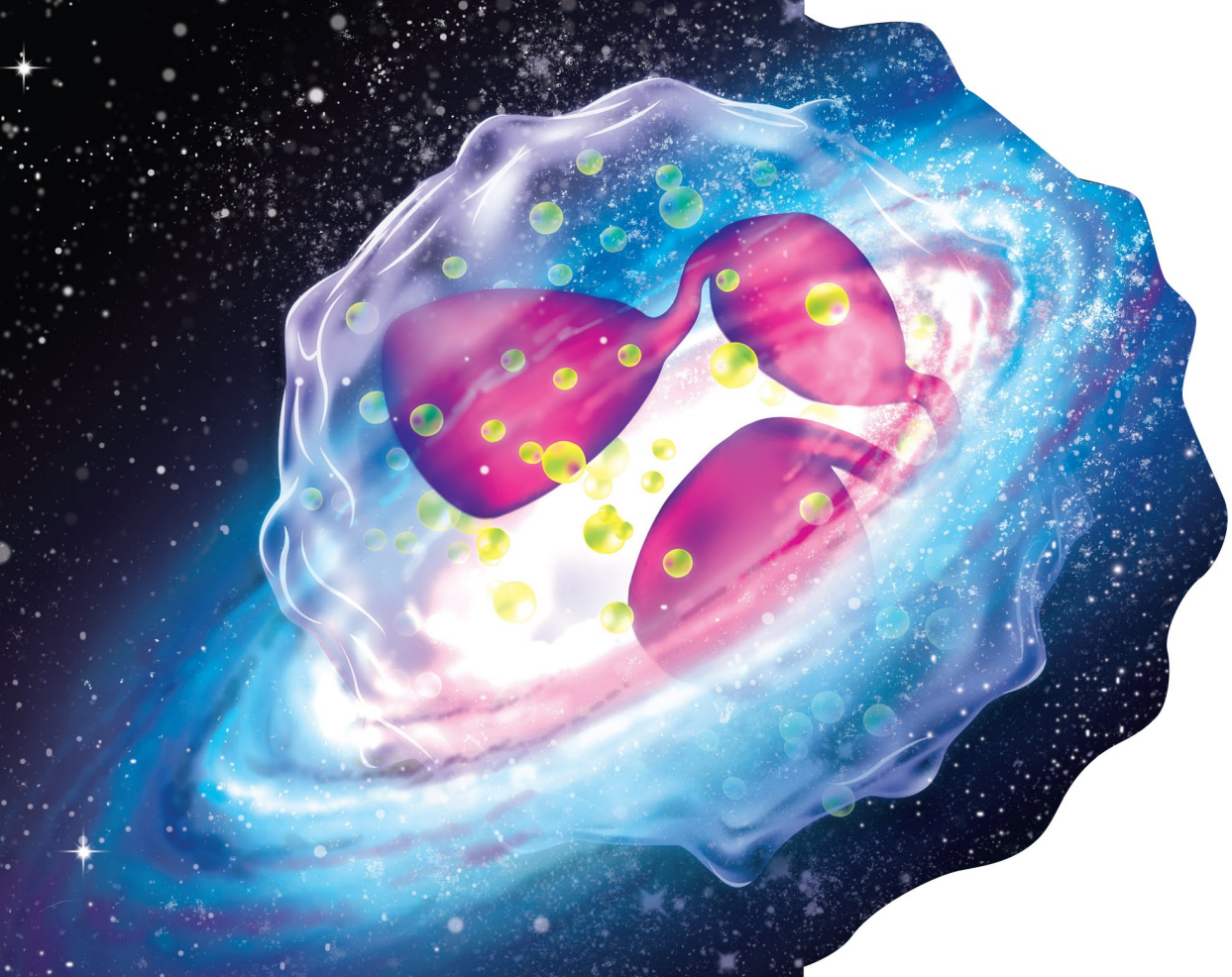
