An Electron Microscope Study of Histiocyte Response to Ascites Tumor Homografts*

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SUMMARY

When the ascites forms of the DBA/2 lymphoma L1210 or the C57BL E.L. 4 lymphoma are injected into C3H mice, host histiocytes (macrophages) accumulate and are responsible for the destruction of a large number of tumor cells. Many of the tumor cells, often apparently intact, are ingested. The ingestion process is rapid and depends upon invagination of an area of the histiocyte with simultaneous projection of cytoplasmic fimbriae which complete the encirclement. The earliest change seen in the enclosed cell is shrinkage; digestion of the cell wall and cytoplasmic elements follows. Phagocytosis accounts for only a proportion of the cells destroyed by histiocytes. Other cells are probably destroyed when their cell membrane is broken down in an area in contact with a histiocyte, apparently permitting fusion of the two cytoplasms.

Weaver and his colleagues (13) observed that host cells were frequently found in close association with tumor cells and described death of both host and incompatible tumor cell after a period of contact. Gorer (9) found that the predominant host cell in the ascites fluid during tumor rejection was the histiocyte. This finding was confirmed, and some quantitative measurements were made by one of us (1) and later by Weiser and his colleagues (4).

During rejection, both host and tumor cell have increased susceptibility to damage by antibody or by exogenous complement, and it appears that, in some instances, host histiocytes in the presence of complement are damaged by antibody directed against the tumor (2). Light microscopy suggested that extremely close union occurs between tumor and host cell and that extracellular digestion is an alternative to phagocytic destruction as a means of eliminating tumor cells from the population (3). During the phase of acute rejection, host histiocytes frequently appear to be degenerate, and there is an abrupt fall in their number.

This report describes some of the structural changes accompanying the ingestion and later digestion of lymphoma or sarcoma cells by histio-

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cytes and details some of the concomitant changes occurring in the histiocytes themselves.

MATERIALS AND METHODS

In a series of experiments 20 × 10⁷ cells from rapidly growing ascites populations of the lymphomas E. L. 4 or L1210 native to C57BL and DBA/2, respectively, were injected into the peritoneal cavity of young adult male C3H/He mice. In another series, 2 × 10⁶ C3H ascites sarcoma MC1M cells were injected into C57BL male mice. The recipients were killed at daily intervals from the 5th to the 10th day after inoculation, and 0.2 ml. of the ascites fluid was withdrawn and mixed immediately with 5 ml. of cold 2 per cent osmic acid in 0.4 M sucrose. The viscous ascites supernatant from the MC1M tumor appeared to give excellent protection to the cells during fixation and subsequent embedding.

After fixation, the cells were lightly centrifuged if gravity sedimentation had not occurred, the supernatant was removed, and the cells were dehydrated by the procedure described by Epstein (6).

The fragments were embedded in prepolymerized methacrylate (95 per cent butyl + 5 per cent methyl containing 1 per cent benzoyl peroxide) and polymerization completed with U.V. irradiation. Sections were cut with glass knives, placed on formvar-coated grids, and examined in an RCA EMU 2B or 3F microscope.
RESULTS

The predominant C3H host cell type after injection of L1210 lymphoma is the histiocyte. During late phases of the rejection of the tumor, these cells may come to make up more than 75 per cent of the ascitic population. Phagocytosis of tumor cells by histiocytes is extremely common, and, unlike the phagocytosis of A strain lymphoma #1 cells seen by Shelton and Dalton (10), leads to the rapid shrinkage and digestion of the ingested cells. From electron microscope preparations and Wright's stained smears, it appears that many intact tumor cells are being ingested. Trypan blue staining of fresh preparations indicated that more than 92 per cent of the ascites population was unstained and therefore presumably viable.

Lymphoma cells.—The lymphoma cells were characterized by large, centrally placed nuclei surrounded by a thin rim of cytoplasm. In many instances the nuclei had deep indentions which gave them a lobulated appearance. Chromatin material was disposed in patches near the nuclear envelope. Nucleolar material consisted of compact masses of dense granules but occasionally assumed a loose, filamentous arrangement. Mitochondria, which were few in number, were primarily of the round or short rod variety, showing typical fine structure. Several large lipide droplets were commonly found in each sectioned cell. The cytoplasm contained a few isolated ergastoplasmic tubules but was filled predominantly with free ribosome particles. Cytoplasmic regions composed of fine fibrils were found infrequently; no virus-like particles or inclusions were encountered (Fig. 1).

