ELECTRON MICROSCOPE OBSERVATIONS ON TINGIBLE BODY MACROPHAGES IN MOUSE SPLEEN

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It is generally accepted that the tingible bodies (Körper) of Flemming (7) found in lymphatic tissue germinal centers represent phagocytized nuclear debris of small lymphocytes (1, 2, 6, 7, 11, and others) or both lymphocyte and erythrocyte debris (15).

Several ideas about the significance of tingible bodies assume that they are small lymphocytes. The "graveyard theory" emphasizes the phagocytosis of pyknotic small lymphocytes (6, 9, 11). This theory was extended by Hamilton (8), Trowell (17), and Sundberg (16) who postulated reutilization of small lymphocytes in lymphocytopoiesis. Andrew (1) stressed the role of degeneration and phagocytosis of small lymphocytes in germinal centers as indicating that these centers are not germinal but reactive, as proposed by Hellman (10). Recently, Ortega and Mellors (13) suggested that intrinsic germinal center cells degenerated after secreting gamma globulin and were phagocytized by tingible body macrophages.

The identification of tingible bodies as small lymphocytes rests on indirect evidence since stained nuclear debris viewed in the light micro-

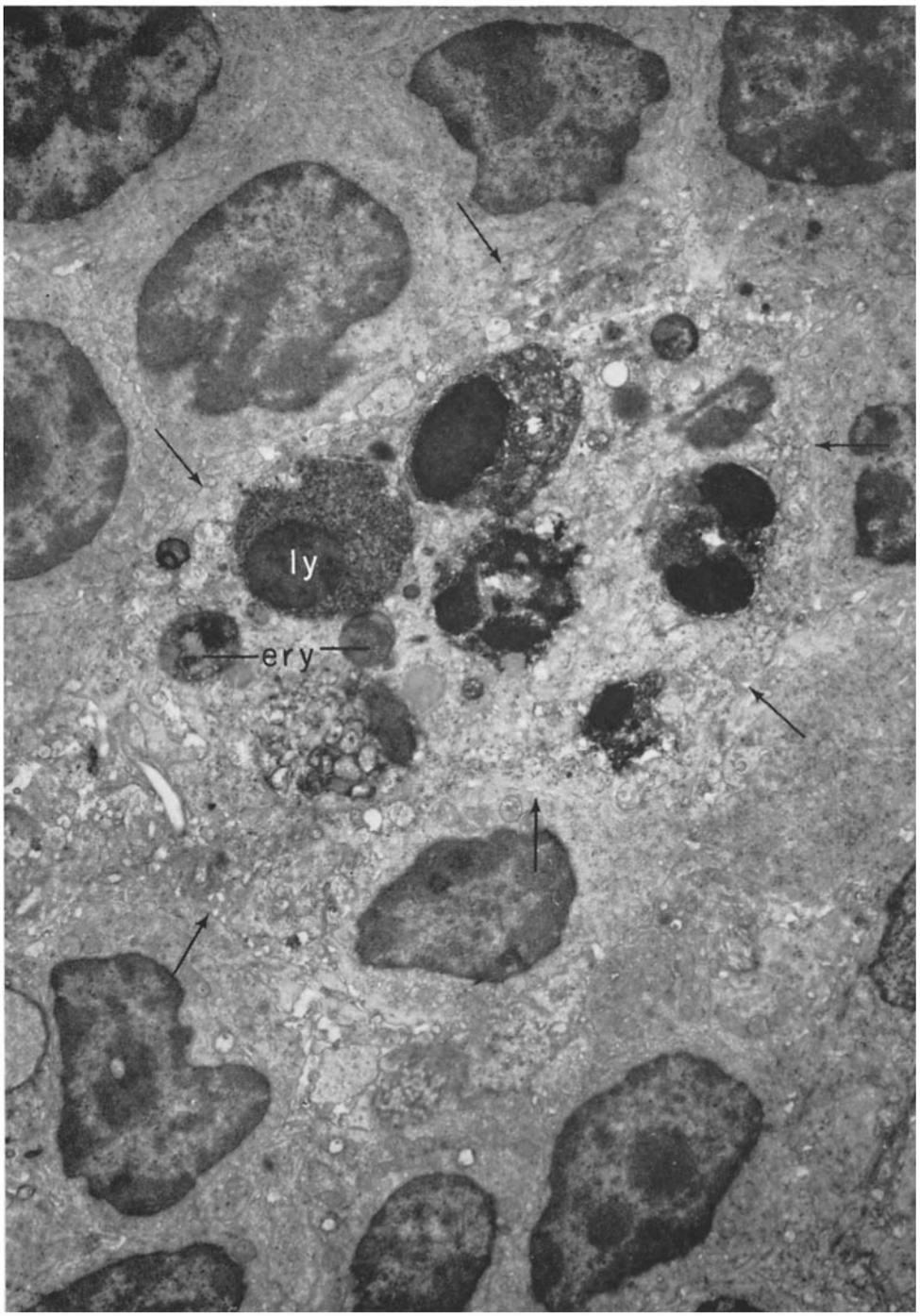


FIGURE 1 A low power electron micrograph showing a tingible body macrophage surrounded by large cells from germinal center 5 days after injection of rat bone marrow. Six phagocytized cells and other debris are visible. Note the eccentric position of the nucleus in two of the ingested cells. The plane of section does not include the macrophage nucleus. Arrows indicate the cytoplasmic boundary of the macrophage. Lymphocyte (*ly*), erythrocyte debris (*ery*). × 5000.