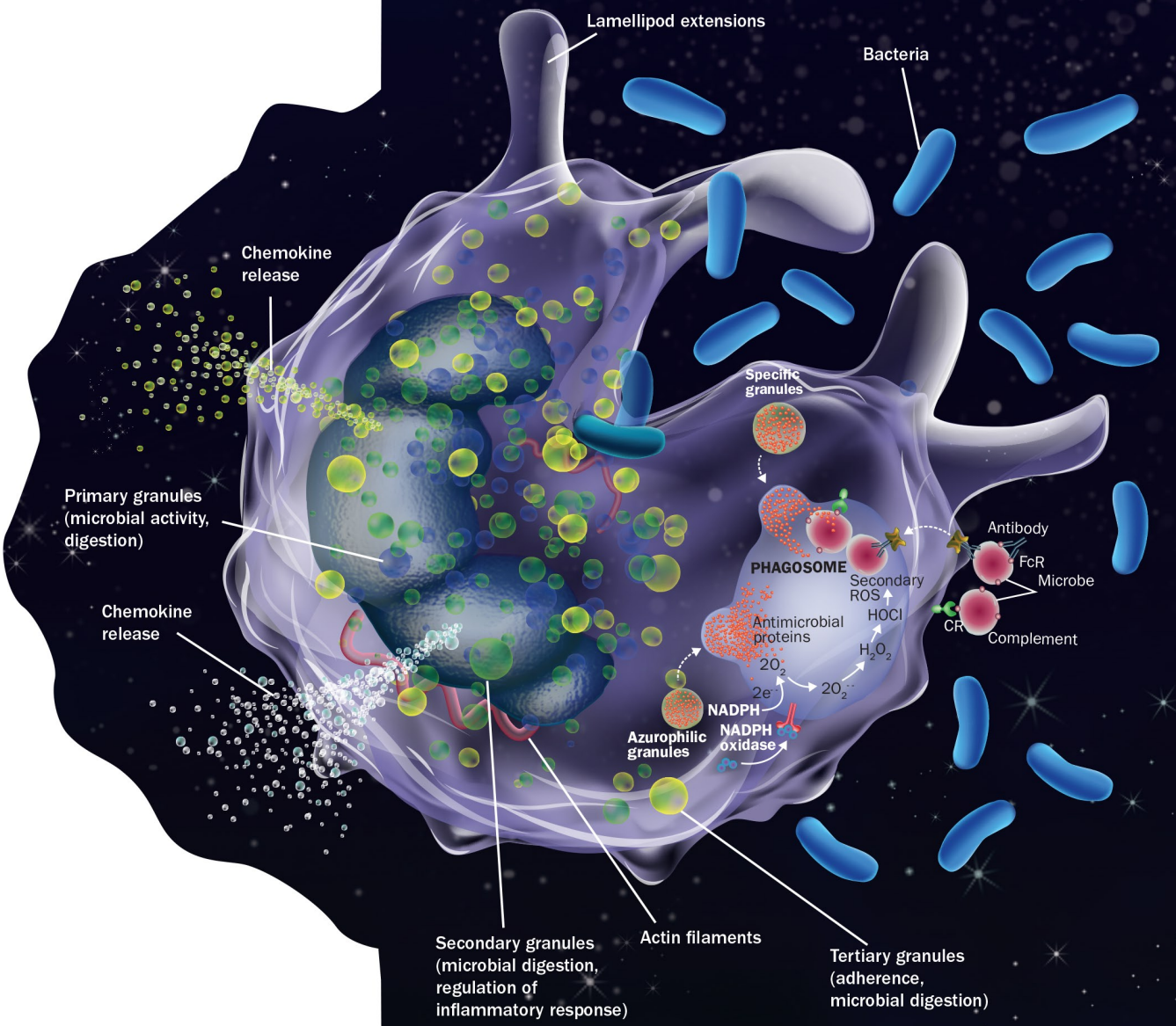
THE POWER OF THE NEUTROPHIL



