"Intermediate" Cell Types and Mixed Cell Proliferation in Multiple Myeloma: Electron Microscopic Observations

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It is generally accepted that the myeloma cell represents a neoplastic plasma cell. The origin of the plasma cell and its relationships to other cells, particularly the lymphocytes, are not as yet completely understood. Lymphocytic,1-10 histiocytic or reticulum cell,2,9-14 and adventitial cell15 origin of the plasma cell have been amply defended and disputed. Excellent discussions of the subject have been written in recent years by Jordan,16 Sundberg,17 Amano,15 Rebuck and LoGrippo,8 Braunsteiner and Zucker-Franklin,10 and Bessis.13,17 Transitional or intermediate forms between the histiocytic reticulum cell,2 the lymphocyte,6,7 the perivascular reticular cell,7 and the plasma cell have been noted in light microscopy studies. Suggestive evidence of transformation from nonphagocytic lymphoid reticulum cells10,18,19 and from fixed reticulum cells14,20 to plasma cells has been found by electron microscopy studies made in hyperimmunized animals. Lymphoplasmocytoid cells have been observed in cultures of small lymphocytes obtained from human peripheral blood.21

The myeloma cell has also been thought to be derived from lymphocytic, lymphoblastic, histiocytic, and other cell precursors. Bayrd,22 in 1948, reviewed the different origins that had been postulated for the myeloma cell and the types of myeloma that had been reported. He concluded that the "so-called types of myeloma were simply variations in differentiation of the plasma cell." However, the hematologist frequently sees cases which clinically fulfill the diagnostic criteria of multiple myeloma but exhibit a bone marrow picture indistinguishable from that of lymphocytic proliferation. These cases are sometimes called "lymphoid myelomas" because of their morphologic picture. Recently Zucker-Franklin23 called attention to the morphologic "overlap" between lymphocytes and plasma cells even when studied with the electron microscope.

It is the purpose of this paper to report certain observations made in regard to the possible histogenesis of the myeloma cell in a light and electron microscope.
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Fig. 1.—Bone marrow smear. The predominant cell element (94.5 per cent in the unconcentrated preparations) is a lymphoid-appearing cell with scanty cytoplasm (Wright; ×1200).

microscopy study of 19 cases of multiple myeloma. Special consideration is given in this report to the proliferation of lymphocytic and reticulum cell elements and to the presence of seemingly "intermediate" cells between the myeloma cell and those other cell elements.

PROCEDURES AND METHODS

Bone marrow was obtained by sternal or iliac crest aspiration and, occasionally, by Vim-Silverman needle biopsy. For study with the light microscope unconcentrated, concentrated ("buffy coat"), and "touch" smears were prepared. Histologic sections (paraffin blocks) were made from the marrow units. The bone marrow smears were stained with Wright and May-Grunewald-Giemsa stains, and the sections were stained with hematoxylin-eosin. Special histochemical stains included methyl-green-pyronine stain.24

For electron microscopy fixation, a small amount of bone marrow was placed immediately after aspiration in a Petri dish containing precooled, veronal-buffered, isotonic 1 per cent osmium tetroxide at pH 7.4.25 The bone marrow fragments were then transferred to a vial containing the same solution for one hour to complete fixation. After fixation the material was dehydrated in alcohols of increasing concentrations and embedded in butylmethyl-methacrylate (4:1) and Epon 812.26 When Vim-Silverman needle biopsy was done, the core obtained was fixed in the same manner, but later decalcification with ethylene-diaminetetra-acetate was done if necessary. A Porter-Blum microtome was used to obtain ultra-thin sections, which were stained with uranyl acetate when Epon was the embedding material. Methacrylate sections were occasionally stained with lead hydroxide.27 "Thick" (1 μ) sections were made and correlation was attempted, by light microscopy, with the matching ultra-thin sections.