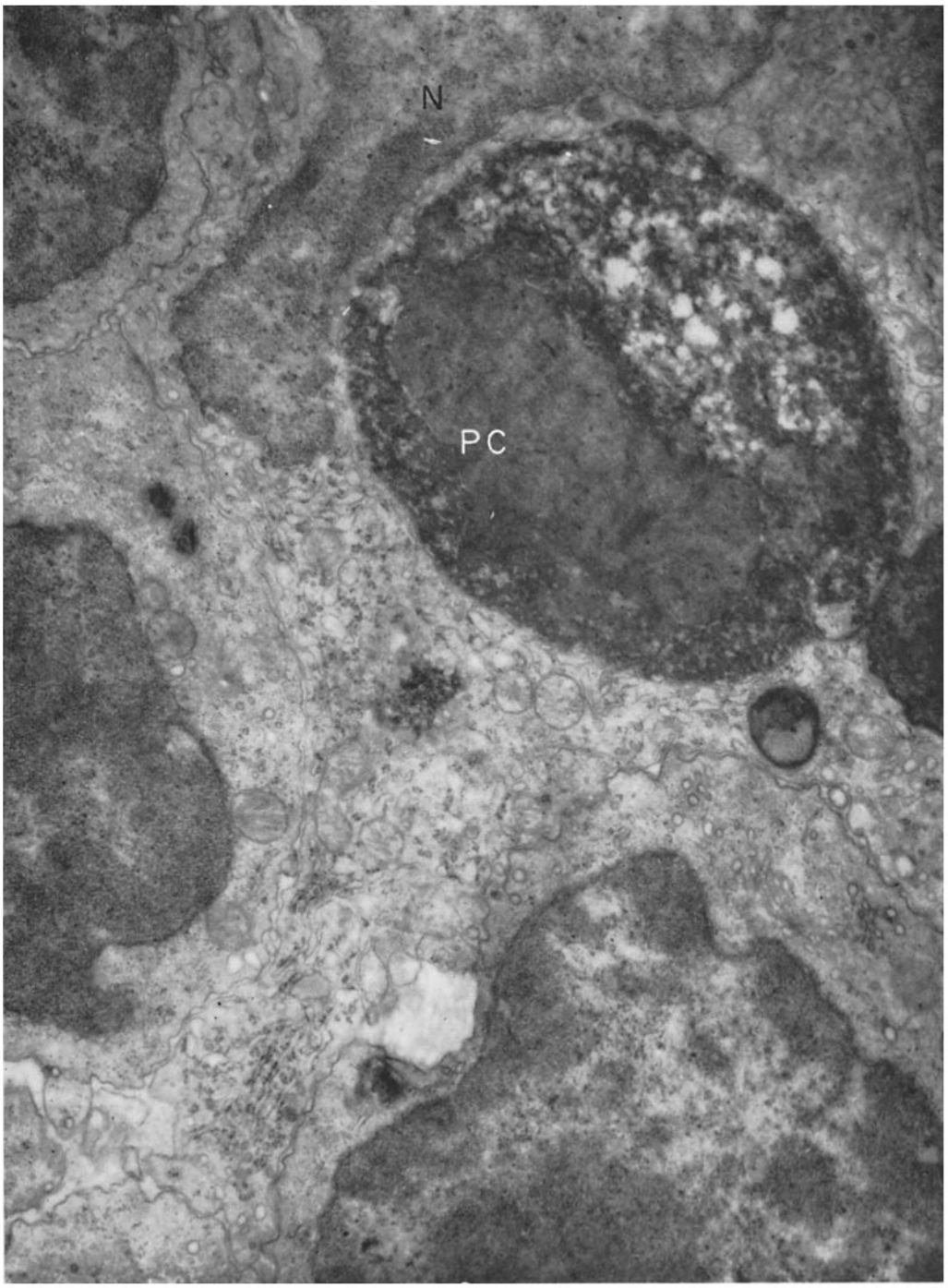


FIGURE 2 Electron micrograph showing a portion of the nucleus (*N*) of a macrophage containing a phagocytized cell (*PC*) with plasma cell characteristics. Note adjacent cells to left and below. × 17,000.

scope does not enable precise morphological characterization of cell types. Electron microscope observations of tingible bodies found in hyperplastic germinal centers of lymphatic tissue following antigenic stimulation have shown many inclusions possessing morphological criteria of plasma cells, *i.e.*, eccentrically placed nuclei and extensive profiles of rough endoplasmic reticulum (ER). The nature of these inclusions viewed in the electron microscope is the subject of this report.

The histological changes in mouse splenic white pulp following intravenous injection of rat bone marrow have been described by Congdon (3) and are the bases for the present electron microscope observations. The experimental plan is identical to that used by Congdon.

MATERIALS AND METHODS

Twelve-week-old male (101 × C3H)F_1 mice were injected intravenously with 100 × 10^6 nucleated rat bone marrow cells as antigen. Spleens were removed from mice killed under ether anesthesia at daily intervals for 7 days following antigen injection. Small bits of tissue were fixed in ice-cold 2 per cent osmium tetroxide buffered at pH 7.5 with 0.2 M acetate veronal. Sucrose was added to the buffered fixative to make a 4.5 per cent (w/v) solution. Tissue was dehydrated and embedded in Epon by the method of Luft (12). Sections were cut on a Porter-Blum ultramicrotome using glass knives and were picked up on Formvar-covered copper grids, that had been lightly stabilized with carbon, and stained with uranyl acetate. The sections were examined in a Siemens Elmiskop I at 40 kv.

Another group of mice was injected intravenously with 1 ml of 10 per cent washed sheep RBC as antigen (5) and the tissue was prepared as described above. Tissue from control, unstimulated mice was also fixed for electron microscopy. Mice of the same strain, age, and sex have been used in all experiments reported.

The term, tingible body macrophage, as used in this report, refers only to those macrophages found in the dark-staining portion of a germinal center (4). This does not imply a physiological or morphological difference between macrophages found in germinal centers and elsewhere in lymphatic tissue, although such differences may exist.