Despite the discovery of neutrophils at the dawn of immunological sciences, their function was shrouded in mystery due to their short life span and experimentally evasive nature.[1] In circulation, neutrophils live for only 6-8 hours and their turnover is astronomically high: Humans produce over 100 billion new neutrophils every day – that is as many neutrophils as there are stars in the Milky Way.[1-3]

The neutrophil's weapons arsenal

The neutrophil's weapons arsenal consists of toxic substances, which must be transported safely through the bloodstream and then focused on a target at the appropriate time.[4] To accomplish such feat without harming the host, nature developed a specialty storage organelle in neutrophils: the granule.[1] Neutrophil granules are formed sequentially during maturation from the promyelocyte stage.[5] There are three different types of granules, and they are filled with pro-inflammatory and antimicrobial proteins.[6] These are azurophilic (primary) granules, which contain myeloperoxidase, specific (secondary) granules, which contain lactoferrin, and gelatinase (tertiary) granules, which contain matrix metalloproteinase 9 (also known as gelatinase B).[6, 7]

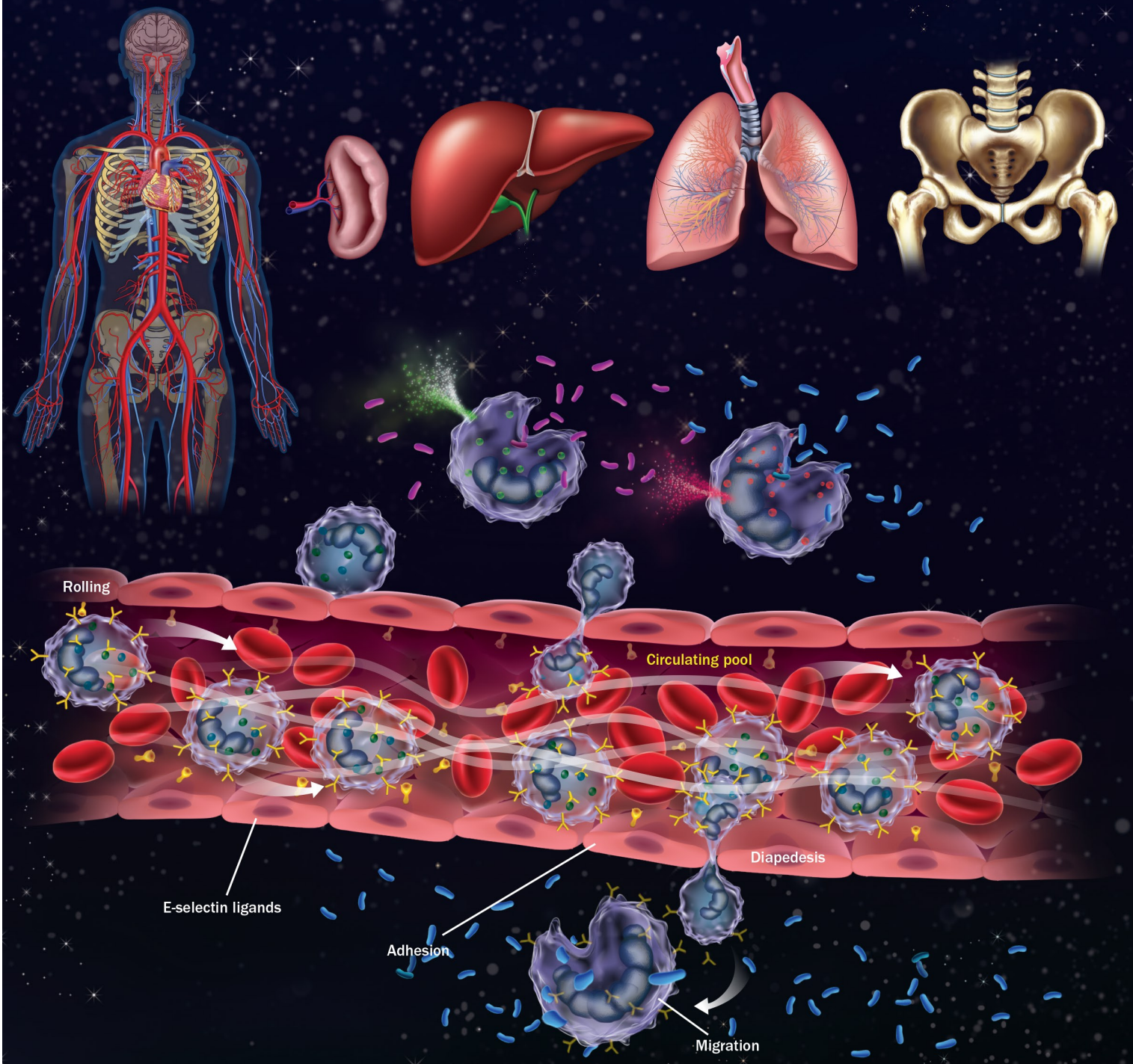


Azurophilic (peroxidase-positive) and specific (peroxidase-negative) granules can be further subdivided. In humans, azurophilic granules can be differentiated into defensin-hi and defensin-low. In addition, human azurophilic granules contain another unique protease, azurocidin. Specific granules can be divided into at least four subtypes: lactoferrin-hi, cysteine-rich secretory protein 3 (CRISP3)-hi, gelatinase-hi and ficolin 1-hi.[8,9] The existence of multiple types of granules can be explained by the fact that some of the proteins cannot exist together in the innate form: for example, neutrophil elastase digests neutrophil gelatinase-associated lipocalin.[7]

Upon activation of a neutrophil, granules are mobilized and fuse with either the plasma membrane of the neutrophil itself or the phagosome, thereby releasing antimicrobial toxins either outside the neutrophil or against a pathogen engulfed in the phagosome.[9, 10]

The neutrophil is the first line of defense

Neutrophils are the most abundant white blood cells in the human circulation and represent the first line of cellular defense against invading pathogens. Neutrophils are first cells to be recruited to a site of infection and inflammation where they deploy powerful mechanisms to eliminate pathogens.[9] Neutrophils form an essential part of the innate immune system against bacterial and fungal pathogens, and also participate in the development of the inflammatory reaction.[11] The innate immune system, also known as non-specific immune system and first line of defense, is a subsystem of the overall immune system that comprises the cells and mechanisms that defend the host from infection in a non-specific manner. Unlike the adaptive immune system (which is found only in vertebrates), the innate immune system does not confer long-lasting or protective immunity to the host.[12, 13]

