In contrast to flagella[1] HPOD fills the intercellular space between lymphocytes and also between lymphocytes and reticular cells. Antigen material is associated directly with the membranes of these cells. From this point of view, consequently, there exists the possibility that unchanged antigen material may react directly with receptors on lymphoid cells without earlier 'processing' in macrophages. Previous in vitro investigations[12] have shown that even a few seconds of antigen contact with lymphoid cells are able to induce a specific transformation and proliferation of these cells.

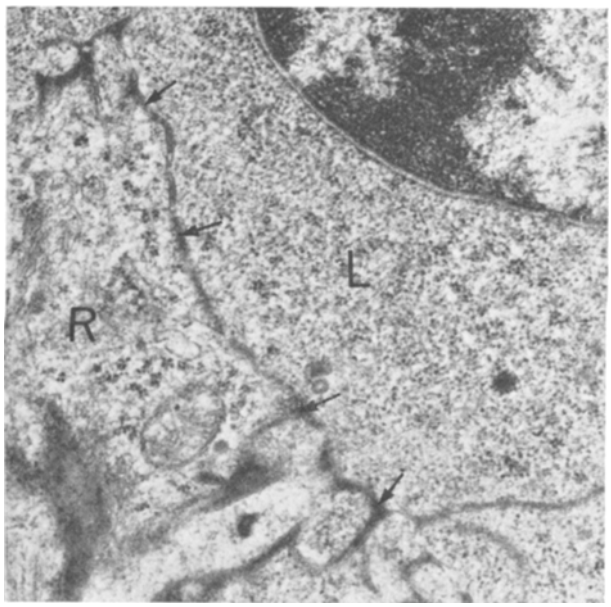

Fig. 2. Lymph node of rabbit. L, lymphocyte; R, reticular cell; ↑, positive reaction in intercellular space, × 35,000.

Zusammenfassung. Drei Stunden nach i.v.-Gabe an Kaninchen ist das Antigen Meerrettich Peroxydase extrazellulär in den Marksträngen der Lymphknoten und der roten Pulpa der Milz elektronenmikroskopisch nachweisbar. Das unveränderte Antigen steht damit noch zu dieser Zeit in direktem Kontakt mit der Zellmembran der lymphoiden Zellen.

T. DIAMANTSTEIN, B. WAGNER and D. GRAF KEYSERLINGK

Klinikum Steglitz of the Free University Berlin, Department of Oto-rhino.-laryngology, Biochemical and Immunological Laboratory, D-1 Berlin 33, and II. Anatomisches Institut of the Free University Berlin, (Germany), 9 July 1969.

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The Cellular Sources of Antibodies in Diffusion Chamber Cultures of Alveolar Exudates

Diffusion chambers (DC) are a valuable tool for the study of immune processes in closed cellular systems[1,2]. The chambers are implanted in new-born or irradiated recipients which are incapable of demonstrable antibody production to the amount of antigen applied. In competent adult recipients DC have a strong adjuvant effect on the recipient's response against soluble antigens[3]. Although new-born recipients do not respond serologically to the antigens enclosed in the DC[1], the cellular events occurring in their lymphatic tissues under the influence of the DC cultures of antibody forming cells deserve closer attention. In the present study lung alveolar exudate cells were cultivated in the DC in newborn rabbits, since 'adult' histiocytes may enhance the recipient's immune capacity[4].

Methods. Alveolar cells were washed out from lungs of young adult chinchilla rabbits in Earle's fluid. The donors were either untreated, or had been immunized intratracheally with $2–4 \times 10^9$ sheep red blood cells (SRBC) 8–30 days previously. The Earle-suspended alveolar cells were mixed with SRBC (ratio 1:2) and 1 ml of the suspension injected into DC made from lucite rings and millipore filters of 0.1 μ porosity. The filled DC were implanted into the peritoneal cavities of 5 days old chinchilla rabbits. Control animals received DC which contained either SRBC or alveolar exudate cells from untreated donors. In the latter case the appropriate amount of SRBC was injected i.p. into the recipients of DC.