MC1M ascites.—The MC1M ascites cells were rounded or oval, with large irregular nuclei, which contained one to three prominent nucleoli. Mitochondria were large and pleomorphic with few cristae, resembling those previously described in the solid MC1M tumor (5). Tubular elements of the ergastoplasm were more numerous than in lymphoma cells, but without evidence of interconnection. Scattered through the cytoplasm were lipide droplets and occasional fibrillar areas (Fig. 2). The Golgi material consisted of stacked cisternae with associated chains of microvesicles. Other cytoplasmic elements not commonly found in ascites cells included granular bodies, possibly lysosomes, similar to those described in bladder epithelia (12), and multivesicular bodies (Figs. 3, 4) of the type found in rat ova (11) and in human amnion cells (7).

Histiocytes.—Typically, histiocytes possessed lobulated nuclei and had villi or protrusions along the cell periphery. Numerous vacuoles were found subjacent to the plasma membrane, suggesting pinocytic activity. The cytoplasmic matrix was less dense than in tumor cells, because of the relative absence of free ribosomes, and contained many ergastoplasmic tubules and small vesicles. Mitochondria appeared as short, dense rods, and an extensive Golgi apparatus was usually seen between the lobes of the nucleus (Figs. 5, 6). Granular bodies of varying sizes, termed round or residual bodies by Palade and probably corresponding to lysosomes, were another common feature of histiocyte cytoplasm. Inactive histiocytes had a few fat droplets arranged in the peripheral cytoplasm, but during periods of maximal reactivity contained numerous irregular lipide particles of varying size scattered throughout the cell.

Phagocytosis.—Lymphoma cells were seen at many different stages of ingestion. The earlier steps must be very rapid, since very few cells are caught in this stage. Thin protoplasmic filaments project around the cell and rapidly envelop it in a film of cytoplasm. The ability to ingest other cells is not confined to histiocytes; on rare occasions a tumor cell is seen attempting phagocytosis. Although none of the tumor cells examined by electron microscopy contained phagocytic vacuoles, very infrequently tumor cells containing phagocytized masses were encountered during light-microscope examination of smears, where the sampling covered many thousands of lymphoma cells. Extensive phagocytosis of dead histiocytes by A strain sarcoma cells has been noted by Gorer (9).

Digestion of lymphoma cells.—Phagocytosis is followed by rapid digestion. An analysis of the various changes following ingestion is shown in

1. L. Journey and M. Goldstein, to be published.
Table 1. A typical course, based on an examination of 70 ingested cells, would seem to be as follows:

A distinct membrane lines a digestive vacuole in about half the examples studied (Fig. 8). The cell undergoing digestion shrinks progressively and is often surrounded by a layer of fluid; sometimes secondary vacuoles are seen nearby. The nucleus shows more advanced degenerative changes than the cytoplasm, and the nuclear membrane usually disintegrates before the cytoplasmic membrane. There is little correlation between the persistence of a vacuolar and the cytoplasmic membrane, but in the more severely degraded inclusions the cell remnant appears to be in direct contact with the histiocyte cytoplasm. Mitochondria of the ingested cell show vesicular changes in about one-third of the examples examined.

Mitochondria in less severely damaged histiocytes often exhibit the same type of vesiculation seen in the ingested cell.

Digestion usually involves a progressive loss of all cytoplasmic and nuclear structures of the lymphoma cell; but occasionally the remnants form a dense, spongy osmiophilic mass, often internally vacuolated (Fig. 10). There is a suggestion from two plates that this mass may be extruded (Fig. 11); in other cases, small fragments appear to be pinched off and may be seen in separate vacuoles.

Phagocytosis and digestion of sarcoma MC1M.—Extensive phagocytosis of MC1M is rather less common than phagocytosis of a lymphoma, but has been observed in some experiments. The tumor cell is large relative to the histiocyte, and simultaneous ingestion of more than one such cell is rare. The sequence of changes appears to differ from the digestion of lymphomas.