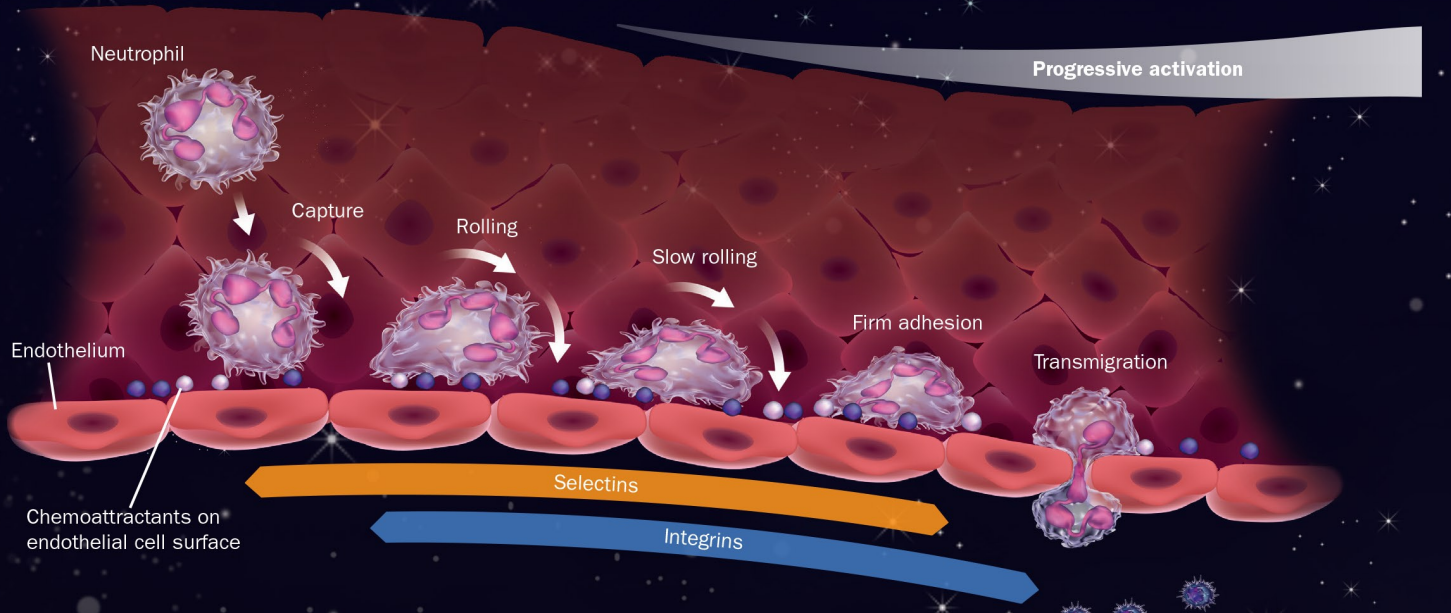


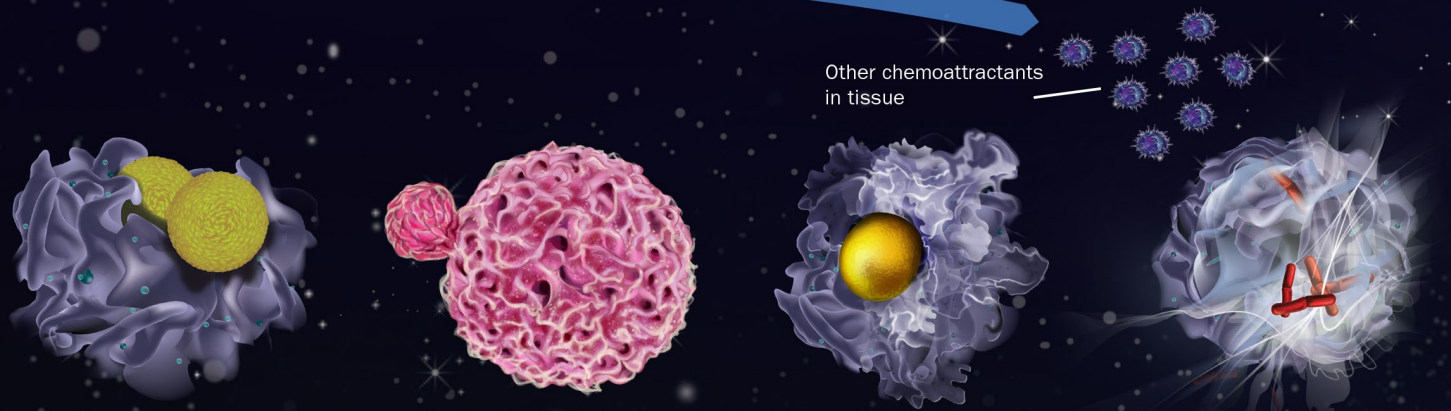
The neutrophil in and outside of circulation

