A STUDY OF INTERACTION BETWEEN LYMPHOCYTES AND TUMOR CELLS WITH THE SCANNING ELECTRON MICROSCOPE

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It was shown with the aid of the scanning electron microscope that in the course of interaction between lymphocytes of BALB/c mice with methylcholanthrene-induced sarcoma and autologous tumor cells the state of the surface membranes of the cells underwent substantial changes. Lymphocytes separated from autologous tumor cells by means of a Millipore filter impermeable to the cells formed long cytoplasmic processes which passed through the pores of the filter and established close contact with the tumor cells. Under these conditions the tumor cells became spherical in shape, they swelled, and "holes" appeared in the surface membrane, leading to lysis of the cell.

Key words: lymphocytes; tumor cells; cytoplasmic processes.

The writers showed previously [3] that lymphocytes of BALB/c mice with a methylcholanthrene-induced sarcoma, if grown in culture in diffusion chambers, inhibited the "spheroid-formation" by tumor cells and had a marked cytotoxic action. Under these circumstances the lymphocytes and tumor cells were separated from each other by a Millipore filter impermeable to the cells and the result could be evidence of the humoral nature of the observed effect. These findings are in good agreement with investigations [4, 5, 9, 16] by workers who consider that the cytotoxic action of lymphocytes activated by antigens of target cells is due to highly active humoral substances of the lymphokinin type, synthesized and secreted by lymphocytes. However, this does not agree with the generally accepted view that direct "physical" contact between lymphocytes and the corresponding target cells is necessary [1, 13].

This contradiction in the data stimulated interest in an investigation, described below, to study the character of interaction between lymphocytes and tumor cells on a membrane filter (from functional and physical aspects) by studying the state of the cell surfaces of the interacting elements, which play an important role in functional activity of the cell.

EXPERIMENTAL METHOD

Interaction of lymphocytes and autologous tumor cells was studied during their culture in "triple" diffusion chambers mounted so that the two types of cells were separated from each other by a Millipore filter. The target cells were cells of a polymorphonuclear sarcoma induced in BALB/c mice by 20-methylcholanthrene. The tumor was minced and broken up with a 0.5% solution of trypsin (Spofa) in Hank's solution. One compartment of the diffusion chamber contained 1×10^6 living tumor cells. These cells formed colony-like structures on the Millipore filter resembling the "spheroid" described by Sutherland and Credie [15]. The second compartment of the diffusion chamber contained 1×10^6 autologous lymphocytes. Lymphocytes of intact syngeneic animals acted as the control.

To prepare the diffusion chambers, Millipore filters (VUFS, Czechoslovakia) with a pore size of 0.2-0.3 μ and a thickness of 100 μ were used. The diffusion chambers were mounted by the usual method and

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implanted into the peritoneal cavitity of intact syngenic animals. On the 3rd day the chambers were removed and the filters were fixed and taken up through alcohols of increasing strength, for 5 min in each strength. After 100%alcohol the filters were dried in air for 30 min and placed in a vacuum spray. The specimen was sprayed with gold with the support tilted at different angles. Specimens prepared in this way were examined in the Stereoscan scanning electron microscope under an angle of 45° to the incident electron beam with an accelerating voltage of 15-20 kV.

EXPERIMENTAL RESULTS

The surface of the lymphocytes of intact syngeneic animals was compared with the surface of lymphocytes from the mice with tumors. The lymphocytes of the latter had considerable cytotoxic activity when cultured with the tumor cells on the opposite side of the Millipore filter. At the same time the surface of the tumor cells was studied when cultured with autologous lymphocytes and with intact lymphocytes from syngeneic animals.

The lymphocytes of the intact BALB/c mice were single cells, not making contact with each other, resembling in appearance simple round "spheres" with a slightly rough surface. They were attached to the Millipore filter by several short, thin processes, given off from the surface of the lymphocyte. Lymphocytes obtained from the lymph glands of mice with a methylcholanthrene-induced sarcoma were characterized by considerable irregularities of the surface and by the formation of numerous, frequently long processes. By means of them the cells made contact with each other. The processes differed greatly in thickness – from thin microfilaments (200-500 Å) to fairly thick cytoplasmic bands (0.1-0.2 μ). Sometimes the length of the bands was greater than the diameter of the cells. In the writers' opinion these bands were identical with the uropodia described by McFarland et al. [7]. McFarland [8] and Rosenstreich et al. [14] consider that the uropodia are "receptor organs" responsible for the possible and varied contacts taking place between lymphocytes and target cells, producing an "explosive" increase in the surface area of the lymphocyte by means of the "microfilaments" [12]. Similar changes in the membrane surface of immune lymphocytes were observed previously by one of the present writers (T.P.E.) in experiments to cultivate sensitized lymphocytes in diffusion chambers implanted in animals inoculated with a tumor [2].

The study of the surface of tumor cells, grown together with normal lymphocytes, in the scanning electron microscope showed that these tumor cells have a mirror-smooth surface and were closely applied to the filter, forming a layer on it (Fig. 1a). The surface of the tumor cells grown together with autologous lymphocytes, on the other hand, was sharply modified. The cells were spherical in shape, very swollen, with a loose surface, and appeared raised above the surface of the filter (Fig. 1b, c, d). Under high power (Fig. 1c) a tumor cell with parts of its membrane destroyed and with protruding cytoplasm, evidently as a result of disorganization of the cell, leading to its lysis, can be seen. In the center of these cells on the membrane there were tiny holes 100 Å in diameter. Similar holes in the red cell membrane as a result of its interaction with hemolysin and complement were described by Durmashkin and Rosse [6]. Humphrey [10] considers that one such hole in the outer layer of the cell membranes is sufficient to cause lysis of the target cells.