After 8 days (cultures of preimmunized cells) or after 9–10 days the DC were removed from anaesthetized recipients. The cultured cells were liberated from the DC by mechanical scraping or by pronase treatment[2]. Neither technique detached all cells from the filters. Recipient's lymph was collected from cisterna chyli (yield $1–1.5 \times 10^7$ viable lymph cells). Then the recipients were killed and the samples of the lymphatic tissues and omentum-derived tissue covering the DC taken out and teased in Earle's fluid (yield $1.2–10 \times 10^7$ viable cells per organ or tissue). All cell samples were washed, suspended in Sevac IV medium and assayed for plaque-forming cells (PFC) by JERNE's technique[5]. The PFC were examined microscopically in situ, after 2.5% glutaraldehyde fixation and methyl green-pyronin staining. The DC fluid and recipient's sera were tested by the standard hemagglutination and hemolysis technique in test-tubes.

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Results. Animals with DC containing only SRBC had very few PFC in DC-attached omentum and lymph (Table I). In controls with DC containing only alveolar cells, no PFC at all were found in most organs. Alveolar cells gave the same background number of PFC as fresh normal alveolar exudates (0.01–0.5 PFC/10^6 cells). No antibodies were found in either DC fluid or recipient's sera, except for a very low titre of hemolysins in some animals.

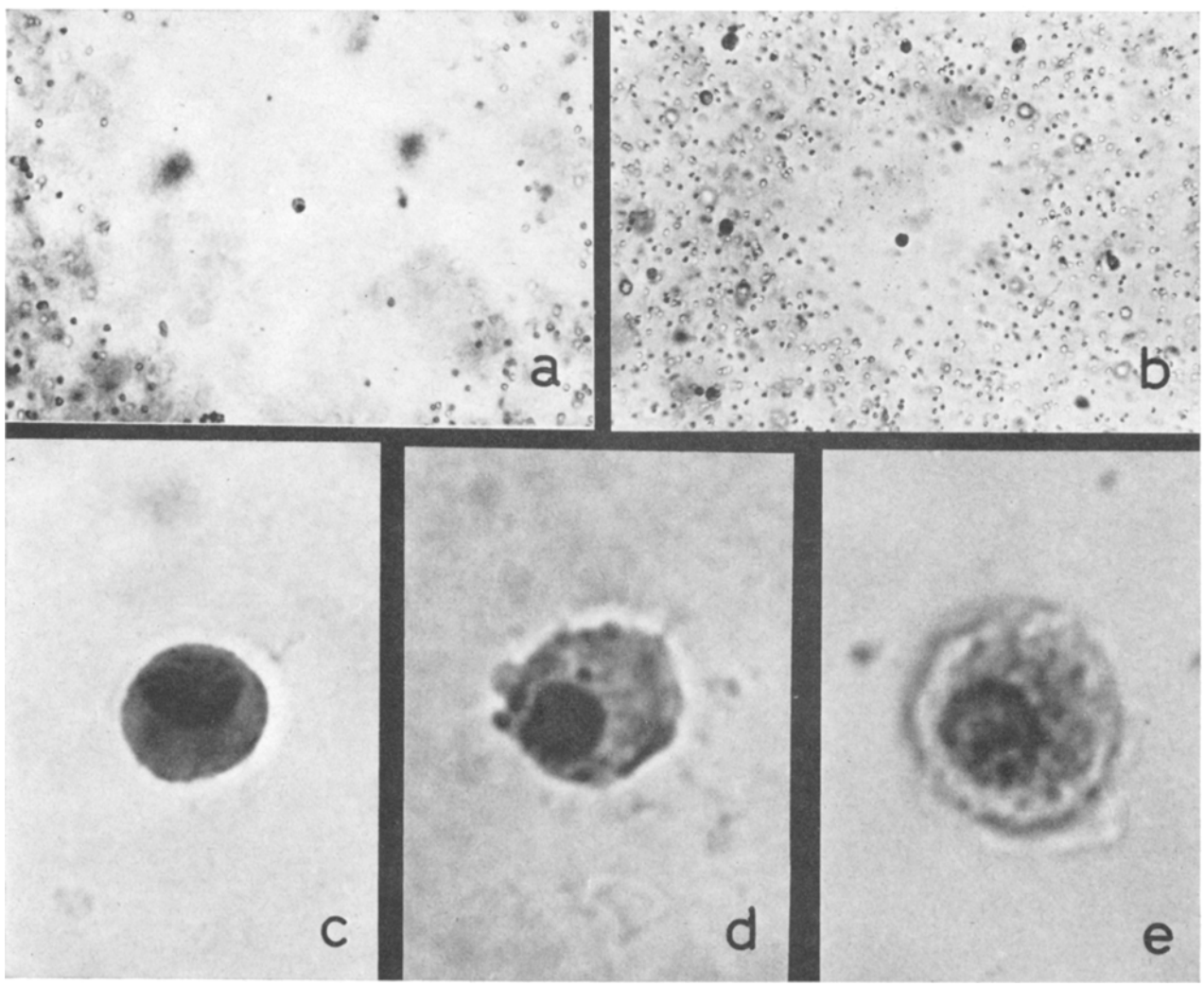
DC cultures of normal or preimmunized alveolar cells with antigen resulted in an appearance of moderate numbers of PFC among the alveolar cells and of a few PFC in recipient's tissues (Tables II and III). The increase of the number of PFC in the omental tissue covering the DC was statistically significant (Wilcoxon's test, at the 5% level of significance). Hemagglutination titres of 1:8–128 and hemolytic titres of 1:16–256 were found in the DC fluids. In the recipient's sera hemagglutinin titres of 1:2–16 were found and hemolytic titres never exceeded 1:32. The antibody titres correlated well with the number of cells cultured in the DC.