The release of neutrophils from the bone marrow is tightly regulated: Chemokines control the passage of neutrophils into circulation (circulating pool), but also maintain a pool of cells ready to be summoned in case of emergency (marginal pool). Under physiological conditions, a marginal pool of mature, segmented neutrophils exists in the bone marrow, spleen, liver and lung. However, how and why neutrophils are concentrated within these organs remains poorly understood. It is hypothesized that these organs hold neutrophil reserves, which can be rapidly deployed to sites of inflammation or infection, a process also known as demargination.[9, 14] Since neutrophils take up to 14 days to develop in the bone marrow and demise rapidly, the marginal pool may be an evolutionary shortcut to increase neutrophil levels rapidly independent of new production.[9, 14]

The neutrophil detects inflammation and infection

At inflammatory sites, bacterial-derived and host-produced inflammatory signals are abundant and, once released into circulation, neutrophils continuously and randomly probe the vessel wall to detect inflamed or "activated" endothelial cells that show the signs of underlying infection.[15] Detection of an inflammatory site triggers a cascade of events eventually resulting in the migration of neutrophils through the vessel wall into the underlying tissue.[16] Detection of an inflammatory site occurs via surface bound glycoprotein P-selectin glycoprotein ligand-1 (PSGL-1) and L-selectin.[17, 18] In the beginning the neutrophil only loosely adheres to the vessel wall, which is followed by a characteristic rolling of the neutrophil along the endothelium wall.[18] After selectin-mediated rolling, neutrophils enter a state of firm adhesion in which they continue to crawl along the vessel wall, a process dependent of β2 integrins and ICAMs, until a preferred site of migration is reached. Then the neutrophil negotiates a path through the cells of the vessel wall into the underlying tissue.[19, 20] Inside the tissue, the neutrophil moves along a gradient of chemokines, such as host-produced interleukin-8 (IL-8 or CXCL8), and also pathogen derived chemoattractants, such as bacterial N-formyl-methionyl-leucyl-phenylalanine (fMLP), to the site of infection in a process known as chemotaxis.[1, 21] IL-8, also known as *neutrophil chemotactic factor*, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection.[22] IL-8 also induces neutrophils to start phagocytosis once they have arrived.[23] In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca^{2+}, exocytosis (e.g. histamine release), and the respiratory burst.[23] Neutrophils are similarly activated by fMLP through the formyl-peptide-receptor-1 (FPR1).[1]





Methicillin-resistant Staphylococcus aureus (MRSA) bacteria (yellow) being ingested by a neutrophil. Credit: NIAID

Neutrophil engulfing and destroying *Mycobacterium tuberculosis*

Neutrophil white blood cell engulfing and destroying a hypha (yellow) from a thrush (*Candida albicans*) fungus

NETs killing *Mycobacterium tuberculosis* (red).

Killing strategies of neutrophils

Phagocytosis

Phagocytosis is the major mechanism to remove pathogens and cell debris. It is an active, receptor-mediated process during which a particle is internalized by the neutrophil into a vacuole called the phagosome.[6, 23, 24] After engulfment, the phagosome acquires its lethal properties through the generation of reactive oxygen species via a NADPH oxygenase-dependent mechanisms, a process called the respiratory burst, or antibacterial proteins (cathepsins, defensins, lactoferrin and lysozyme) through the fusion of granules with the phagosome membrane.[1, 9, 24]

Respiratory burst

The respiratory burst involves the activation of the enzyme NADPH oxidase, which produces large quantities of reactive oxygen species.[1, 25]

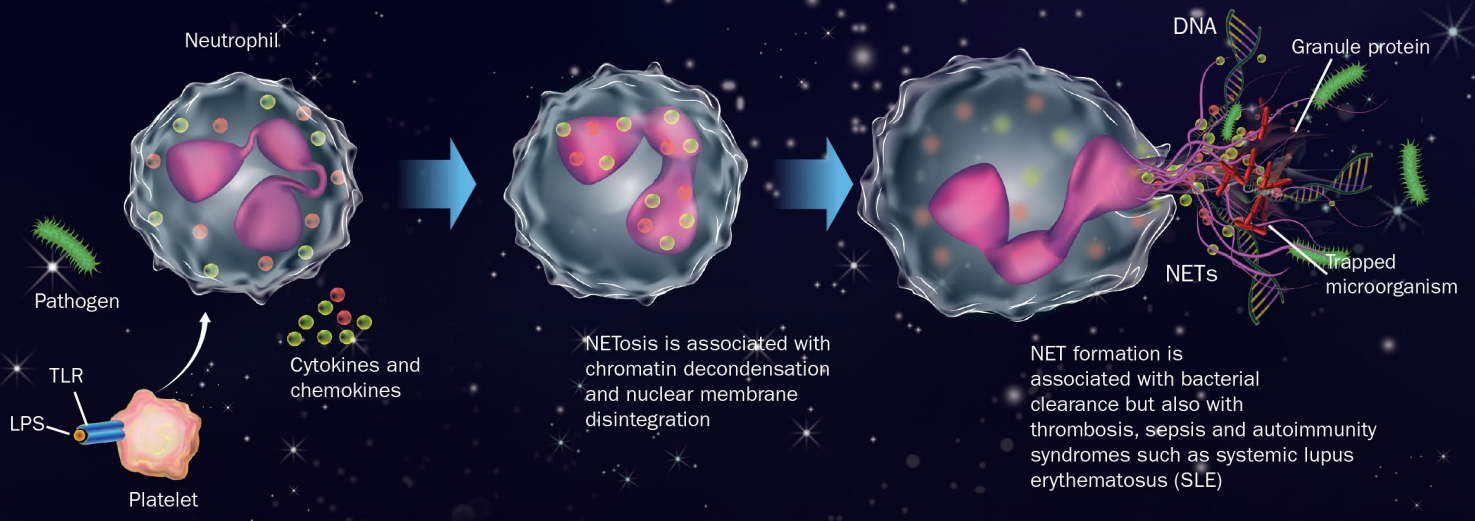
The main products of the neutrophil respiratory burst are strong oxidizing agents including hydrogen peroxide, free oxygen radicals and hypochlorite.[25] Superoxide decays spontaneously or is broken down via enzymes known as superoxide dismutases (Cu/ZnSOD and MnSOD), to hydrogen peroxide, which is then converted to hypochlorous acid HClO, by the green heme enzyme myeloperoxidase. It is thought that the bactericidal properties of HClO are enough to kill bacteria phagocytosed by the neutrophil, but this may also be a step necessary for the activation of proteases.[25]