The histiocytes themselves often show signs of digestive breakdown, especially in the cytoplasm. The cause of this is unknown; it could be from an excess of activated enzymes released from the degenerating ingested cell or could result from an intracellular antigen-antibody reaction. Of 38 phagocytic histiocytes examined, nineteen showed some evidence of loss of cytoplasmic detail. In eleven of these, the damage was marked, and in a few, most of the cytoplasmic structures including mitochondria, ergastoplasm, and Golgi have disappeared, the cell appearing remarkably empty (Fig. 9). These changes are not fixation artifacts, since adjacent cells may show adequate fine structural details, and the severely affected histiocytes often contain a number of adequately fixed lymphoma cells in varying stages of digestion. The mitochondria in less severely damaged histiocytes often exhibit the same type of vesiculation seen in the ingested cell.

Table 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Structure studied</th>
<th>Outer membrane present</th>
<th>Degeneration</th>
<th>Vesicular mitochondrial membrane present</th>
<th>Vacuolar membrane present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingested cell</td>
<td>Cytoplasm</td>
<td>22</td>
<td>Severe: 35, Moderate: 31, None or slight: 4</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Nucleus</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histiocyte</td>
<td>Cytoplasm</td>
<td>38</td>
<td>11, 8, 19</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

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A thin rim of cytoplasm surrounds the apparently intact Sarcoma 1 cell (Fig. 12). The nucleus begins to degenerate, but the nucleoli remain clearly visible (Fig. 13). The cytoplasmic and nuclear membranes appear to remain intact, but small vesicular bodies may be seen between the cytoplasmic membrane of the tumor cell and the vacuolar membrane of the histiocytes. These appear to be budded off from the latter (Figs. 14, 15) but may be separated from it during subsequent shrinkage of the ingested remnant. Degenerative

Fig. 1.—L1210 tumor cell with large, dense nucleus. Several compact mitochondria and irregular lipide droplets are distributed in the cytoplasm. Other cytoplasmic elements include numerous microvesicles and free ribosomes, with no evidence of ergastoplasmic tubules. Stained with lead hydroxide. X×42,000.
Fig. 2.—Part of an MC1M ascites cell showing the loosely arranged nucleolar granules. Mitochondria appear as large ovals with few cristae. Scattered through the cytoplasm are lipid droplets, many rough-surfaced ergastoplasmic tubules, and free ribosomes. X16,000.
Fig. 3.—Part of the cytoplasm of an MC1M cell containing several inclusion bodies (arrows) within which are found dense vesicles. X16,000.

Fig. 4.—Higher magnification of the multi-vesicular bodies revealing details of the enclosed heavy-walled vesicles. X52,000.
Fig. 5.—Micrograph depicting structural components of a histiocyte before ingestion of tumor cell. Although the cell appears binucleate, it may represent the plane of section through a single, lobulated nucleus (n). Lipid droplets are distributed in the peripheral cytoplasm. Typical Golgi elements are localized between the nuclear lobes. Mitochondria and many ergastoplasmic elements are displaced throughout the cytoplasm. In one area (arrow) the histiocyte appears to be sending out villi to establish contact with a neighboring L1210 tumor cell. $\times 7,200$. 
FIG. 6.—Higher magnification of a histiocyte which includes part of the nucleus and details of mitochondria, stacked membranes, and microvesicles of the Golgi and granular bodies (lysosomes?) of varying size. Stained with lead hydroxide. ×16,000.
FIG. 7.—Micrograph of an L1210 tumor cell (T) sandwiched between two histiocytes (H-1 and H-2). Histiocyte in upper part of picture has apparently established protoplasmic contact, and the second has extended filamentous processes (arrows) to engulf the tumor cell. Nuclei (n) of the histiocytes are indicated. \( \times 12,600 \).
Fig. 8.—Histiocyte containing two ingested cells (*c* and *c*-2). Tumor cell (*c*-1) represents an early stage of assimilation; the nucleus and swollen mitochondria are still recognizable. The other (*c*-2) represents a more advanced stage of digestion in that the cell remnant has reended from the membrane-bound vacuole, and there is marked vacuolization of the cytoplasm and dense nuclear material. Smaller phagocytic vacuoles (arrows) have pinched off and migrated into the surrounding cytoplasm. The nucleus (n) and cytoplasmic elements of the histiocyte appear relatively normal. X11,240.

