The Surface Ultrastructure of Normal and Leukemic Rat Lymphocytes*

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In studies on the nature of the malignant state, there has been increasing interest in the external surface of cancer cells. Coman and his co-workers (1, 2, 5) established the fact that the intercellular adhesiveness of malignant cells was lower than that of their normal prototypes and that this difference probably played a major role in the invasive characteristics of cancer cells. Since adhesiveness is a surface phenomenon, it seemed reasonable that some morphological difference in surface structure might be demonstrable at the ultramicroscopic level, and, in 1955, Coman and Anderson (3) reported a difference between the surface ultrastructure of malignant and normal rabbit epithelial cells.

It is possible that these structural changes are directly related to the decreased adhesiveness of cancer cells. On the other hand, these surface alterations may be independent of adhesiveness and reflect some other characteristics of neoplastic cells.

To examine these possibilities, a cell system was needed in which the adhesive characteristics of the normal and malignant cell types were the same, thus separating adhesiveness from other aspects of neoplasia. Normal and leukemic lymphocytes provide such a system, since the adhesiveness of both cell types is low; that is, they both readily detach from their fellows and enter the circulation as individual cells. Consequently, a comparison was made of the surface ultrastructure of normal and leukemic lymphocytes from both the blood and lymph nodes of Wistar rats.

MATERIALS AND METHODS

The rats employed in this study were young females of the PA strain in which a transplantable lymphocytic leukemia (LK2) was being carried. The leukemia was passed by intraperitoneal injection of 0.1 cc. of whole blood. The animals died in 14 days with lesions in the liver, spleen, thymus, and mediastinal lymph nodes, and with peripheral white cell counts of 150,000–300,000 (95–99 per cent lymphoid forms). The percentage of small lymphocytes in the peripheral blood varied from 25 to 50 per cent, with the remainder consisting of large prolymphocytes and "blast" forms. Sections of mediastinal lymph nodes revealed sheets of small and large lymphocytes interspersed with varying numbers of reticulum cells with large vesicular nuclei.

Control lymph node specimens were obtained from normal rats of the same strain. Cells from normal rat thymus and bone marrow and from the lymph nodes and thymus of normal C57 mice were also examined. Wright's-stained blood smears and hematoxylin and eosin-stained sections were made of all specimens to compare the electron microscopic findings with those of the light microscope.

Cells were prepared for electron microscopy by a modification of Coman and Anderson's "chromium-carbon replica" technic (3). Cell suspensions from lymph nodes were obtained by sieving through #40 stainless steel mesh into Earle's balanced salt solution (pH 7.5). Circulating leukocytes, obtained from heparinized blood collected by cardiac puncture, were separated by slow centrifugation (350–500 r.p.m.) after the addition of phytohemagglutinin (6). Both types of cells were then washed once with balanced salt solution, fixed for 5 minutes in 1 per cent osmic acid, and then washed 3 times with distilled water.

The suspensions of fixed cells were spread on clean mica and air-dried. Shadow-casting with chromium and carbon was then carried out according to the technic of Coman and Anderson (3). A moderately heavy layer of chromium was evaporated onto the specimens at an angle of 15°, followed by a light carbon film.

The chromium-carbon films with cells attached were then floated from the mica onto 50 per cent nitric acid and transferred through distilled water.
to 10 n sodium hydroxide. After 3–6 hours at room temperature, all the organic cellular material had been digested away, leaving only the chromium-carbon film replicas. These were run through three changes of distilled water and then picked up on copper mesh for examination in the electron microscope.

RESULTS

Normal lymph node.—The preparations contained easily identifiable red blood cells and small lymphocytes and varying numbers of larger cells. The red blood cells varied in size from 4 to 6 μ and showed a surface ultrastructure similar to that described by Coman and Anderson (3), and by Hillier and Hoffman (4): a granular surface with evenly distributed particles ranging in size from 100 to 200 A. The small lymphocytes varied from 6 to 8 μ in diameter and were hemispherical in shape. This latter characteristic resulted in a shadowing effect on the whole cell, with piling up of chromium on the “front” surface and very little chromium deposition on the “back.” Consequently, very little fine detail was present on either of these surfaces; but enough detail was retained at the top and lateral margins of these cells to permit evaluation of their true surface ultrastructure. This was again finely granular but showed considerable variation in size and distribution of the surface granules, which ranged from 100 to 300 A in diameter and were distributed unevenly in closely packed, small clumps and linear aggregates without any uniform pattern (Fig. 1).