Degranulation

In a process known as degranulation, the antibacterial proteins are released from the neutrophil granules either into the phagosome or into the extracellular milieu, resulting in killing and digestion of either intra- or extracellular microorganisms.[9]

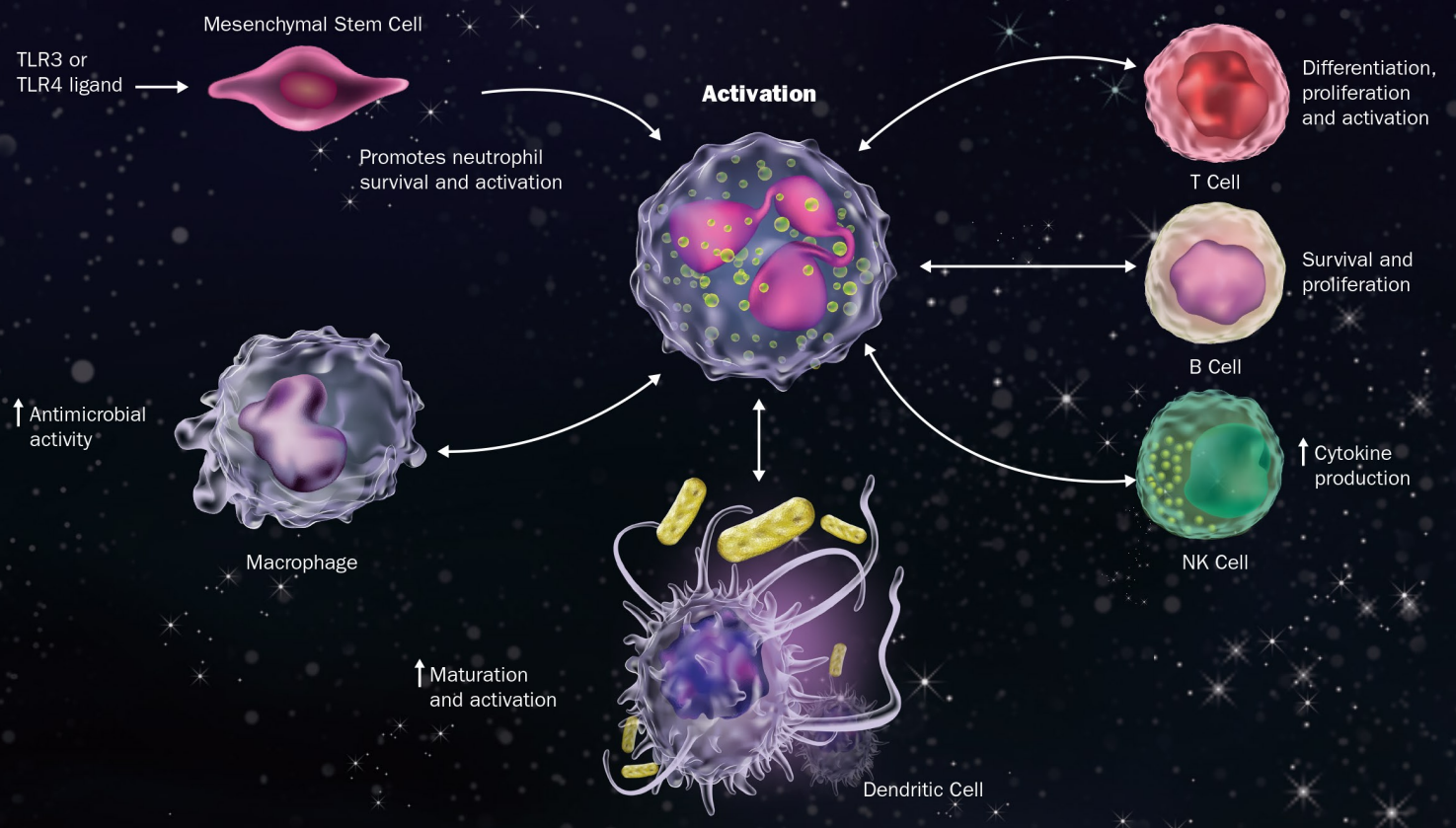
Neutrophil extracellular traps (NETs)

