Cell Reactions to Metastatic Tumors in the Regional Lymph Nodes
—Light and Electron Microscopic Studies—

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ABSTRACT

Cell reactions to metastatic tumors in the regional lymph nodes were studied by light and electron microscopy in 20 cases; i.e. reactive hyperplasia (3), tuberculosis (3), metastatic carcinomas from the breast (4), from the stomach (2), from the lung (2), metastatic epidermoid carcinoma (2), metastatic melanoma (2), and reticulum cell sarcoma (2). The lymph node response was usually germinal center predominence type and the pyroninophilic cell response was a similar pattern of nonspecific germinal centers with prominent reactive hyperplasia. In two cases of undifferentiated tumors, one from the breast and another from the lung, large numbers of pyroninophilic cells were found within the tumor tissue. However, the majority of lymphoid cells surrounding tumor cell or tumor masses were pyronin negative lymphocytes. Electron microscopic observations revealed that the cells surrounding tumor cells were mostly medium sized lymphocytes, occasionally blast cells and mature plasma cells. The contact border between the tumor cells and the surrounding cells was mostly tight and smooth, but occasionally loose with irregular processes, and widely separated in the case with plasma cells. Degenerative changes of adjacent cytoplasm of either the tumor cells or the lymphocytes were not frequent, but in some instances focal degeneration of adjacent cytoplasm, particularly on the side of the lymphocytes, was noted.

INTRODUCTION


Attempts to demonstrate prognostic significance from the regional lymph node histology were made by Black et al (1953, 1954), Black and Speer (1958), Tsakraklides et al. (1953) and Tsakraklides et al. (1974). They

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reported that sinus histiocytosis and proliferation of lymphocytes are an indicator of a better prognosis, thus pointing out the importance of cell-mediated immunity.

Despite very strong evidence shown by animal experiments and in vitro studies to indicate that the regional lymph node cells play a very important role in tumor immunity, in vivo observation on the morphologic evidence of tumor cell and lymphoid tissue interaction in the regional nodes is lacking. Present studies are intended to investigate cell types surrounding metastatic tumor cells in the regional nodes and their interaction with tumor cells by light and electron microscopy.

MATERIALS AND METHODS

Lymph nodes from 20 cases; i.e. reactive hyperplasia (3), tuberculosis (3), reticulum cell sarcoma (2), metastatic carcinoma from the breast (4), the stomach (2), the lung (2), epidermoid carcinoma (2), and metastatic melanoma (2), are studied. For the light microscopic observations, the tissue was fixed in 10% neutral formalin and embedded in paraffin. About 6µ thick sections from a paraffin block were stained with hematoxylin and eosin, methyl green pyronin, and PAS methods to make a histologic diagnosis and to determine the distribution and amount of pyroninophilic cells. Degree of fibrosis and the pattern of lymphoid hyperplasia as well as the degree of sinus histiocytosis were also checked. For the electron microscopic studies, a portion of lymph node was cut into pieces of 1 mm³ size and double fixed with 4% glutaraldehyde and 2% osmium tetroxide, and then processed with routine epon embedding methods. Sections from epon blocks 1µ thick were cut and stained with methylene blue-fuchsin double stain to orient tissue and confirm the presence of both tumor and lymphoid tissue elements. The ultrastructural observations were concentrated to identify the cell types surrounding tumor cells, their morphologic characteristics and contact border with the tumor cells. For comparison with the findings of tumor cases, three cases of non-specific reactive hyperplasia and three cases of tuberculous lymphadenitis were included.

RESULTS

1. Light microscopic findings:

The regional lymph nodes with metastatic tumors showed mostly reactive hyperplasia with various degrees of germinal center formation (Fig. 1): very prominent in cases of metastatic breast carcinoma, slight in cases of metastatic carcinoma from the lung and epidermoid carcinoma, and no germinal center formation in the cases of metastatic carcinoma from the stomach. The activity of the germinal centers was most prominent when the tumor replaced between 40 to 70% of the nodes, and less prominent when the replacement was below 20%. The nodes without tumor metastases showed mild germinal center reaction in the case of metastatic breast carcinoma, but prominent in cases of metastatic stomach carcinoma. Sinus histiocytosis was either absent or very mild in all of the cases irrespective of the type of tumor. Fibrosis varied by case, but increased as the amount of the metastatic tumor mass increased.

