



Changes in the cytoplasm of neutrophil granulocytes

MQZH 2016-2

Distribution of neutrophil granulocytes

Apart from mature neutrophils circulating in the blood, the body keeps two reserves ready with an approximate 10-fold cell quantity in the bone marrow and adhered to the vessel walls in larger venules (marginal pool). These cells can be quickly mobilized when needed.

If the cells in the reserve units has been depleted and there is a continued need for more cells, more immature forms, such as unsegmented or individual precursor cells, are also increasingly released from the bone marrow into the peripheral blood. This is then referred to as a „reactive left shift“ in granulopoiesis.

NETs

(neutrophil extracellular traps)

Researchers from the Max Planck Institute in Berlin were able to observe this process in neutrophils for the first time in 2004. The process is also referred to as NETosis and leads to cell death within three hours.

Step 1

First the structure of the cell changes, the nuclear envelope disintegrates, the nuclear chromatin decondenses, and the granules dissolve.

Step 2

Then the nucleic acids of DNA in the cell nucleus mix with anti-bacterial enzymes from the granules within the cell.

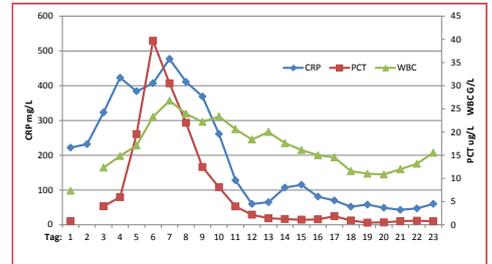
Step 3

Lastly, the cell contracts again until the cell membrane ruptures and the highly active mixture is released in form of a net. This becomes a deadly trap for microorganisms.

Introduction

Neutrophils play a central role in the innate, non-specific immune defense. If the neutrophils are activated under the influence of cytokines (e.g., GCSF), changes in cell morphology occur, which are also described as „toxic signs.“ The importance of neutrophils in immune defense becomes apparent in patients with severe neutropenia (absolute neutrophil count <0.5 g/L). Within days and weeks, they develop potentially life-threatening infections.

Our current proficiency testing survey specimen 2016-2 H3B is from a patient with sepsis. CRP (411, reference <5 mg/L), PCT (22, reference <0.1 µg/L), and the white blood cell count (24, reference 3.0-9.6 g/L) were also measured. The course shows that all three analyzes significantly increase with sepsis. After successful antibiotic therapy, PCT decreases the fastest.

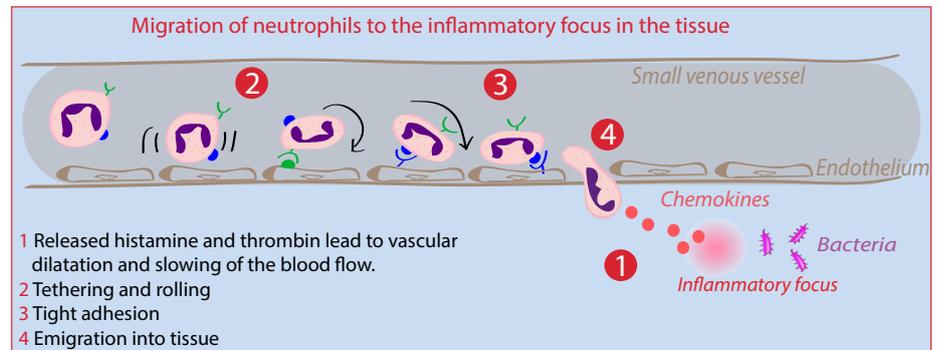


Development and breakdown of neutrophil granulocytes

Neutrophil granulocytes develop from multipotent myeloid stem cells, to myeloblast-promyelocyte-myelocyte-metamyelocyte-unsegmented neutrophils and to mature segmented neutrophils. Mature neutrophils remain in the peripheral blood for approximately 6-12 hours. Physiologically they leave the bloodstream, die after 2-4 days (apoptosis = programmed cell death) and are degraded, mainly in the spleen and liver, by macrophages.

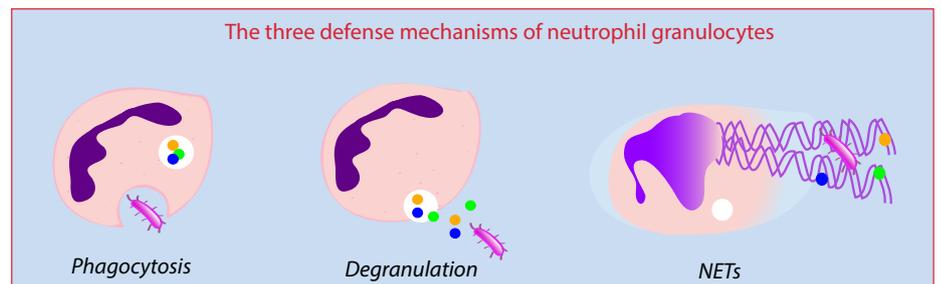
Function of the neutrophil granulocytes

Attracted by chemokines and by changes of blood vessels and blood flow velocities, neutrophils attach to the vascular endothelium. Finally, they migrate through endothelial spaces into the surrounding tissue in order to arrive at the site of inflammation.



