Phagocytized Plasma Cells in Mouse Spleen
Observed by Light and Electron Microscopy

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An intense plasmacytopoiesis occurs in lymphatic tissues following antigenic stimulation, particularly in a secondary response. A less intense plasma cellular reaction is observed following a single antigen administration. The plasma cells evoked in response to antigen injection have been shown to contain antibody, or gamma globulin, or have been correlated with antibody production by histologic techniques.

Although the structure, origin, and function of plasma cells have been the subjects of numerous studies, relatively little attention has been given to the fate of plasmacytes. Maximow and Jordan believed that they simply degenerated, although these observations were not correlated with the disappearance of plasma cells in an immune response. Fagraeus noted that the number of immature plasma cells observed in the spleen 3 to 4 days after stimulation exceeded those of mature plasmacytes found 7 to 10 days later. Likewise, a loss or disappearance of plasma cells from their sites of proliferation and differentiation has been reported by other investigators.

As indicated by Nossal, plasma cells must either die or undergo a change of morphology and migrate away from their tissue sites of proliferation since these cells are not normally present in blood or lymph in significant numbers to account for their migration as recognizable plasma cells. Recently, however, Hall and Morris reported the presence of considerable numbers of plasma cells in efferent lymph draining from stimulated popliteal lymph nodes in sheep.

De differentiation of some immature plasma cells was suggested by Fagraeus to explain their decrease in number. Pyknotic plasma cells were noted by Ehrich et al. as evidence of cell death and disintegration. Wissler et al. suggested that the majority of antibody-forming cells (presumably immature plasma cells) transformed into small lymphocytes in the rat spleen although a few became typical mature plasma cells. Using the electron microscope, Thiéry described cytoplasmic fragmentation and loss. In another report, the suggestion was made that small lymphocytes might result from cytoplasmic shedding. In a preliminary report of another electron microscopic study, phagocytized plasma cells have been reported in reticular macrophages of mouse spleen germinal centers.

To study the disposition of plasma cells evoked during an immune response, light and electron microscopic observations were made on mouse...
Spleen fixed at daily intervals following a single intravenous injection of either sheep erythrocytes or rat bone marrow. Consistent evidence was obtained showing that many plasma cells were phagocytized during the log phase of antibody production when the antibody titer in the circulation increases exponentially.

**Materials and Methods**

Twelve-week-old male (101/Cum  2 × C3H/Anf Cum  2 ) F1 mice, known as (101 X C3H) F1 mice, were injected intravenously with 1 ml of Tyrode's solution containing 10^8 rat bone marrow cells or 1 ml of Tyrode's containing 2 × 10^9 sheep erythrocytes. Five groups of 10 animals each were injected in different experiments, and spleens were removed at daily intervals following stimulation for routine histologic and electron microscopic study. One-half of each spleen was fixed in Zenker-formol for paraffin embedding and hematoxylin and eosin staining. The remaining half was cut into small pieces and fixed approximately 1 hour in ice-cold 2 per cent osmium tetroxide buffered at pH 7.3–7.4 with 0.2 M veronal acetate and 4.5 per cent sucrose (w/v) added.

Osmium-fixed tissue was embedded in Epon, and thin sections were cut with glass knives on a Porter-Blum ultramicrotome. Sections were picked up on 300-mesh uncoated copper grids and stained in uranyl acetate prior to examination in a Siemens Elmiskop I electron microscope.

**Results**

Histologic changes in the mouse spleen following the intravenous injection of sheep erythrocytes or rat bone marrow are essentially similar. Quantitatively, sheep erythrocytes provoke a more intense plasma cellular reaction. On days 2 and 3, many immature plasma cells are located in the red pulp, particularly in the areas adjacent to white pulp, and in the marginal zone of white pulp. On subsequent days, plasma cells tend to localize in groups, sometimes associated with blood vessels and frequently along trabeculae. Microscopically, the number of plasma cells decreases so that by day 10, relatively few mature plasmacytes are observed in random sections of spleen.

The presence of pyknotic plasma cells in the mouse spleen 6 days after a single intravenous injection of sheep erythrocytes is clearly seen in figures 1 and 2 with conventional methods of light microscopy. That these pyknotic cells represent phagocytized plasma cells in many, if not all cases, can be seen in electron micrographs (figs. 3–6). Phagocytized plasma cells are found in both red (figs. 1, 3, 4) and white pulp (figs. 2, 5 and 6), particularly in the latter case in the marginal zone bordering the red pulp.

