± 1.0	1.1
	24	10 ± 3.0	12 ± 3.0	1.6
200 μg PPD	4	11 ± 2.2	13 ± 2.2	2.5
with incomplete	7	13 ± 1.7	11 ± 1.7	3.0
Freund's adjuvant	24	16 ± 1.7	11 ± 1.7	1.4
10^6 of living bacillus	4	143 ± 5.7	11 ± 1.4	131.6
BCG with complete	7	80 ± 1.4	16 ± 2.2	126.0
Freund's adjuvant	24	27 ± 3.8	17 ± 1.0	5.6

[a] Mean obtained from 9 cultures. [b] Tuberculin concentration in culture medium 1/5000.

Table II. No. of multinucleated cells in cultures of peritoneal cells from normal and egg albumin sensitized animals

Groups of 6 guinea-pigs immunized with	h of culture	Multinucleated cells No. ‰[a]		*t* index
		Cultures with antigen added[b]	Cultures without antigen added	
Saline in incomplete	4	16 ± 5.2	13 ± 3.4	1.8
Freund's adjuvant	7	19 ± 4.0	15 ± 4.2	3.0
	24	15 ± 3.5	12 ± 3.6	3.0
50 μg egg albumin	4	19 ± 5.2	18 ± 4.3	0.6
in incomplete	7	19 ± 4.7	20 ± 4.6	0.7
Freund's adjuvant	24	22 ± 3.0	20 ± 3.8	2.0
50 μg egg albumin	4	49 ± 6.8	13 ± 4.2	18.0
in complete	7	58 ± 8.4	20 ± 3.7	14.0
Freund's adjuvant	24	28 ± 3.2	14 ± 4.6	14.0

[a] Mean obtained from 12 cultures. [b] Egg albumin concentration in culture medium 20 μg/ml.

Résumé. On a ajouté des antigènes aux cultures in vitro des cellules mononucléaires obtenues du péritoine des 3 groupes de cobayes, le premier étant normal, le deuxième présentant une sensibilité immédiate et le troisième une hypersensibilité retardée à la tuberculine ou à l'ovalbumine. L'addition de l'antigène spécifique aux cultures des cellules obtenues des cobayes avec hypersensibilité retardée a provoqué la formation de cellules avec 2 ou plusieurs noyaux. Ceci suggère une relation étroite entre l'hypersensibilité retardée et la formation de cellules multinucléaires.

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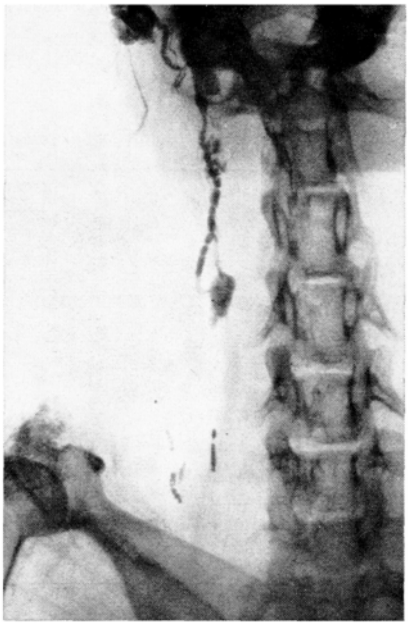
Prolongation of Skin Homograft Survival by Local Intralymphatic Radioisotope Injections

The importance of regional lymphatic tissue in antibody formation was first demonstrated by MCMASTER and HUDACK[1] and confirmed by EHRICH and HARRIS[2]. It was also shown that selective reduction of lymphocytes is a means of homograft survival[3]. Attempts were therefore made to achieve a prolongation of homograft survival by selective reduction of lymphocytes with the intralymphatic irradiation of the lymphoid tissues[4,5]. These procedures involved an introduction of large doses of radioisotopes. In the present study an attempt was made to produce a prolongation of homograft survival by selective irradiation of regional lymph nodes with small doses.

In the rabbit, lymphatics draining from the ear before their confluence with the cervical veins pass through only 2 agglomerations of lymphoid tissue: the retroauricular and the cervical. These lymph nodes can be injected from the central lymphatic channel of the ear. Six rabbits therefore received an intralymphatic injection of 1 mC ^{111}Ag colloid (particle size 10–100 nm) into their right ear, 7 animals were injected with ^{131}I-Lipiodol ultra fluid (radioactive dose 0.5 mC). The volume of injected fluid was 0.3–0.5 ml. Twenty-four h after the injection a skin homograft was made on the injected right ear. The skin flap removed from the right side was transplanted onto the non-irradiated left ear of the same animals (technical control). The dimensions of the grafts were 15 × 15 mm. In 6 controls the grafting procedures were duplicated without isotope injection.

The homografts were rejected in the controls on the 7th to 9th day (mean 7.8 ± 0.4 days), in the ^{111}Ag-injected animals on the 11th to 14th (mean 12.5 ± 0.7) day, and in the ^{131}I-Lipiodol-injected on the 12th to 16th (mean 13.9 ± 0.5) day. Moreover, in this series, in 3 out of 7 animals the rejection was incomplete and the reaction was limited to only a small part of the transplant.

The distribution of the injected radioisotope was studied by the injection of 3–4 μC ^{131}I-Lipiodol or ^{111}Ag-colloid diluted to 0.4–0.5 ml with non-radioactive Lipiodol or colloid solution. The animals were killed 1 h after the intralymphatic injection. The regional lymph nodes, the spleen, liver and lungs were removed and digested with 20% sodium hydroxide and the radioactivity measured



Retroauricular and cervical lymph nodes of a rabbit injected with ^{131}I-lipiodol ultra fluid.

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Tissue recoveries after intralymphatic injections of ^{111}Ag-colloid and ^{131}I-Lipiodol in the rabbit, expressed as % of injected dose.

	Lymph nodes		Liver	Spleen	Lungs	Injection site	Total
	Auricular	Cervical					
^{111}Ag	28.4 ± 5.9	23.0 ± 6.2	14.0 ± 0.5	0.01 — 1.7	0.02 — 0.1	8.6 — 18.7	69.4 ± 5.3
^{131}I	4.6 ± 0.97	2.8 ± 1.2	–	–	24.3 ± 2.2	17.1 ± 6.0	45.6 ± 8.3