An RCA-EMU-3E electron microscope was used. Sections from at least 4 different blocks were studied, and an average of 150 photographs were taken from each case at initial magnifications of 1600 to 24,000.
Electron Microscopic Observations: Correlation with Light Microscopy Findings

Lymphocytic and "Lymphocytoid" Plasma Cell Proliferation

In the series of 19 cases of multiple myeloma there were 3 in which light microscopic examination of the bone marrow revealed the presence of numerous "lymphocytoid" cells, cells with a more definite "plasmocytoid" appearance, and cells with combined features. Two of 3 cases were particularly striking in this regard (Figs. 1 and 2). In these cases bone marrow sections, under light microscopy, showed significant infiltration of lymphoplasmocytic elements with very scanty cytoplasm (Figs. 3 and 4). The picture resembled that seen in lymphoproliferative disorders.

The study with the electron microscope revealed a spectrum of cells ranging from typical lymphocytes to cells with a plasmocytic appearance (Figs. 5 to 16). The endoplasmic reticulum varied in amount from lymphocytes with virtually no endoplasmic reticulum to plasmocytoid cells with a moderate amount of this organelle. In some instances a morphologic sequence could be constructed from one to the other (lymphocyte to plasma cell). A characteristic example of this cellular spectrum found in the same patient is shown in Figures 5 to 13.

In a significant number of cells in one of the cases, the endoplasmic reticulum formed rounded or "globular" cisternae. These cells also showed a tendency to form a pseudosyncytium with loss of cell boundaries. Sometimes a combination of lamellar and "globular" types was noted in the same cells.

Fixed reticuloendothelial cells were conspicuously noted in numerous instances and in different types of myeloma (Figs. 17 and 18). Often these
Fig. 3.—Same case as in Figure 1. Bone marrow paraffin section. There is a marked increase in lymphoplasmocytic elements (hematoxylin and eosin; ×1200).

Fig. 4.—Same case as in Figure 2. Vim-Silverman needle biopsy. Bone marrow (paraffin section) showing infiltration by lymphoplasmocytic cells (hematoxylin and eosin; ×600).

Reticuloendothelial cells appeared surrounded by myeloma cells in a manner suggestive of the plasmocytic island of Undritz and Sundberg (Fig. 19). Between the fixed reticulum cell and the more differentiated myeloma cells there were elements that could qualify as a second type of “intermediate” forms. These cells may be called reticular plasma cells, plasma cells with reticular features, or reticulum cells with a plasmocytic appearance (Fig. 20).
Figs. 5–10.—Same case as in Figures 1 and 3. Electron microscopy. Cell types observed in a case of “lymphoid” myeloma. Cells range from typical lymphocytes (Figs. 5 and 6) to cells with plasmocytic features (Figs. 9 and 10). Between these two extremes are some cells with intermediate characteristics (Figs. 7 and 8) (Epon; Fig. 5 ×4900, Fig. 6 ×7450, Fig. 7 ×7750, Fig. 8 ×7450, Fig. 9 ×4900, Fig. 10 ×4900).

Perivascular Plasma Cell Proliferation

Normally, plasma cells tend to grow around blood vessels. This phenomenon is particularly conspicuous in the so-called reactive plasmocytosis. Capillaries were seen surrounded by myeloma cells in the present electron microscopy study (Fig. 21). Reticulum cells and lymphoid cells were also seen in areas adjacent to capillary walls.
Fig. 11.—Same cell as in Figure 6. This cell is a typical lymphocyte with minimal endoplasmic reticulum in its cytoplasm. The nucleus (N) occupies a large area of the cell section; m = mitochondria.

Fig. 12.—Same cell as in Figure 8. This cell, like that in the previous illustration, has a relatively large nucleus (N). The scanty cytoplasm, however, shows a well-developed endoplasmic reticulum (arrows); m = mitochondria.
Fig. 13.—Same case as in Figures 1, 3, and 5 to 10. This cell corresponds to that depicted in smaller magnification in Figures 9 and 10. It has plasmocytic features as demonstrated by some endoplasmic reticulum (arrows), large numbers of mitochondriae (m) and a very prominent Golgi area (GA). The nucleus (N) has an ovoid configuration and is located toward one of the sides of the cell (Epon; x 15,500).

