

The Polymorphonuclear Neutrophilic Phagocyte

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Patients who have too few functioning mature polymorphonuclear neutrophils frequently develop fatal bacterial or fungal infections despite our best efforts to prevent and treat those infections. Recently new facets of white cell function which enable us to better understand both normal and abnormal states have been found. Several review articles about polymorphonuclear neutrophils (also called neutrophils and granulocytes—the latter term includes eosinophils and basophils) have been published recently¹⁻⁷ and the reader is referred to these for more comprehensive coverage of the field.

Development and Deployment of Neutrophils.

Cells that have the potential to develop into the polymorphonuclear neutrophil series or into the monocyte-macrophage series are present in bone marrow. Those destined to be neutrophils go through a sequence of maturation steps lasting about two weeks. The early developing cells have a very rigid cell surface, are poorly motile, and cannot effectively engulf foreign particles. As they develop, granulocytes become progressively more flexible, more able to ingest foreign particles, and more efficient at phagocytosis and killing microbes.

Mature polymorphonuclear neutrophils are constantly released from the marrow into the circulation. Their half-life in the blood is about six to eight hours. After leaving the blood, polymorphonuclear neutrophils migrate to the tissues where they may live for several days before being destroyed. Neutrophils are

destroyed by fixed mononuclear phagocytes in such organs as the liver, spleen, lungs, and bone marrow, and others are excreted in the fecal stream. Hundreds of millions of granulocytes enter the bloodstream from the marrow and leave the bloodstream for the tissues each day, but only a small percentage of the body's total number of granulocytes circulates in the blood at any one time. This circulating pool contains between 3% to 5% of the total granulocyte population. The vast majority of mature neutrophils are extravascular in tissues, or waiting to be released from the bone marrow. It is this latter pool that is the source of most of the increased numbers of circulating white cells in patients with acute bacterial infection. There appear to be separate regulators of neutrophil proliferation, maturation, and release into the circulation.

Morphology and Motility.

The mature neutrophil is about 12 to 15 microns in diameter and contains a multilobed nucleus. The characteristic of the cell responsible for the name "granulocyte" is the presence of multiple granules containing enzymes and preenzymes in the cytoplasm. Other organelles are less conspicuous and very little in the way of ribosomal structure or mitochondria can be seen. There is a large amount of cytoplasmic glycogen which can be utilized as an energy source. Both microtubules and microfilaments can be seen in the cell in special preparations.

Neutrophils are actively motile when in contact with a solid surface: they crawl rather than swim. Motile polymorphonuclear neutrophils look very different from cells seen on a stained blood smear. They usually have a broad front with a thin edge of cytoplasm (the lamellipodia) followed by a slowly ta-

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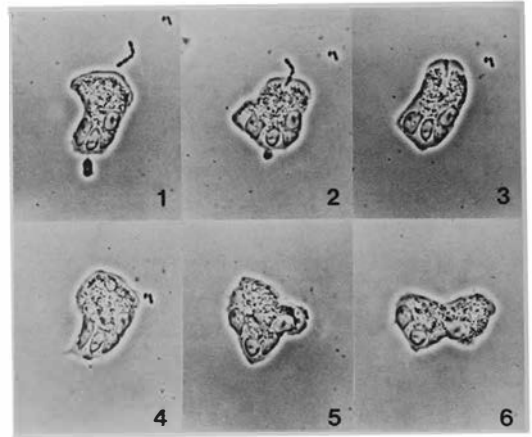
pering, roughly triangular-shaped body and a knob-like tail. Granules are not seen in the leading edge, but granules do move in the cytoplasm of the cell where they seem to travel in roughly-defined channels. There is evidence to support the concept that microtubules and microfilaments are responsible for cell movement.

Phagocytosis.

After mature neutrophils leave the bone marrow and enter the circulation, their mission is to leave the vascular system and migrate to areas where they are needed such as an area of infection or injury. In the first step in this process, called margination, neutrophils circulating through capillaries near the site of injury become sticky and adhere to endothelial cells lining small blood vessels. Anti-inflammatory drugs such as aspirin and prednisone inhibit granulocyte adherence. The phagocytes leave the blood vessels by crawling through the junction between endothelial cells. The neutrophils are then directed towards the site of microbial invasion by chemotaxis, a process in which factors from the region of microbial invasion attract neutrophils towards the greatest concentration of these factors.

After neutrophils have migrated to tissue invaded by microbes, they attempt to ingest susceptible microorganisms and destroy them. The first step is recognition of the microorganisms as foreign to the phagocyte and a target for phagocytosis. With some organisms high titers of specific antibody are required for this recognition while in others the so-called "natural antibody" or low level of antibody usually present in normal serum is enough to stimulate phagocytosis. The antibody itself may promote phagocytosis, but usually the combination of antibody plus complement factors is optimum for promoting phagocytosis. Only immunoglobulins of the IgG class are opsonic.

When a neutrophil engulfs a microbe, the leading edge of the lamellipodia makes initial contact (Figure) and pseudopods are sent out, cupping the microorganism. This cup closes by means of membrane fusion which results in the microorganism being enclosed in a membrane-bound space. This space, called the phagosome, is bounded by the external cell membrane which becomes internalized in the process. The energy for phagocytosis and locomotion is derived largely through anaerobic glycolysis, and cells are able to move and engulf organisms in an anaerobic atmosphere.



Figure—A series of phase contrast photomicrographs of a human polymorphonuclear neutrophil ingesting two clumps of *Streptococcus pyogenes* (original magnification, $\times 1200$). Photomicrograph by James Sullivan and Gerald Mandell.

Leukocyte Bactericidal Activity.

During and after phagocytosis, granules in the cytoplasm move towards phagosomes which contain ingested particles. The contents of the granules are extruded into the phagosome as the granule membrane fuses with the phagosome membrane. The phagosome, now containing lysosomal and other enzymes from the granules, is called the phagolysosome. Degranulation enables these potent enzymes to contact ingested microbes in the phagosome without exposing the cytoplasm of the neutrophil to the possible deleterious effects of the enzymes. Granules "disappear" from the cell during this reaction, hence the term degranulation.

Along with the events described above, changes occur in the metabolism of the neutrophil, the most dramatic of which is a marked stimulation of oxygen consumption. The products of this oxygen consumption are oxidized forms of oxygen such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), both of which possess antibacterial activities. The antibacterial activity of hydrogen peroxide is markedly enhanced by the presence of myeloperoxidase and a halide. In the phagolysosome containing the ingested microbe one finds myeloperoxidase, hydrogen peroxide, and a halide, which in concert can kill the microorganisms. Bacterial death may be the result of a reaction which culminates in oxidation or halogenation.

nation, or both, or vital structures on the bacterial surface.

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