RESULTS

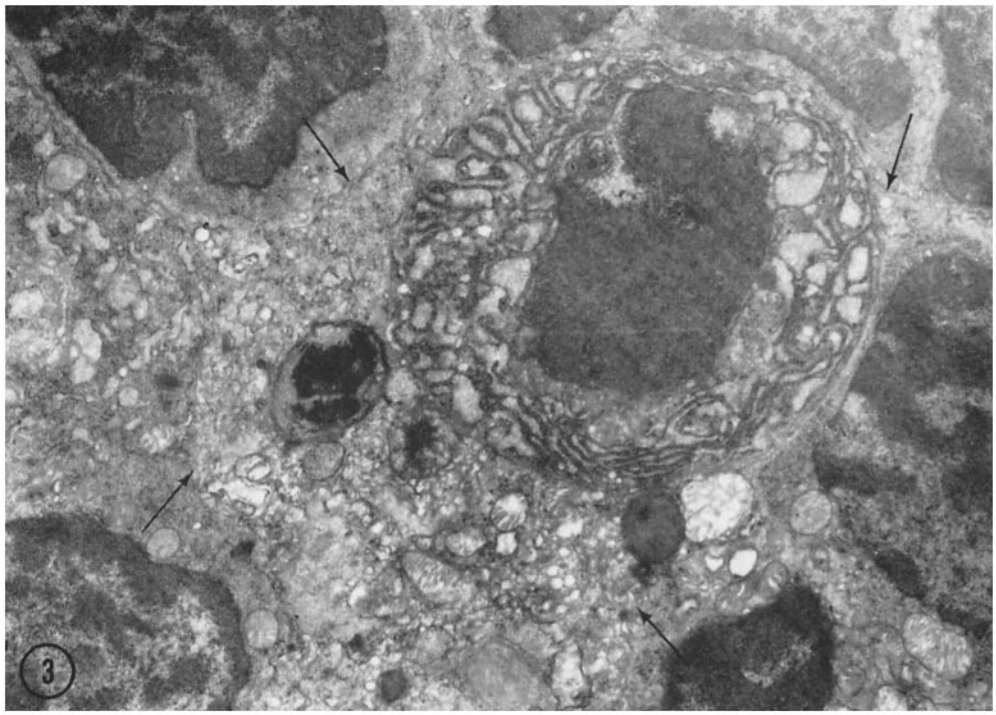
Tingible body macrophages range in size from 20 to 30 μ or larger and contain a variable number of inclusions (Fig. 1). The nucleus is 12 to 15 μ in diameter, and usually contains two dense, nucleolar-like areas and peripheral dispersed chromatin. Mitochondria, profiles of both smooth and rough ER, and a well developed Golgi region are present in the macrophage cytoplasm.