COMMENT

In the lympho-proliferative and plasmo-proliferative disorders, there is a spectrum of clinical entities with chronic lymphocytic leukemia and lymphosarcoma at one end and multiple myeloma at the other. Between these two extremes are cases with “intermediate” clinical and morphologic manifestations. Azar and associates and Osserman and coworkers have reported cases of lympho-proliferative disorders associated with myeloma-type serum proteins. Waldenström's macroglobulinemia has been defined in an electron microscopy study as a lymphocytic proliferative disorder; in another study, lymphocytes and “lymphoid plasma cells” were observed in this condition. Lymphomatous disorders with intracytoplasmic crystals of protein nature in lymphoplasmocytoid cells have been reported.

As noted previously, cell lineage has been demonstrated between the fixed reticuloendothelial cell or the lymphoid reticulum cell and the plasma cell. Electron microscopy studies of the fixed reticulum cell revealed an elongated or spindle-shaped cell with an ovoid or elongated nucleus, with scanty cytoplasm and with rather poorly developed endoplasmic reticulum. Bernhard and Granboulan found, in inflammatory lymph nodes, reticulum cells with
Fig. 14.—Same case as in Figures 2 and 4. Cell with scanty cytoplasm, some endoplasmic reticulum, and relatively large centrally located nucleus (N); m = mitochondria (Epon; ×9900).

Fig. 15.—Same case as in Figures 2, 4 and 14. This myeloma cell has slightly more abundant cytoplasm and endoplasmic reticulum than the cell in the previous illustration. It has more definite plasmocytic characteristics. Note the relative abundance of Golgi profiles (G) and the presence of cytoplasmic extensions indicated with arrows. N = nucleus; m = mitochondria (Epon; ×10,100).
Fig. 16.—Same case as in Figures 2, 4, 14 and 15. Although this myeloma cell has only a moderate amount of cytoplasm and may give a "lymphoid" appearance, it has a well-developed endoplasmic reticulum (arrows) and Golgi area (GA). N = nucleus; m = mitochondria; NI = nucleolus (Epon; ×16,200).

Figs. 17 and 18. Fixed reticulum cells. Note small amount of rough endoplasmic reticulum in scanty cytoplasm (Epon; Fig. 17 ×3500, Fig. 18 ×3450).
Fig. 19.—Same case as in Figures 1, 3, and 5 to 13. In center of field is elongated reticulum cell (RC) with moderately developed endoplasmic reticulum and dense bodies. This cell is surrounded by myeloma cells, some of which have scanty cytoplasm (Epon; ×7200).
Fig. 20.—Same case as in Figures 2, 4, 14 and 15. "Reticular" type of myeloma cell or reticulum cell with "plasmocytic tendency." Note moderately developed endoplasmic reticulum and Golgi apparatus (GA); m = mitochondria (Epon; ×20,100).

more developed endoplasmic reticulum, resembling plasma cells. These authors referred to the cells with more definite plasmocytic features as "reticular plasma cells" or "reticulum cells with plasmocytic tendencies." The "stimulated" reticulum cell and the reticular plasma cell seem to be similar, particularly in regard to the amount of "rough" endoplasmic reticulum.
The observations previously discussed favor the existence of a close relationship between the plasma cell and the reticulum cell and lymphocyte.

The findings in the present study further suggest a close association of those cell lines and give support to the idea of a mixed reticulolymphoplasmocytic proliferation in certain cases of multiple myeloma. Dameshek and Gunz54 have observed this mixed proliferation both in myeloma and in Waldenström’s macroglobulinemia. In regard to myeloma they state that “some cases seem to be mixtures of proliferating reticulum and plasma cells with apparent transitional forms being quite prominent.”