The remainder of the cells in the preparations were larger in size (8–20 μ) and invariably flatter than the small lymphocytes. From a study of the stained sections, it was apparent that these larger cells included immature lymphoid cells, reticulum cells, macrophages, and plasma cells. Most of these cells had a surface ultrastructure similar to that of the normal small lymphocytes with 100–300 A particles distributed in irregular clumps and short chains (Fig. 3). Between 10 and 20 per cent of the cells, however, showed quite different surface characteristics. These were large, extremely flat cells, often somewhat broken but usually oval in shape, with one or more rounded elevations 1 μ in diameter. The surface showed varying numbers of small craters which were uniformly 800–1000 A in diameter and were formed by a number of 100–200 A granules arranged around a central depression (Fig. 2). These craters appeared both singly and in clumps and seemed to be randomly distributed. Their numbers varied from one or two to more than 50 per cell. Portions of the surface of these “crater cells” appeared quite smooth, with a uniform, heavy deposit of chromium. Elsewhere, the surface granules appeared more evenly distributed and somewhat more prominent than on the other cells of the normal lymph node, although their size range (100–300 A) was the same. The frequency of these “crater cells” in the lymph node preparations varied directly with the number of reticulum cells observed in the hematoxylin and eosin-stained sections but did not appear to be related to the number of macrophages or plasma cells in the nodes.

To gain some idea of the relative adhesiveness of the “crater cells” as compared with that of the lymphocytic elements in the nodes, several preparations were made by gently shaking pieces of node in salt solution for comparison with preparations made by the usual method of pressing pieces through a sieve. The frequency of “crater cells” in the preparations made by shaking was 3–10 times less than in preparations made from the same node by sieving.

Normal blood.—Small and large lymphocytes, as well as erythrocytes, were similar in configuration and surface ultrastructure to those observed in the normal lymph nodes. Large cells, presumably granulocytes, showing a grossly nodular surface and an ultrastructure essentially similar to that of the lymphocytes were also observed, but only one cell with the craters described above was found in numerous specimens of normal rat blood.

Leukemic lymph node.—The cells in these preparations varied from 6 to 20 μ in diameter. The majority of the cells were large, flattened, and showed some fraying at the edges, suggesting that they were more fragile than those from the normal node. Regardless of size, their surface ultrastructure was indistinguishable from that of the cells of the normal node. The surface particles of both large and small leukemic lymphocytes were of the same size range and similarly distributed as on the normal cells (Fig. 4).

A number of typical large “crater cells” were observed in the preparations from leukemic nodes; but, in general, they were much less frequent than in the preparations from normal lymph nodes. However, as in the normal nodes, their frequency in the leukemic preparations varied directly with the number of reticulum cells observed in the stained sections.

Leukemic blood.—These preparations resembled those from the leukemic nodes, except that “crater cells” were only very rarely observed, and there was a greater frequency of small lymphocytes.
All photographs were taken with a Philips Electron Microscope, Model 100-A, at 80 kv, with a screen magnification of 20,000 X.

FIG. 1.—Portion of a small lymphocyte from a normal rat lymph node. The whole cell was 6 µ in diameter. The hemispherical shape of the cell has resulted in a heavy deposit of chromium on the front surface (upper left) and very little chromium on the back (lower right). Good surface detail is retained at the top and lateral margin of the cell. The surface ultrastructure consists of unevenly distributed particles varying from 100 to 300 A in size and arranged in irregular clumps and short chains.

FIG. 2.—Portion of a "crater cell" from a normal lymph node. The whole cell was ovoid (14 × 11 µ) and flat, and showed several 1-µ elevations on its surface (lower right). The surface is studded with 800-1000 A craters (arrow) consisting of several 100-200 A particles arranged around a central depression. The cell surface shows smooth areas covered by a heavy chromium deposit; elsewhere the surface granules are somewhat more prominent than on the small lymphocyte but similar in size. The evidence indicates that these "crater cells" are probably reticulum cells.

