Antibody Production by Lymphocytes after *in vitro* Contact with Bacterial Antigen and Transfer to New-born Rabbits

THERE is as yet no direct evidence of antibody formation in lymphocytes. Neither descriptions of cytological changes of lymphocytes in lymphatic tissues during antibody production[1], nor the proof of release of γ-globulin from lymphoid cells[2], nor the demonstration of antibodies in tissue cultures of pure lymphocytes immunized *in vivo*[3] could reveal whether lymphocytes are producers or only carriers of antibodies. An attempt was made, therefore, to obtain antibody production after contact of lymphocytes from rabbit lymph with antigen *in vitro*.

We used the method of cell transfer to 2–5-day-old rabbits, which are not capable of active antibody response[4]. 2–4 ml. of lymph were collected under sterile conditions from the cisterna chyli of adult rabbits (1,500–3,000 gm.). With three exceptions, cells from each donor separately were washed three times with Hank's solution, suspended in 1–2 ml. of this solution and mixed with bacterial antigen (to *Brucella suis*, *Salmonella paratyphi B*) in the proportion of 2–6 microbes to each cell. 2×10^6 to 80×10^6 viable cells (according to the trypan-blue test) were injected intraperitoneally into each recipient. The differential counts of ethanol-fixed smears stained with methyl green–pyronine from thirteen donors showed the following estimate of the constitution of transferred lymph: blasts (probably lymphoblasts) $8 \cdot 78 \pm 0 \cdot 95$ per cent; prolymphocytes $17 \cdot 41 \pm 1 \cdot 13$ per cent; lymphocytes $72 \cdot 59 \pm 1 \cdot 38$ per cent; macrophages and monocytes $0 \cdot 76 \pm 0 \cdot 22$ per cent; granulocytes $0 \cdot 46 \pm 0 \cdot 17$ per cent. In five recipients splenectomy was performed 5 hr. before cell transfer.

In order to exclude possible participation of the recipient's tissues and to enable quantitative analysis of cell changes, in some cases the lymph cells in Hank's solution were transferred in diffusion chambers ('Plexiglass' cylinders with bases of 'Millipor' membranes of 0·8-μ porosity, content 0·4–0·6 ml.). These chambers were placed in the peritoneal cavity of recipient rabbits under ether anæsthesia.

New-born rabbits of the same litters served as controls. They received: (1) isolated spleen cells with antigen under the same conditions; (2) lymph cells without antigen; (3) cell-free lymph supernatant with antigen and (4) antigen alone in corresponding quantity. Thirty-two test animals and thirty-one controls from sixteen litters were used.

It was found that after transfer of lymph cells, antibodies appear in about 90 per cent of intact recipients (Table 1) after 2–4 days, the peak being attained after 5–8 days. There is a direct relation between the quantity of transferred cells and the antibody titre. Results on splenectomized animals suggest that, after direct injection of cells into the peritoneal cavity, antibody formation is due mainly to cells localized in the spleen. Cells in a diffusion chamber give relatively lower titres in the serum, but antibodies persist for a longer period and in higher titres inside the chamber (20 days, 1 : 16). Spleen-cell transfer results in 100 per cent antibody formation.

Other animals were examined histologically. Proliferation of the lymph cells injected directly occurs chiefly on the omentum and in the spleen within a few days. A prompt reaction of macrophages develops, together with granulation and fibrous tissue formation in the surrounding area. Clumps of immature and mature plasma cells and pyroninophil reticular cells are formed in the marginal zones of layers of transferred cells. The average spleen weight in test animals from the fifth to the twelfth day after transfer is $1 \cdot 336 \pm 0 \cdot 104$ gm./kgm. (ten rabbits), in controls with antigen only $0 \cdot 939 \pm 0 \cdot 098$ gm./kgm. (ten rabbits). In the chambers during the initial proliferation a rapid fall in the number of blast cells and shedding of cytoplasm of lymphocytes was observed. There is a definite increase in macrophages (22 per cent in 8 days), pyroninophil cells resembling reticular cells (up to 1·6 per cent), immature and mature plasma cells (up to 3·2 and 4 per cent in 4–8 days), cells crowded with Russel bodies and eosinophil crystals (up to 28·8 per cent in 8 days). In controls without antigen a slight degree of plasma cell development occurred. After 45 days, only macrophages and eosinophil crystals can be found in the chambers. Details will be given elsewhere.

Table 1

Mode of transfer	No. of cells ($\times 10^6$)	No. of recipients	Distribution of agglutinating titres						
			0	2	4	8	16	32	64
Lymph cells intraperitoneally	2–20	5	1	1	1	2	—	—	—
Lymph cells intraperitoneally	20–80	13	2	—	1	3	2	4	1
Lymph cells in diffusion chambers	2–20	9	—	—	4	2	3*	—	—
Lymph cells intraperitoneally after splenectomy	20–80	5	5	—	—	—	—	—	—
Spleen cells intraperitoneally	20–80	16	—	—	1	3	5	4	3
Lymph cells intraperitoneally without antigen	20–80	6	6	—	—	—	—	—	—
Antigen alone or with cell-free lymph supernatant	—	9	9	—	—	—	—	—	—

* 75×10^6 cells in two cases

It is concluded that lymphocytes from cisterna chyli can form antibodies after transfer to new-born animals, the metabolic change being connected or followed by morphological transformation of some of the less-mature cells into the macrophage and plasma-cell types which, however, may not be responsible for the amount of antibodies formed.

MIROSLAV HOLUB

Division of Immunology,
Institute of Biology of the
Czechoslovak Academy of Science,
Prague, 6.

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Surface Antigens of *Haemophilus pertussis*

IT has been shown by Frappier and Guérault[1,2], and Frappier, Guérault and De Repentigny[3], that the antigen of *Haemophilus pertussis* which protects mice against intracerebral infection could be washed off the bacteria by saline (0·85 per cent sodium chloride). As this protective effect in the mouse is accepted as an indicator of the efficiency of vaccines in protecting children against whooping-cough it would appear that saline washings afford a ready means of obtaining the immunizing antigen of the

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