Pyroninophilic cells were scattered throughout lymphoid tissue except at the mantle zone of the secondary follicles, and also were occasionally found within the tumor cell mass. The germinal centers always contained various amounts of pyroninophilic cells, particularly at the peripheral portion. The pyroninophilic cells at the germinal centers were immature with large
nuclei (Fig. 2). Small numbers of pyroninophilic cells were found at the interfollicular diffuse cortex and paracortex. These pyroninophilic cells were either mature or intermediate types of plasma cells. Considerable numbers of pyroninophilic cells were found at the medullary sinusoidal wall. They aggregated usually in groups, and were mostly mature plasma cells (Fig. 3). Histiocytes within the sinuses were negative to pyronin. Various numbers of pyroninophilic cells were found within and near the thickened capsule, and they were mostly mature plasma cells. Occasionally pyroninophilic cells were present within tumor tissue, and were very prominent in one case of metastatic undifferentiated carcinoma from the lung (Fig. 4).

There were also considerable numbers of pyroninophilic cells in the fibrous septa, and most of them were mature plasma cells (Fig. 5). In one case of reticulum cell sarcoma, the majority of tumor cells showed intense pyroninophilia (Fig. 6). No appreciable number of pyroninophilic cells were seen within or around the tubercles.

2. Electron microscopic findings:

Ultrastructural characteristics of reactive hyperplasia and tuberculous lymphadenitis showed proliferation of various stages of lymphoid cells. Their contact border was tight and smooth without interdigitation or specialized structures (Fig. 7).

Ultrastructure of tumor cell surroundings showed either tumor cells, lymphoid cells or plasma cells. The contact border between tumor cells showed specialized structures, such as desmosomes, microvilli, and canaliculi according to the type of tumors. These structures were mostly copies of the primary tumor, and maintained the nature of the original tumor at the metastatic site.

The contact border between tumor cells and lymphoid cells were of three types. The first and most frequent one was tight straight contact without noticeable change of the cytoplasm of either lymphoid cell or tumor cell (Fig. 8, 9, 10). The second type showed an irregular surface of the lymphocytes and the tumor cells with wider intercellular spaces, but without noticeable degenerative change of the cytoplasm of either cell (Fig. 11). The third type showed marked degeneration of the cytoplasm of the lymphocytes with shrinkage and wide separation from the tumor cells, and various degrees of focal cytoplasmic degeneration of the adjacent tumor cells, especially in melanoma and epidermoid carcinoma (Fig. 12, 13, 14). The lymphoid cells which were in contact with tumor cells were mostly medium sized, mature forms or occasionally blast forms (Fig. 8, 9). The contact border between the tumor cells and the plasma cells showed wide separation in most of the cases and the surface of the plasma cells showed irregular protrusions (Fig. 15). Most of the plasma cells were well differentiated, and only occasionally an intermediate form was noted (Fig. 16). There was no noticeable cytoplasmic degeneration of either plasma cells or adjacent tumor cells. In the cases which showed large numbers of pyroninophilic cells within the tumor tissue there were also many plasma cells admixed with tumor cells and lymphoid cells (Fig. 17), and the cases of reticulum cell sarcoma with intense cytoplasmic pyroninophilia showed an abundant amount of polysomes (Fig. 18).

DISCUSSION

Prognostic significance of regional lymph node histology in cancer patients was reported by Black et al. (1963), Black et al. (1954), Black and Speer (1958), Tsakraklides et al. (1973) and Tsakraklides et al (1974). Findings
by Black and his associates emphasized that lymphocytic infiltration in primary tumors and sinus histiocytosis in the regional lymph nodes are morphologic signs of better prognosis, and Tsakraklides and his associates stressed that the lymphocyte predominant type of regional lymph node response is a sign of a better prognosis while the lymphocyte depletion type of nodal reaction is indicative of a poor prognosis. These studies identified morphologic features to cell-mediated and humoral immune responses of the host to tumors.

In animal experiments, Fisher and Fisher (1973) reported pyroninophilic cell response in regional nodes, and an increase of follicles and sinus histiocytosis in distant lymph nodes, and they attached significance of immunoblastic response of regional lymph nodes to tumor immunity. Godfarb and Hardy (1975) studied immunologic activity of the regional lymph nodes, distant nodes and spleen following the transplantation of murine mammary adenocarcinoma and found that initiation and progression of immunologic activity was found in the regional lymph nodes, and response in the distant nodes and spleen developed later. Kidd and Toolan (1950) reported that infiltration of lymphocytes in the transplanted tumor tissue brought about destruction of the tumor and raised the question whether the lymphocytes actually kill the cancer cells or lymphocytic infiltration means immune reaction. Hammond and Rolley (1970), Fisher and Fisher (1972), and Perez et al. (1973) reported the important role of the regional lymph nodes in the inhibition of tumor growth in animal experiments.

The inhibitory function of the regional lymph nodes against tumors is considered to be achieved by the cell mediated immunity of lymphocytes from vitro studies of Gewant et al. (1971), Deodhar et al. (1972), Fisher et al. (1974), Hellström and Hellström (1974) and Ellis et al. (1975). They demonstrated this effect by the clumping of lymphocytes around the tumor cells, the cytotoxic effect of the lymphocytes, the blast transformation of lymphocytes, and lymphocyte migration inhibition assay, when tumor cells and regional lymph node lymphocytes are mixed in culture.