It will be clear from Fig. 1d that a very slender bifurcated process of a lymphocyte approaches the tumor cell after penetrating through a pore of the Millipore filter from the opposite side, thereby creating direct contact between the lymphocyte and the tumor target cell.

Consequently, the lymphocytes of mice with a methylcholanthrene-induced sarcoma, prevented under the conditions of the diffusion chamber from migrating toward the target cells and becoming adsorbed on their surface, form cytoplasmic processes by which they not only adhere to the Millipore filter, as is observed with lymphocytes from intact mice, but they pass through the pores of the filter (0.23 μ) and make contact with the tumor cells on the opposite side of the filter. This conclusion is also confirmed by the results of investigations by other workers [17] who made a microscopic study of transverse sections of Millipore filters and showed that the cytoplasmic processes will penetrate through pores of the filter 0.2 μ in diameter and 25 μ thick. The filter used in the present experiments cannot thus prevent direct contact between lymphocytes and tumor cells.

These results indicate that during interaction between lymphocytes and autologous tumor cells the state of the surface membrane of the cells is substantially modified. The surface of the lymphocytes of mice with tumors, when placed in diffusion chambers with autologous tumor cells, was sharply changed: long cytoplasmic processes were formed by means of which the lymphocytes made contact with tumor cells on the opposite side of the filter. The contacts observed do not rule out the possibility that not all lymphocytes make contact with tumor cells; evidently some of them (those first committed to the tumor antigen)

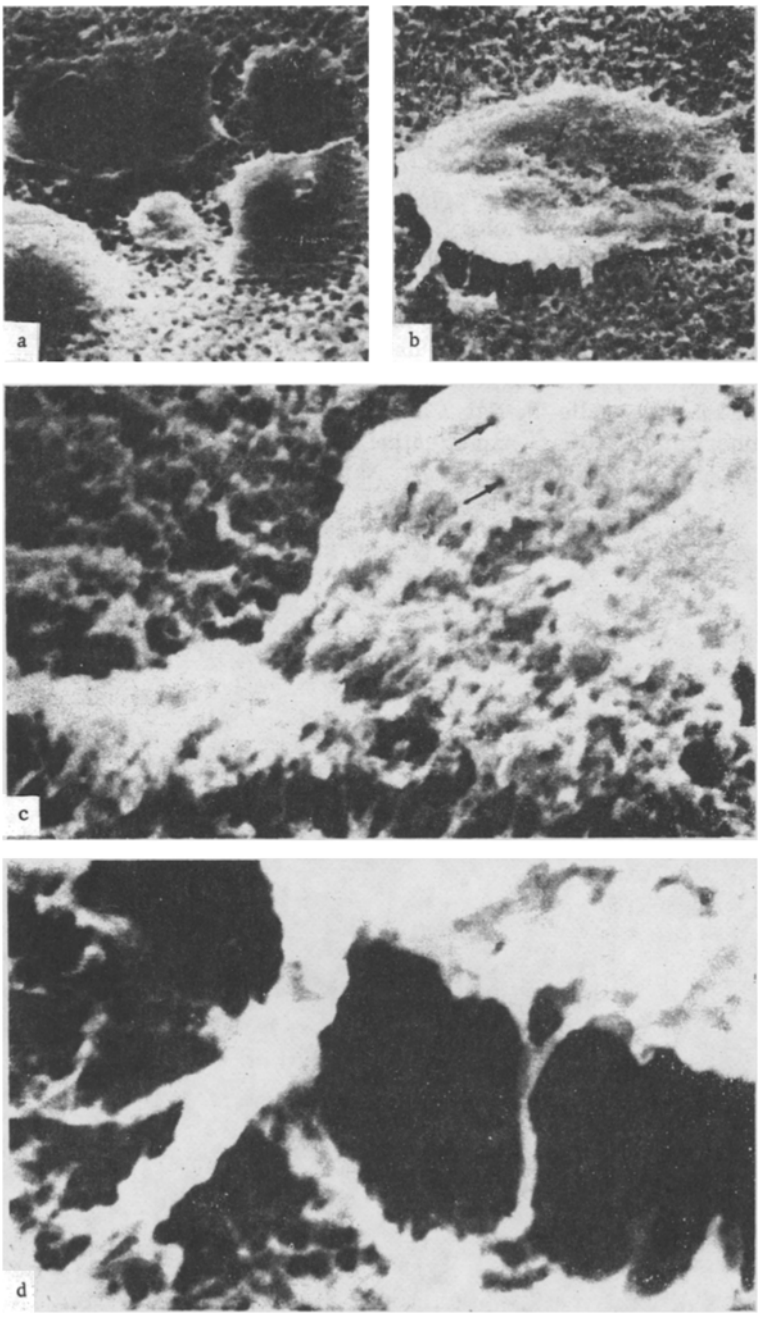

Fig. 1. Surface of tumor cells and lymphocytes in diffusion chamber: a) surface of tumor cells on 3rd day of culture in diffusion chamber: lymphocytes of intact syngeneic animals on opposite sides of Millipore filters (45°; 2000×); b, c) surface of tumor cells on Millipore filter with autologous lymphocytes on opposite side (45°; 10,000×); d) contact between autologous lymphocytes and tumor cells (45°; 20,000×).

establish such contact. These lymphocytes may be regarded as "triggers" of the immune response. They synthesize and secrete both harmful factors [4, 9] and also substances extending the immune response (mitogenic factor, transfer factor [4, 11]) whereby those lymphocytes that have not established direct contact with the tumor cell are made to participate in the cytotoxic reaction.

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