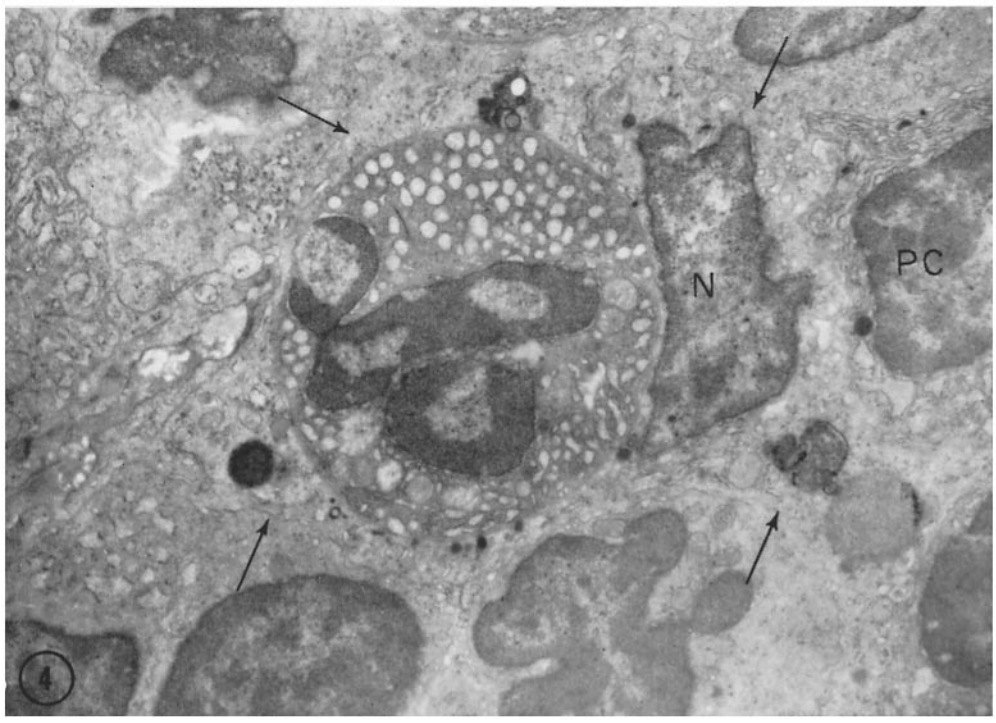
The inclusions represent not only nuclear but also cytoplasmic debris in varying stages of lysis (Figs. 1 to 4). Two phagocytized cells clearly possessing plasmocytic characteristics, shown in Figs. 3 and 4, are in the early stages of breakdown. Macrophages shown in Figs. 1, 2, and 4 are from regenerating germinal centers 5 days after injection of rat bone marrow. At this time numerous plasma cells are present in the white pulp. Various stages of degenerating phagocytized plasma cells have been found in this material, and as many as five plasmocytes have been seen within a single macrophage. The possibility that phagocytized plasmocytes are injected donor cells is invalidated by the observation that sheep erythrocytes used as antigen give a similar result (Fig. 3).

These observations do not exclude the possibility that other cell types form the debris constituting tingible bodies. Lymphocyte debris and phagocytized erythrocytes (Fig. 1) are tentatively identified in macrophages from germinal centers. Congdon and Goodman (4) also report granulocytes in tingible body macrophages in the first few hours following antigenic stimulation.

FIGURE 3 Electron micrograph of macrophage showing very early stage of plasma cell breakdown. Other inclusions are in cytoplasm of macrophage adjacent to plasma cell. Portions of nuclei of seven adjacent cells are surrounding the macrophage. Arrows indicate the cytoplasmic boundary of the macrophage. × 11,000.

FIGURE 4 An electron micrograph showing a plasma cell in a later stage of disintegration than seen in the Fig. 3. Compare nuclear appearance with that of the plasma cell in Fig. 3. Portion of macrophage nucleus (*N*) is visible as well as other dense inclusions in the cytoplasm. Note normal plasma cell (*PC*) in contact with macrophage. Arrows point to cytoplasmic border of the macrophage. × 8000.




N
PC

DISCUSSION

The functional significance of plasma cell phagocytosis by tingible body macrophages is unknown. Removal of plasma cells in this manner might account for both their disappearance from the tissue sites of proliferation following antigenic challenge and the failure to find significant numbers in circulating blood. In addition, phagocytosis of plasma cells could merely represent the removal of dying or defective cells from the population of cells proliferating during an immune response. Since quantitative data are not available, the importance of this type of plasma cell removal must be inferred.

In the absence of selective phagocytosis of one cell type by tingible body macrophages, an argument might be raised against the reutilization scheme proposed by Hamilton (8) and Trowell (17). In our opinion, phagocytosis of plasmocytes reflects plasma cell proliferation rather than lymphocyte production from the population of cells comprising germinal centers after antigenic stimulation. This is in agreement with the suggestion by Ringertz and Adamson (14) and Congdon and Goodman (4) that the centers form either antibody-producing plasma cells or lymphocytes, depending upon an antigenic stimulus. This idea is also consistent with the functional proposal by Ortega and Mellors (13) that depleted germinal center cells are phagocytized following protein synthetic activity.

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