The morphologic evidence that lymphocytic infiltration plays an important role in tumor destruction in vivo was reported by Kidd and Toolan (1950). They stated that lymphocytes penetrate the tumor tissue, transform morphologically, become intimately in contact with individual cancer cells, curving like a crescent partly about the tumor cells, and finally destroy the tumor cells. Fisher and Fisher (1972) observed lymphoid response at the tumor-kidney interface following subcapsular implantation of tumors in immune animals, but no appreciable lymphoid response in non-immune animals. They could not, however, find ultrastructural alterations of tumor cells exhibiting direct contact with lymphocytes.

Ultrastructural studies of lymphocyte-target cell interaction were made by Malchow et al. (1968), Able et al. (1970), and Richters and Sherwin (1974). Malchow et al. (1968) reported that when target cells were exposed to sensitized heterogenic lymphocytes, the lymphocytes first underwent mitoses followed by differentiation into medium and large lymphocytes, and came into close contact with the target cells by their uropod, leading to eventual destruction of the target cells, whereas exposure to allogeneic lymphocytes in the presence of phytohaemaglutinin lymphocytic contact and motility caused target cell death rapidly without transformation or mitosis of lymphocytes. Able et al. (1970) reported that when sensitized and nonsensitized lymphoid cells were incubated with allogeneic
target cells, sensitized lymphocytes became attached to the target cells leading to rounding, swelling, and lysis of target cells, whereas nonsensitized lymphoid cells rarely become attached to target cells. On electron microscopy, lymphoid cells attaching to target cells were of the large variety, containing numerous polysomes, smooth and coated vesicles and little endoplasmic reticulum. The sensitized lymphoid cells attached to the target cells by two distinct types of contact; close contact over extensive areas and interdigitating spike-like projections. Swelling and vacuolization was observed in the target cells which was probably due to surface alteration by contact with the sensitized lymphoid cells. Nonsensitized lymphoid cells showed no attachment to target cells or close contact with target cells. Richters and Sherwin (1974) reported diverse spike-free and spike bearing cytoplasmic processes including uropod attachments when tumor bearing axillary nodes from metastatic breast carcinoma were cultured.

Fine structural changes of lymphoid tissue following primary and secondary antigenic stimulation were reported by Movat and Fernando (1965) and following homografts by Adrè-Schwartz (1964). According to Movat and Fernando (1965) pyroninophilic blast cells appear diffusely in the cortex, migrate toward the medullary cords, and mature into plasma cells following primary stimulation. Germinai centers react later and pyroninophilic blast cells in the germinai center do not mature into plasma cells. On electron microscopy, large pyroninophilic cells were either immunoblast (rich in free ribosomes without endoplasmic reticulum) or plasmablast (with development of endoplasmic reticulum). They stated that immunoblasts developed from lymphocytes in the diffuse lymphoid tissue of the lymph node. Electron microscopic study of lymph node response after skin homograft in rabbits by Adrè-Schwartz (1964) reported the appearance of large, round and ribosome rich cells (hemocytoblasts) during the first set reaction and a striking proliferation of plasma cells during the second set reaction.

The light microscopic findings of our study indicate that the usual lymph node response is a germinal center predominant type of response and the sinus histiocytosis is not a prominent finding. The pattern of pyroninophilic cell response was similar to that of the germinal center prominent reactive hyperplasia, namely immature blastic cells in germinal center, intermediate type in diffuse cortex and mature plasma cell type in medullary cords. Pyroninophilic cell response was weak in the nonmetastatic nodes and the nodes with massive metastasis, but marked in the nodes of 40 to 70% replacement. In two cases in which large numbers of pyroninophilic cells were found within the tumor tissue, the tumors were a rather undifferentiated type of irregular spreading without solid nodular mass formation or a fibrous wall around the tumor cell mass. In all cases, pyroninophilic cells are only a small part of the cells around a tumor cell or mass, and the majority of the cells were pyronin negative small lymphocytes. Both pyronin positive and negative cells were loosely adjacent to the tumor cells and without close contact. This is probably due to fixation artifact. Richters and Kaspersky (1975) studied surface immunoglobulin positive lymphocytes in human breast cancer tissue and homolateral axillary lymph nodes. In the lymph nodes, they found that patients with nodal metastasis showed a higher percentage of IgM positive lymphocyte population in comparison to patients without nodal metastases. In the primary tumor tissue, 5 out of 10 cases showed no surface immunoglobulin
positive cells, and in the remaining cases various proportions of IgM or IgG positive lymphocytes were found. It is not clear whether the response of pyroninophilic cells or surface immunoglobulin positive cells in the regional nodes and primary tumor tissue are indication of immune response to tumor antigen or some other source of stimulation.