In 2004, Brinkmann et al described a striking observation that activation of neutrophils causes the release of web-like structures of DNA; this represents a third mechanism for killing bacteria.[27] These neutrophil extracellular traps (NETs) comprise a web of fibers composed of chromatin and serine proteases that trap and kill microbes extracellularly.[27, 28, 30] It is suggested that NETs provide a high local concentration of antimicrobial components and bind, disarm, and kill microbes independent of phagocytic uptake. In addition to their possible antimicrobial properties, NETs may serve as a physical barrier that prevents further spread of pathogens. Trapping of bacteria may be a particularly important role for NETs in sepsis, where NETs are formed within blood vessels.[26] NETs immobilize pathogens, thus preventing them from spreading but also facilitating subsequent phagocytosis of trapped microorganisms. They are also thought to directly kill pathogens by means of antimicrobial histones and proteases.[28, 29]

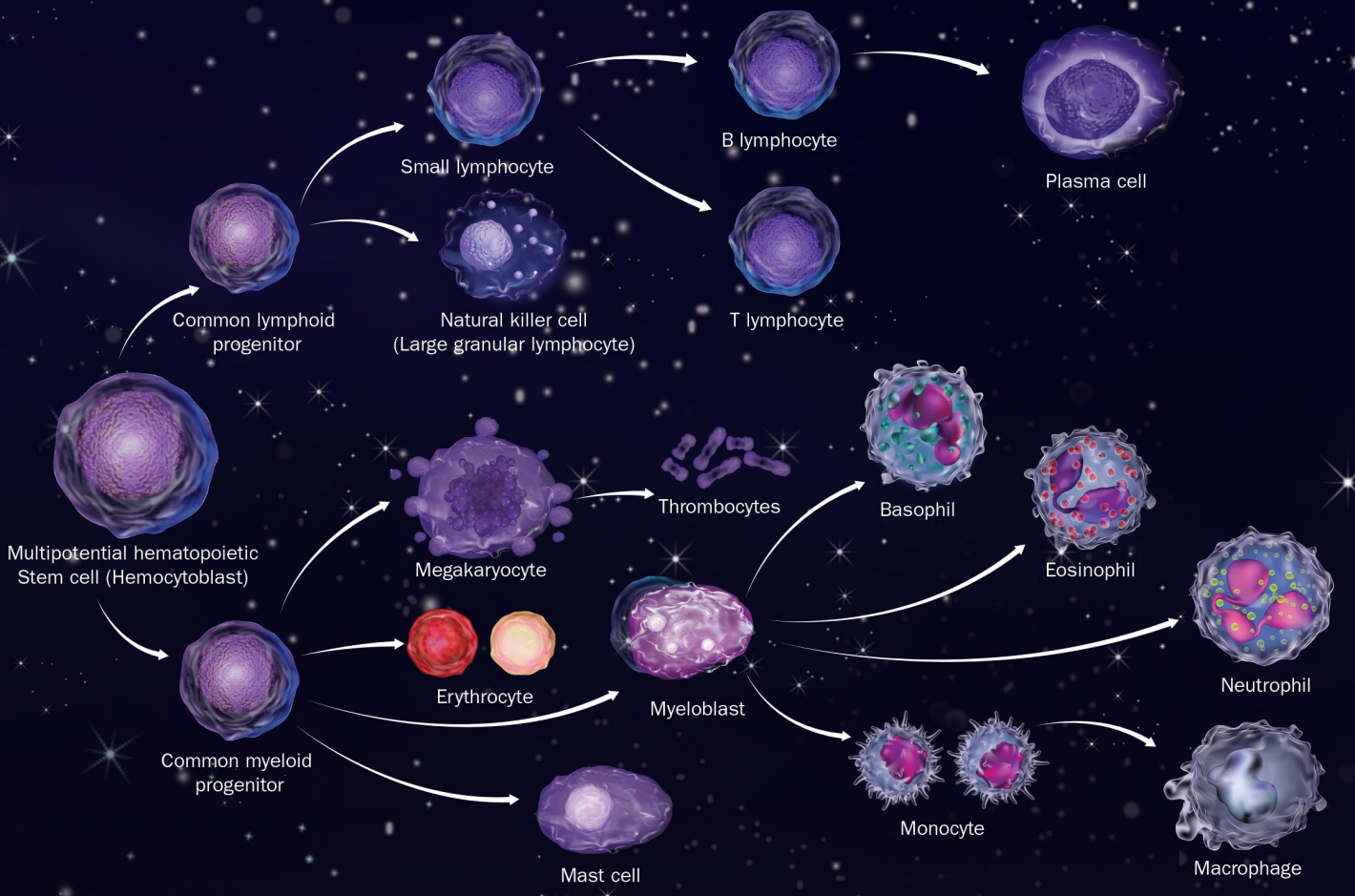


The neutrophil orchestrates other immune cells

Neutrophils have long been thought of as simple suicide killers at the bottom of the hierarchy of the immune system with no influence on shaping the adaptive immune response.[31] Research has since uncovered a role far more intricate. Interestingly, neutrophils issue instructions to practically all other immune cells.[24, 30] As one of the first cell types to arrive at sites of infection, neutrophils secrete cytokines and chemokines that are not only critical in the inflammatory response, but also trigger the adaptive immune response.[32] The cytokines released by neutrophils are often synthesized de novo.[1] Although neutrophils transcribe little after leaving the bone marrow, once activated, they can undergo a transcriptional burst that results in the synthesis of signaling molecules.