Human Germinal Centers Lymph Nodes Responses to Leukemogenic Viruses in vitro

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The responses of the lymphoid system of different species of animals to various antigens are well documented. In our experiments we made an approach to study the responses of human lymph nodes and human peripheral heparinized whole blood, to the presence of animal leukemogenic virus (Rauscher) in vitro. This study revealed that human lymph node and whole blood respond to the Rauscher leukemia virus with a pattern of formation of large basophilic blast-like cells demonstrating mitotic activity, large mononuclear cells with intranuclear inclusion bodies and plasma cells.

Materials and Methods

In conventional tissue culture methods bone-marrow cells and lymphocytes have a tendency to transform into spindle-shaped cells. To prevent this phenomenon and also to avoid the possible influence which could occur by using different additions in the conventional media (horse serum, chicken embryo extract, etc.), we have developed a new tissue culture method (Jankay and Cole, 1960; Jankay, 1964). The principal point of this method is that we are using autologous or homologous heparinized whole blood as tissue culture medium. With this method, no spindle cell formation takes place (Jankay and Kurnick, 1962). During the cultivation period, the blood with the tissue fragments is kept in continuous motion. This continuous motion prevents sedimentation of the blood cellular elements. A gas phase of 95% air with 5% CO₂ provides the cultures with oxygen.

The feeding, oxygenation and removal of the metabolic waste products from the culture, is accomplished by continuous dialysis through a semi-permeable tube which contains the blood with the tissue fragments. Heparinized homologous pooled plasma is used as dialysing fluid. Patients without blood dyscrasias, malignancies or infections, are selected as lymph node and blood donors. In these cultures, autologous blood has been used. Fresh surgical specimens of scalenus and mesenteric lymph nodes were obtained by surgery. They were cut into about 2 mm thick fragments and placed with 10 ml of autologous heparinized whole blood into the semi-permeable cellophane dialysing tube. 0.1 ml of Rauscher leukemia virus suspension was added to each 10 ml of blood. For control, lymph node cultures and also blood cultures without lymph node and without virus addition, were done from the same aliquots. The same

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set of cultures were also done using 6 R X-irradiation. After 5 days of cultivation, the cultures were terminated. The tissue fragments were fixed and paraffin sections made. For blood, smears were made and stained with Wright stain.

Results

Histologic examination of the 5 days cultivated lymph nodes shows the maintenance of the original architecture. The nodes cultured with virus show hyperplasia of the reticulum cells. In the capillary vessels, endothelial proliferation is seen. Within the nuclei of many reticulum cells, there are spherical bodies having a homogenous appearance and a light purple color, showing the characteristics of viral inclusions. The reticulum cells with inclusion bodies are not limited to the germinal center and the follicle area, they are also seen in the medullary part of the lymph node. Plasma cell formation is seen in the germinal center and also in the medullary area.

The blood smears obtained from the cultures without lymph node, but with virus addition, show 2 typical cell transformations.

Fig. 1

Fig. 1. Transformed large blast-like cell in mitosis

Fig. 2. One blast-like cell with deeply basophilic cytoplasm and one large transformed mononuclear cell with intranuclear inclusion body. Note the well preserved erythrocytes

1. Large mononuclear blast-like cells with well defined cell membrane, deep basophilic cytoplasm, round nuclei with mitotic activity (Fig. 1).

2. Large mononuclear cells with intranuclear inclusion body, similar to that seen in the lymph node cultures. This type of cells does not undergo mitosis. These two char-