Neutrophils employ three different mechanisms to eliminate pathogens:

- **Phagocytosis:** Engulfment of the pathogen into the cytoplasm - killing with substances inside the granules in the phagolysosome.
- **Degranulation:** Release of the granule content into the surrounding area. Killing of the pathogen outside of the cell.
- **NETs:** Disintegration of the nuclear envelope and granules mixing of the contents in the cell rupture of the cell membrane and release of a DNA net that is fatal for the microorganism-cell death





Spotlight on hematology

Toxic signs

Toxic signs in neutrophils appear with severe infections, after trauma, and extensive burns. Therapeutic administration of cytokines (e.g., in the context of chemotherapy or for treatment of neutropenia) also results in similar changes.

The simultaneous occurrence of toxic vacuoles, neutrophilia, and left shift (increased unsegmented, or other myelopoietic precursors) are indicative of sepsis with 95% sensitivity.

Alder-Reilly anomaly

It can appear in connection with congenital mucopolysaccharidoses (lysosomal storage diseases). Already in infancy these can lead to skeletal deformities and severe organ damage and therefore frequently to death.

Chédiak-Higashi anomaly

Congenital disease with albinism, hepatosplenomegaly (jaundice), neurological disorders and recurrent, purulent infections of the skin and respiratory tract.

May-Hegglin anomaly

The May-Hegglin anomaly is a very rare congenital point mutation in the MYH9 gene. It results in thrombocytopenia and thrombocytopathy and bright basophilic inclusions in all leukocytes.

About

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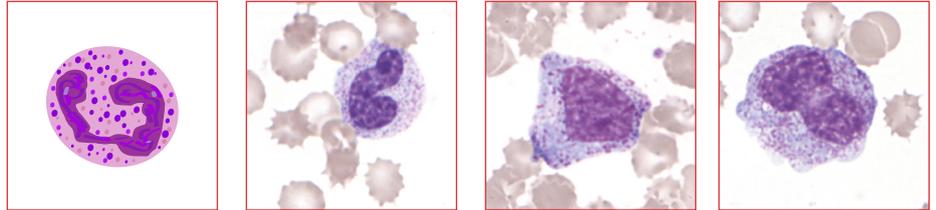
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Morphological changes in the cytoplasm of neutrophils

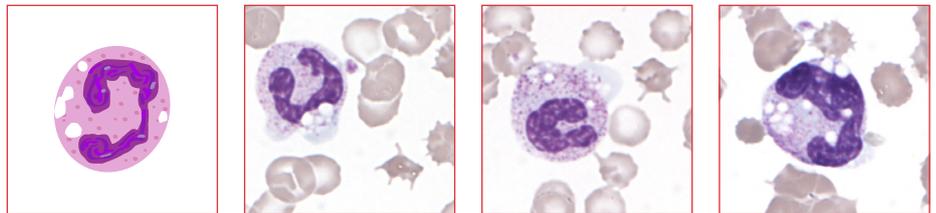
Toxic granulation

An increased cytokine-mediated stimulation of myelopoiesis leads to an increased production of lysosomal enzymes. Morphologically this leads to enlarged, dark basophilic (rarely also azurophilic) granules, which correspond to the primary (azurophilic) granules of myeloid precursors.



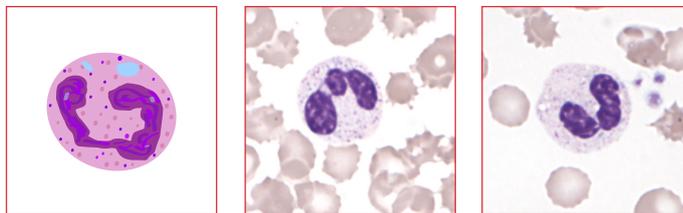
Vacuolization

Vacuoles are sharply delimited „holes“ in the cytoplasm of cells. They form during phagocytosis and digestion of pathogens in the cytoplasm of neutrophils. Toxicity-induced vacuoles are large and can be confluent, therefore threatening the cell membrane to rupture.



Basophil streaking «Döhle bodies»

These are small oval or oblong basophilic sites in the cytoplasm. They have in part distinct, more frequently irregular margins. Basophilic streaks are remnants of the rough endoplasmic reticulum, which normally disappears during cell maturation. When the cell maturation is shorter due to increased stimulation of myelopoiesis they can still be present in the cytoplasm.



Differentiation: Toxic signs versus artifacts and congenital disorders

	Toxic signs	Artifacts and inherited disorders
Rough Granulation	Dark basophilic to azurophilic, different sizes, irregular distribution. Only in neutrophils. Eosinophilic granulation normal.	Staining artifact staining time too long time or pH (buffer) too low Alder-Reilly anomaly Azurophilic granules of uniform size, regular distribution. Occurrence in all leukocytes possible (rarely in monocytes). Eosinophilic granulation more basophilic. Granules can be surrounded by a bright „halo.“ No vacuoles or basophilic spots. Chédiak-Higashi anomaly Huge, often round, reddish, blue or green-gray stained granules. Possible in the cytoplasm of all leukocytes and other body cells. Anemia, neutropenia and thrombocytopenia. No vacuoles or basophilic spots.
Vacuoles	Large vacuoles, >2 vacuoles per cell, possibly confluent	Cellular degeneration through storage in EDTA > 2 hours; smaller vacuoles and < 2 per cell
Basophilic streaking («Döhle bodies»)	Oval or oblong basophilic spots. Only in neutrophils. Tc and other Lc normal.	May-Hegglin anomaly The inclusions appear in all leukocytes, they are larger and more strongly stained. Thrombocytopenia with giant forms. No vacuoles or toxic granulation.