Fig. 9.—Histiocyte containing the remnants of four phagocytized cells (*c*). The histiocyte cytoplasm appears empty with a few lipide droplets and secondary phagocytic vacuoles but no mitochondria, indicating the impending disruption of the cell. X8,100.
Fig. 10.—Portion of a histiocyte in the L1210-C3H system containing two dense vacuolated masses which probably represent the final stages of phagocytic digestion. X8,400.

Fig. 11.—Micrograph showing portions of a histiocyte (H) and two E.L.4 tumor cells. The dense mass at center (arrow) appears as if it has just been expelled by the histiocyte. X8,400.
FIG. 12.—MC1 M tumor cell immediately after ingestion by a histiocyte. Note that the tumor cell is completely enclosed by the histiocyte and that its structures appear unaffected. X10,000.
FIG. 13.—A later stage in the phagocytosis of a MC1M cell showing initial deterioration of nuclear contents and vacuolization of mitochondria. X7,600.
FIG. 14.—Detail of the vesicles (arrows) that accumulate in the vacuolar space between the cytoplasmic membrane of the MC1M tumor cell (top) and the vacuolar membrane of the ingesting histiocyte (bottom). The vesicles appear to be budded off from the vacuolar membrane. $\times 22,000$.

FIG. 15.—Another example of vesicle accumulation (arrows) at the boundary of the ingested tumor cell in a later stage of digestion. The cytoplasmic membrane and internal structures of the MC1M cell have been largely destroyed while the vesicles remain intact. $\times 22,000$. 
Fig. 16.—A more advanced stage in the digestion of two MC1M cells in which few elements are still recognizable. ×9,000.
Fig. 17.—Histiocytes with large empty vacuoles from which the tumor cell contents were presumably resorbed were frequently encountered in the MC1M in C57 system. Several smaller vacuoles containing dense osmiophilic material are also present. X9,000.
Fig. 18.—Area of contact between a histiocyte and an MC12 cell. There is interdigitation of cytoplasmic processes of the two cells and vesicle formation and excessive infolding of the cell membrane of the histiocyte. There is no breach in the continuity of the cytoplasmic membranes. ×20,000.
Fig. 19.—An example in which villi of the histiocyte (H) have developed into open bridges (arrows) and continuity between the cytoplasm of histiocyte and tumor cell (T) has been established. X24,000.
changes become extreme and lead to shrinkage and liquefaction of the ingested cell (Fig. 16). A fluid-filled vesicle remains which probably collapses, discharging the contents to the outside (Fig. 17). In contrast with the cytoplasmic degeneration commonly seen following digestion of lymphoma cells, the fine structure of the histiocyte cytoplasm has remained intact during digestion of the sarcoma cells.

Extracellular reactions.—Phagocytosis is not the only function of histiocytes. With some tumors such as the C3H sarcoma MC1M, phagocytosis is, in fact, generally unusual, possibly because of the large size of the tumor cells. Even with lymphomas such as L1210 or E. L. 4 which are sensitive to antibody and small enough to be readily engulfed, extracellular digestion appears to take place.

Goldberg and Green found that heterospecific immune globulin would produce a spongelike texture of localized regions of the cytoplasmic membrane with a number of fimbrial processes which appeared to interdigitate with similar processes from other cells during agglutination (8). Similar but less pronounced changes have been found on the cytoplasmic membranes of some host histiocytes in our preparations. In these mice, antibody is being produced against the tumor; none would be expected to be formed against the host. It has been suggested by one of us (3) that a local change in the surface membrane of the host cell occurs following contact with graft antigen. Increased permeability from local degeneration of the cytoplasmic membrane would permit digestive enzymes to come into direct contact with tumor cells; progressive digestion would then occur.

Considerably more evidence is needed before such a hypothesis can be accepted. Electron micrographs show a localized change of the type reported by Goldberg and Green (8) as being due to antibody in the area in contact with the target cell. Extracellular digestion was seen in a number of sections.

Most of the surface of the histiocyte shown in Figure 5 is relatively smooth, with an occasional small projection. There is an area (arrow) where the membrane is piled up, and numerous villi project to meet an opposing lymphoma cell. Figures 18 and 19 show a similar process at a higher magnification. In the cells shown in Figure 19 there appears to be a definite breach in the wall of both cells. Degenerative changes are seen in the cytoplasm of the tumor cell shown in Figure 19.

REFERENCES
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