Phagocytized plasma cells were found as early as 3 days (fig. 3) after a primary antigen injection and on each successive day thereafter to day 7. The frequency with which phagocytized plasmacytes were observed in thin sections increased from day 3 to day 6. Observations on day 10 did not reveal recognizable phagocytized plasma cells. On day 10, a few mature plasma cells, some with very distended cavities of the ergastoplasm, and plasma cells with Russell bodies, Mott's cells, were observed. Thus, under the conditions of these experiments, the major phagocytic activity involving plasma cell removal in the spleen occurred between days 3 and 7.
Fig. 1.—A photomicrograph of splenic red pulp 6 days after sheep erythrocyte injection. In this plane of focus, six pyknotic plasma cells (arrows) can be seen. Several typical plasma cells are observed in the section.

Fig. 2.—A photomicrograph of the marginal zone of white pulp bordering red pulp 6 days after sheep erythrocyte injection. Two pyknotic plasma cells adjacent to macrophage nuclei are shown by arrows. Note the nuclear appearance of the cell to the right and compare with figure 5.
PHAGOCYTIZED PLASMA CELLS IN MOUSE SPLEEN

No pyknotic plasma cells were observed with the electron microscope that were not phagocytized. The appearance of the ingested cell in figure 4 indicates that nuclear lysis (figs. 5, 6) may occur after the cell has been phagocytized. Initial nuclear disintegration following phagocytosis has been reported for engulfed chicken red blood cells21 and phagocytized ascites tumor cells.24 The arrangement of nuclear chromatin in a crescent shape (fig. 5) is frequently observed (see also fig. 2). A compact, shrunken cytoplasm with swollen mitochondria (figs. 3-6) and nuclear lysis are indicative of cellular disintegration.

DISCUSSION

Although the results presented support the concept that highly differentiated plasmacytes represent terminal cells,18 it should be emphasized that no data are available from this electron microscope study for alternative explanations given in the literature, e.g., plasma cell migration,6,14 transformation,4 or dedifferentiation9 to account for their disappearance. It is unlikely that one could, by morphological study alone, document dedifferentiation or transformation into other cell types. High resolution microscopy, however, can amply document the fact that phagocytosis of plasma cells does occur in the spleen.

Unfortunately, electron microscopic studies are difficult to evaluate in terms of the order of magnitude of plasma cell removal by phagocytosis. Also, as cellular breakdown occurs, the recognition of a plasma cell by established criteria of fine structure, i.e., extensive lamellar ergastoplasm filling the entire cytoplasm, Golgi apparatus, and eccentric nuclear position, is extremely difficult. Therefore, the number of recognizable phagocytized plasma cells observed at any one time may not reflect the true magnitude of this process.

A good correlation exists between the log phase of antibody appearance in the circulation,5,18 which occurs between days 3 and 6, and phagocytosis of plasma cells in the experiments reported in this paper. The earliest observed phagocytosis was on day 3 and the most extensive removal in this manner was on day 6. Jerne et al.25 employing an agar plaque technic, reported a 90 per cent decrease in the number of antibody-producing cells in the mouse spleen between 4 and 7 days after a single injection of sheep erythrocytes. The fate of these cells was not determined. Schooley,26 in an autoradiographic study, suggested a plasma cell mean-life of 8–12 hours after the last division in the stimulated lymph node. However, no conclusion could be drawn from his study as to the fate of plasma cells for, as in the present study, migration of cells played an unassessed role.

It would be interesting to know whether the phagocytized plasma cells described in this report contain antibody. The detailed mechanism of antibody release by plasma cells is unknown. Phagocytosis of apparently intact plasma cells may suggest a breakdown by macrophages as a step in the process of antibody degradation as considered by Askonas and Hum-
Fig. 3.—An electron micrograph of a plasmacytic islet. The macrophage (Mac) nucleus (N) with two phagocytized plasma cells (Pc) and other dense inclusions can be seen surrounded by plasma cells (P1, P7). Picture represents spleen 3 days after injection of sheep erythrocytes.

Another possibility is the removal of plasma cells that had already released their specific antibody. An additional explanation could be that only defective, nonfunctional plasma cells are being removed from the population of rapidly proliferating cells. The last explanation does not seem likely in view of the considerable num-
Fig. 4.—An electron micrograph of a splenic macrophage in red pulp 4 days after injection of sheep erythrocytes. A phagocytized plasma cell (Pc), dense inclusions, and a vacuole (V) of unknown origin are present in the macrophage (Mac). The macrophage nucleus (N), a plasma cell (P), an erythrocyte (ery), and portion of an erythroblast (eb) are indicated.

bers of pyknotic, phagocytized plasma cells observed, an observation that parallels their disappearance from the spleen in these experiments.

Plasma cell death, as reported in this study, is conceivably related to the necrosis and atrophy of mouse lymph nodes and spleen white pulp following X-irradiation and foreign bone marrow transplantation as discussed by
An electron micrograph of a plasma phagocytized by a reticular macrophage (Mac) associated with a bundle of reticular fibers (R) is shown. The nucleolus (No), swollen mitochondria (M), and remnants of the Golgi apparatus (G) of the ingested plasma cell are indicated. The macrophage nucleus (N) and two lymphocytes (Ly) are also labeled.