Whether the phenomenon of mixed cell proliferation is the result of the independent activity of the different cell lines or the consequence of a process of cell differentiation from reticuloendothelial cells and lymphocytes to plasma cells cannot be definitely stated. The morphologic evidence, however, is suggestive of a process of cell transformation.

The present study also demonstrated the difficulty faced by the morphologist in classifying certain cells as lymphocytes or plasma cells even by electron microscopy. This problem is also experienced with reticulum cells. Since there are no established criteria as to the amount of endoplasmic reticulum needed to call a cell a plasma (or myeloma) cell, terms like “lymphoid” and “reticular” plasma cell should continue to be used in reference to cells with combined or intermediate features.

The plasmocytic island of Undritz25 and Sundberg4 is an anatomic structure formed by the aggregation of plasma cells around a histiocyte in a manner analogous to the erythroblastic island of Bessis.35 The plasmocytic island

**Fig. 21.—Capillary surrounded by myeloma cells (Epon; ×5000).**
has been suggested to have functional significance.\textsuperscript{7,14} It is thought that the reticulohistiocytic cell which has phagocytosed the antigenic elements would operate as a “feeding cell” to the surrounding plasma cells supplying them with the substances (or the “information”?) needed for the production of antibodies.\textsuperscript{7,14,86} Therefore, the histiocyte of the plasmocytic island would operate in the same manner as the histiocyte of the erythroblastic island which supplies the iron to the erythroblasts. In the present study plasma-myeloma cell islands were observed. Their significance, whether anatomic (from the standpoint of histogenesis) or functional, is open to speculation.

**SUMMARY**

Bone marrow studies of multiple myeloma revealed, in some cases, a conspicuous proliferation of “lymphoid” cells, virtually indistinguishable by light microscopy from those seen in lympho-proliferative disorders.

Electron microscopy demonstrated a variety of cells ranging from typical lymphocytes to cells with plasmocytoid features. Between these two types of elements there were cells with intermediate characteristics.

In addition, in several cases of myeloma the presence of fixed reticuloendothelial cells and “reticular” plasma cells (or reticulum cells with plasmocytoid features) was frequently noted.

The presence of reticulum cells and lymphocytes and of cells apparently “intermediate” between these cellular elements and plasma cells, as judged from electron microscopic observations, is suggestive morphologic evidence of a phenomenon of cell transformation and evidence of a mixed cell proliferation in certain cases of multiple myeloma.

**SUMMARIO IN INTERLINGUA**

Studios del medulla ossee ab patientes con myeloma multiple ha revelate in certe casos un conspicue proliferation de cellulas “lymfoide” que es practicalmente indistinguisibile per microscopia optic ab illos vidite in disordines lympho-proliferative.

Microscopia electronic demonstrava un varietate de cellulas ab lymphocytos typic usque ad cellulas con characteristicas plasmocytoid. Inter iste duo typos de elementos, altere cellulas con characteristicas intermedie esseva observate.

In plus, le presentia de fixe cellulas reticuloendothelial e de plasmocytos “reticular” (o de cellulas reticular con characteristicas plasmocytic) esseva frequentemente notate in plure specimen ab subjectos myelomatic.

Le presentia de cellulas reticular e de lymphocytos e de cellulas apparentemente “intermedie” inter ille elementos cellular e plasmocytos stipulabile a base de observationes per microscopia electronic pote esser prendite como evidentia morphologic pro un phenomeno de transformation cellular e como evidentia de un proliferation de cellulas mixte in certe casos de myeloma multiple.

**ACKNOWLEDGMENT**

We are indebted to Dr. Gertrude L. Pease for her invaluable help.
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REFERENCES


21. Bartfeld, H., and Juliar, J. F.: "Immunological organisation" and activity of human peripheral white-blood-
32. Bessis, M., Breton-Gorius, J., and Binet, J. L.: Etude comparee du plasmocyto
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