On the electron microscopic observations, the tumor-surrounding cells were mostly medium sized lymphocytes and only small numbers were mature plasma cells. Histiocytic or blastic cells were extremely rare. The contact between tumor cells and the surrounding lymphoid tissue was mostly very tight and smooth without spikes in large areas, and in only occasional cases the contact surface was irregular due to cytoplasmic projections of both lymphoid cells and tumor cells creating an intercellular space, but no structure suggestive of uropod (found on tissue culture) was noted.

The contact between plasma cells and tumor cells, on the other hand was loose with a wider intercellular space and irregular cytoplasmic projections. Changes in the cytoplasm of either the tumor cells or the contact lymphocytes and the plasma cells were not observed in most of the cases. However, in a few cases the cytoplasm of both the tumor cells and the lymphocytes underwent degenerative changes, but this was more marked in lymphocytes and led to shrinkage and separation from the tumor cells. This probably indicates that damage occurs on both sides when the struggle takes place between tumor cells and lymphocytes, and it may be more severe for the lymphocyte sometimes. The meaning of the constant presence of plasma cells around tumor cells is not clear, as to whether they play a part in humoral immunity against tumor antigen or are the end-result of immune response.

REFERENCES


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Fig. 1. Reactive hyperplasia with a prominent germinal center. Methyl green pyronin stain. ×100.

Fig. 2. Peripheral portion of the germinal center showing immature blast cells with pyroninophilic cytoplasm (arrow). Methyl green pyronin stain. Oil immersion ×1,000.

Fig. 3. Medullary cord form reactive hyperplastic node showing large numbers of pyroninophilic cells with characteristics of plasma cells. Methyl green pyronin stain, ×1,000.

Fig. 4. Several pyroninophilic plasma cell within pale staining undifferentiated tumor cell masses. Methyl green pyronin stain, ×1,000.

Fig. 5. Many pyroninophilic cells in fibrous septa. Methyl green pyronin stain, ×1,000.

Fig. 6. Reticulum cell sarcoma showing intense pyronin positive cytoplasm of tumor cells. Methyl green pyronin, ×1,000.
Fig. 7. Electron microphotograph of reactive hyperplasia showing various developmental stages of lymphoid cells with tight and smooth contact borders. ×8,300.

Fig. 8. Two tumor cells (TC) in the upper portion and two lymphoblast (LB) with abundant free ribosomes showing close and smooth tumor cell-lymphoblast contact in the left upper portion (arrow). Metastatic carcinoma from the stomach. ×10,700.
Fig. 9. Contact between lymphoblast (LB) and tumor cell (TC) at the left with focal cytoplasmic degeneration of tumor cell. Metastatic carcinoma from the lung. ×10,700.

Fig. 10. Contact between small lymphocyte (L) and tumor cell (TC). Two cells are in close contact without degenerative changes at right, and focal cytoplasmic degeneration at left (arrow). Metastatic carcinoma from the lung. ×10,700.
Fig. 11. A large lymphocyte (L) is in contact with a tumor cell (TC) with indented cell surface and widened intercellular space. No degenerative change in the cytoplasm of either cell is noted. Metastatic epidermoid carcinoma. ×16,000.

Fig. 12. A lymphocyte (L) in contact with tumor cells (TC) shows marked cytoplasmic degeneration and lysis. ×10,700.
Fig. 13. A case of metastatic epidermoid carcinoma shows marked cytoplasmic degeneration of both lymphocyte (L) and tumor cell (TC). The contact border is indistinct. ×16,000.

Fig. 14. A lymphocyte (L) trapped in tumor cells (TC) shows marked degenerative change and separation from surrounding tumor cells. Large amount of tonofilaments (TO) are noted within the tumor cells. Metastatic epidermoid carcinoma. ×10,700.
Fig. 15. A well developed mature plasma cell (P) is in contact with a tumor cell (TC) with wide space in between and an irregular cell surface. No degenerative change is noted. Metastatic carcinoma from the breast. ×10,700.

Fig. 16. A tumor cell (TC) is in tight contact with proplasma cell (PRB) and lymphocyte (L) with smooth surface. No degenerative change is noted in all three cells. Metastatic carcinoma from the lung. ×10,700.
Fig. 17. A case of undifferentiated tumor from the lung shows a mixture of cells; tumor cells (TC), lymphoblasts (LB), lymphocyte (L) and plasma cells (P). ×4,500.

Fig. 18. A case of reticulum cell sarcoma with intense pyroninophilia shows a large amount of polyribosomes in the cytoplasm of the tumor cells (TC). ×10,700.