Congdon and Goodman, in their experiments, cessation of antibody-forming cell proliferation is followed by necrosis of these cells. Phagocytosis of the dying cells has not been studied.

A plasmacytic islet consisting of plasma cells surrounding a macrophage first described by Undritz, is shown (fig. 3) with two phagocytized plasma
Fig. 6.—An electron micrograph showing a phagocytized plasma cell (Pc) with swollen mitochondria (M) and remnants of endoplasmic reticulum (er) in an advanced stage of digestion. Dense bodies are seen in the macrophage (Mac) cytoplasm and four lymphocytes (Ly) surround the macrophage. Reticular macrophage 7 days after rat bone marrow injection.

cells in the macrophage cytoplasm. That these islets have some physiological significance has been suspected because of their similarity to erythroblastic islets, described by Bessis and Breton-Gorius, in which there is presumably a transfer of ferritin molecules from macrophage to erythroblast. There was no evidence in this study of digestive breakdown of macrophage cytoplasm
as reported by Journey and Amos for histiocytes which had phagocytized acites tumor cells. Neither do any of the apparently intact phagocytized plasma cells appear viable in contrast to the ingested lymphoma cells described by Shelton and Dalton.

The presence of elongated profiles of rough endoplasmic reticulum in splenic macrophage cytoplasm indicating a high rate of protein synthesis by these cells is of unknown significance in the immune response. Dense bodies surrounded by a membrane (figs. 3, 4, 6) are numerous in the macrophage cytoplasm. A possible link to lysosomes (see review by Novikoff) is indicated in view of the high acid phosphatase activity of splenic macrophages. Pleomorphic inclusions may also represent a fragmentation of intact cellular material, as suggested by Shulz and Karrer in alveolar lung macrophages, and in erythrophagocytes by Essner.

The fate of macrophages which have ingested plasma cells is also of interest. In this connection, Roberts et al. reported peritoneal exudate cells obtained from previously sensitized donors caused a typical secondary response when transferred to X-irradiated recipients and stimulated with specific antigen. It was suggested that macrophages gave rise to classical plasma cells. That phagocytosis of plasma cells might govern the fate of a macrophage is speculative. Speirs has advanced the hypothesis that reticuloendothelial cells phagocytize eosinophils and transform into antibody-producing cells. It should be emphasized that, as stated by Wissler et al. and Speirs, more than one cell type may be involved in an immune response, and the precise relations of the various cells involved are far from clear.

**Summary**

A correlative light and electron microscopic study is reported of pyknotic, phagocytized plasma cells in the mouse spleen following a single intravenous injection of sheep erythrocytes or rat bone marrow. Phagocytized plasma cells were observed in both red and white pulp between 3 and 7 days after antigen injection. A few mature plasma cells and plasma cells with Russel bodies were found 10 days after stimulation with no evidence of recognizable phagocytized plasma cells. Phagocytosis clearly plays some role in the removal of plasma cells from the spleen under the conditions employed in this study, although the possibility that significant numbers of plasmacytes migrate out of the spleen is considered. A correlation of phagocytized plasma cells with the log phase of antibody appearance in the circulation is indicated.

**Summario in Interlingua**

Es reportate un comparative studio, a microscopia optic e electronic, de pyknotic phagocytatisate plasmocytos in le splen murin post solitari injectiones intravenose de erythrocytos ovin o de medulla ossee de ratto. Phagocytatisate plasmocytos eseva observate in pulpa rubie e blanc inter 3 e 7 dies post le injection del antigeno. Un certe numero de matur plasmocytos e de plasmocytos con corporis de Russel eseva trovate 10 dies post le stimulation, sin recognoscibile evidentia de phagocytatisate plasmocytos. Phagocytosis ha clarmente un rolo in le elimination de plasmocytos ab le splen sub le conditiones...
que existeva in le presente studio, sed le possibilitate que numeros significative de plasmocytos migra ex le splen es etiam prendite in consideration. Un correlation de phagocytisate plasmocytos con le phase logarithmic del apparition de anticorps in le circulation es indicate.

**ADDENDUM**

A paper (Blinzinger, K., and Hager, H.: Electronenmikroskopische Beobachtungen an phagozytierten nekrotischen Infiltratzellen bei Spätstadien der experimentellen Coli-Meningitis. Verb. Deutsch. Ges. Pathol. 47, 331, 1963) has come to the author's attention. Alterations of phagocytized plasma cells in late stages of experimental *E. coli* meningitis are reported which are identical to changes observed in the present study.

**REFERENCES**

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