The majority of PFC in the alveolar exudate cell cultures had the morphologic appearance of activated lymphocytes, plasma cells and blasts, however 5–20% of all PFC had a morphology of histiocytes (Figure) A number of histiocytic PFC was found also among the omental hemolysin forming cells.

Discussion. The observation made with a soluble antigen[1] has thus been repeated with a corpuscular antigen: rabbit lung alveolar exudates do contain immunocompetent cells which can differentiate even in the DC culture conditions into antibody secreting stages.

Table I. Chambers with 4×10^7 SRBC only

PFC per 10^6 viable lymphoid cells in recipient's organs				
Tissue covering DC	Lymph nodes mediastinal	Lymph nodes mesenteric	Spleen	Thoracic duct lymph
<0.06	<0.08	<0.01	<0.01	<0.1
0.1	<0.08	<0.01	<0.01	<0.1
0.3	0.1	<0.01	<0.01	0.1
0.03	–	–	<0.01	0.2



Plaque-forming cells from diffusion chamber cultures of rabbit lung alveolar cells. (a) Hemolytic plaque formed by a lymphoid cell. (b) Plaque formed by a cell with histiocytic morphology. (c) Plaque-forming plasma cell. (d) Central cell of the plaque a. (e) Central cell of the plaque b. Methyl green – pyronin stain. Magnification × 200 (a, b) and × 3000 (c–e).

The antibody secreting cells include histiocytic forms. As discussed in detail elsewhere[6], these antibody secreting histiocytes are primitive, less differentiated forms, not typical macrophages. Hemolytic plaques produced by these cells are smaller than 'lymphoid' plaques, but, on the other hand, are not due to the liberation of a passively acquired antibody[6]. No evidence for an active contribution of the new-born recipients of DC to the overall serological response was found. However, in the omental tissue covering the DC, a significant increase in the number of antibody producing cells can be detected. Omentum per se is a potent immunocompetent area[7]. The mechanism by which the antibody response is induced in the omental cells remains a matter of speculation. The concentration of both antibodies and possibly antigenic fragments diffusing from the DC[2] should be higher in this area than in the distant lymphatic organs. Antigen alone, in the presence of or diffusing from the DC, does not induce any significant response. It might well be that the antibodies formed by the competent cell population inside the DC potentiate the omental cells for the triggering effect of antigen. Nucleotides from the decaying cultured cells and factors liberated during the secondary contact with antigen which increase the mitotic activity of lymphoid cells[8] may have some adjuvant effect[9].