FIG. 3.—Portion of a large (immature) lymphocyte from a normal lymph node. The whole cell was round, relatively flat, and 10 µ in diameter. The surface ultrastructure is essentially the same as that of the small lymphocyte (Fig. 1) with 100-300 A particles unevenly distributed over the surface.

FIG. 4.—Portion of a large lymphoid cell (10 µ) from a leukemic lymph node. Except for slightly greater fraying of the edges, the leukemic cell was similar in shape to the normal cell (Fig. 3). The surface ultrastructure is indistinguishable from that of the normal.
Fig. 5.—Portion of a large (11 μ) lymphoid cell from the circulating blood of a leukemic rat. The cell is similar in shape and surface ultrastructure to cells from normal and leukemic nodes (Figs. 3 and 4).

Fig. 6.—Portion of a “crater cell” from the circulating blood of a leukemic rat. Except for some breakdown of cell borders, this cell resembles the typical “crater cells” of the normal lymph nodes in both shape and surface ultrastructure. Comparison with Figure 2 shows some variation in the chromium deposition on the two cells, but the craters (arrow) and surface granules are essentially similar. These cells rarely appeared in the circulating blood.
The surface ultrastructure of all cell types was identical with that previously described for normal and leukemic cells (Figs. 5 and 6).

Other organs.—Preparations from normal rat thymus and bone marrow, and from mouse lymph nodes and thymus, all contained lymphoid cells with a surface ultrastructure similar to that described above, as well as varying numbers of typical "crater cells."

DISCUSSION

In the studies reported, no consistent difference between the surface ultrastructure of normal and leukemic rat lymphocytes was found. These results indicate that, in the cell system employed here, in which the adhesive characteristics of the normal and malignant lymphocytes are the same, the malignant state may exist without any concomitant alteration of the cell surface ultrastructure.

On the other hand, the findings suggest that structural changes in the cell surface may specifically reflect differences in intercellular adhesiveness. In our preparations, the only cells which had a different surface ultrastructure, the so-called "crater cells," were also much more difficult to shake out of lymph nodes than were the other cellular elements, suggesting that the intercellular adhesiveness of the "crater cells" is greater than that of the other cells in the node.

The identity of these "crater cells" is of some interest. By a process of elimination, they have been tentatively identified as reticulum cells, the undifferentiated stem-cell precursors of the other hematopoietic elements. For example, they were not found in preparations from the circulating blood of normal animals, and, therefore, they were not granulocytes or monocytes; they were found in significant numbers in thymus preparations, and, therefore, they were not plasma cells or macrophages, which are rarely seen in this organ; and their presence in large numbers in bone marrow preparations eliminated the possibility that they were immature lymphocytic forms. Furthermore, their frequency in lymph node preparations, both normal and leukemic, varied directly with the number of reticulum cells observed in stained sections of these organs. Thus, the evidence indicates that the "crater cells," with their special surface characteristics, are indeed reticulum cells and that their intercellular adhesiveness is greater than that of the other cell types in the lymph nodes.

The occasional appearance of "crater cells" among the immature cells in the circulating blood of leukemic rats is somewhat difficult to explain. It may simply indicate that, despite their increased adhesiveness, under the special conditions of the leukemic state, even these primitive undifferentiated cells may occasionally enter the circulation.

SUMMARY

The surface ultrastructure of normal and leukemic rat lymphocytes has been compared by electron microscopy. Large and small lymphocytes, both normal and neoplastic, from blood and lymph nodes, all showed a similar fine surface structure consisting of unevenly distributed particles 100-500 A in diameter.

Large cells, presumably reticulum cells, with 1000 A craters on their surface, were observed in all preparations except those from the circulating blood of the normal rat. Shaking experiments indicated that the intercellular adhesiveness of these "crater cells" was greater than that of the other cells in the nodes.

In the cell system used, the adhesiveness was low in both the normal and neoplastic cells. The failure to observe a difference in surface ultrastructure suggests that a change to the neoplastic state is not necessarily accompanied by an alteration in the cell surface—at least in rat lymphocytes.

Of all the cells examined, only reticulum cells showed a surface difference; and these cells also showed a greater adhesiveness. This suggests that the ultrastructure of the cell surface may be directly related to intercellular adhesiveness.

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