Table II. DC cultures of alveolar cells from normal donors (primary response)

Donor cells in DC		Recipient's organs – PFC per 10^6 viable lymphoid cells				
Original number $\times 10^6$	PFC per 10^6 cells	Tissue covering DC	Lymph nodes mediastinal	Lymph nodes mesenteric	Spleen	Thoracic duct lymph
13	40	1.7	–	–	<0.01	–
20	25	0.3	0.1	<0.01	0.02	0.2
25	56	0.5	0.06	<0.01	0.2	0.3
25	62	0.4	<0.06	<0.01	0.1	<0.1

Table III. DC cultures of alveolar cells from pre-immunized donors (secondary response)

Days after donor immunization	Donor cells in DC		Recipient's tissues – PFC per 10^6 lymphoid cells			
	Original number $\times 10^6$	PFC per 10^6 cells	Tissue covering DC	Lymph nodes mesenteric	Spleen	Thoracic duct lymph
8	10	12	0.8	0.4	0.2	0.2
15	11	40	3.7	0.8	0.2	0.2
15	8	5	0.8	0.03	0.01	<0.08
30	8	17	1.0	0.02	0.03	<0.1
30	9	60	1.2	0.1	0.6	0.3
30	10	37	1.1	0.9	0.8	0.2

Zusammenfassung. Alveolarexsudatzellen von Kaninchen wurden zusammen mit Schaferythrozyten in Diffusionskammern gebracht und diese in neugeborene Kaninchen implantiert. Antikörperbildende Zellen wurden in den Diffusionskammern und im Omentum der Rezipienten festgestellt.

M. HOLUB

Department of Immunology,
Institute of Microbiology of the Czechoslovak Academy of Sciences,
Praha 4-Krč (Czechoslovakia), 5 March 1969.

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Distribution of C_{19}-Steroid Conjugates in Plasma Protein Fractions

In a previous communication[1], the association between sulphoconjugated C_{19}-steroids and certain plasma proteins under in-vivo conditions has been reported. In order to extend such information to a possible interaction between endogenous C_{19}-steroid glucuronosides and similar plasma proteins, 27.7 ml plasma, obtained from a 46-year-old female subject 10 min after i.v. administration of 0.201 µg 7α-^{3}H-3β-hydroxy-5-androstene-17-one (dehydroepiandrosterone) with 101×10^6 dpm, were submitted to preparative zone electrophoresis. The latter was performed on PVC in Longsworth buffer of pH 8.6 and an ionic strength of 0.1 at a field strength of 4.4 V/cm and led to a satisfactory separation of albumin, α_1-, α_2-, β- and γ-globulins. The corresponding zones were eluted with 0.15 *M* sodium chloride and the eluates reduced to 5–7 ml by ultrafiltration. Following the exhaustive extraction of free steroids with chloroform, total conjugates were extracted with 10 vol. ethanol-acetone (1:1 v/v). For separation of conjugates into steroid sulphoconjugates and glucuronosides ion exchange chromatography of the filtrate on DEAE-Sephadex A-50 and repeated thin-layer chromatography of resulting fractions on silica gel G in chloroform-methanol-ammonia (10:10:0.2 v/v) and chloroform-methanol-ammonia (20:5:0.2 v/v) proved adequate[2]. Aliquots of the different conjugate fractions, as well as of the free fraction were assayed for ^{3}H-activity prior to cleavage of conjugates by solvolysis[3] or incubation with β-glucuronidase (Ketodase, Warner Chilcott, Morris Plains, N.J., USA). Free and liberated C_{19}-steroids were isolated by multiple thin-layer chromatography, converted into 2,4-dinitrophenyl-

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