[33] The initial neutrophil cytokine response results in the attraction of monocytes, macrophages, dendritic cells, natural killer cells, and lymphocytes through complex interactions. The most abundantly produced cytokine, IL-8, primarily serves to recruit other neutrophils but also other granulocytes, causing them to migrate toward the site of infection.[34] Similarly, neutrophil-derived proinflammatory IL-1β and TNF-α induce other cells, such as endothelial cells, to produce additional IL-8.[22, 35] In addition to cytokines, neutrophils release other signaling mediators, including granule contents, lipids, and ROS such as hydrogen peroxide.[34, 36-38] They also communicate via cell to cell contact.[32]



Hematopoiesis



Stages and timing of neutrophil development

Mitotic Phase
5–7 days
(Proliferation/differentiation phase)

Myelocyte

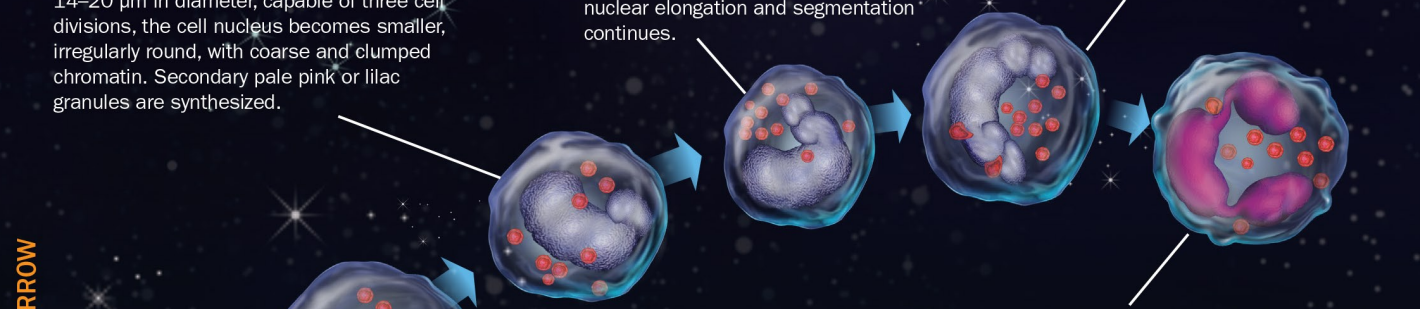
14–20 µm in diameter, capable of three cell divisions, the cell nucleus becomes smaller, irregularly round, with coarse and clumped chromatin. Secondary pale pink or lilac granules are synthesized.

Metamyelocytes

Smaller than myelocytes, the nucleus is bean shaped, and the chromatin is condensed peripherally. Granules are small, fine, blue-black, or gray throughout the cytoplasm. Lose the ability to divide but nuclear elongation and segmentation continues.

Band form

Also known as the "stab or juvenile" form, has a horseshoe shaped nucleus, evenly arranged clumps of chromatin and pink granules, considered fully functional.



MARROW

BLOOD

Polymorphonuclear or segmented neutrophils

13 µm in diameter, the nucleus is segmented with 3–5 lobes, nuclear chromatin is coarse and clumped, cytoplasm is pink with fine azure granules. Primary granules are peroxidase positive. Secondary granules are peroxidase negative.

Promyelocyte

Larger than 20 µm in diameter, presence of many violet or azurophilic granules bound by a membrane.

Myeloblast

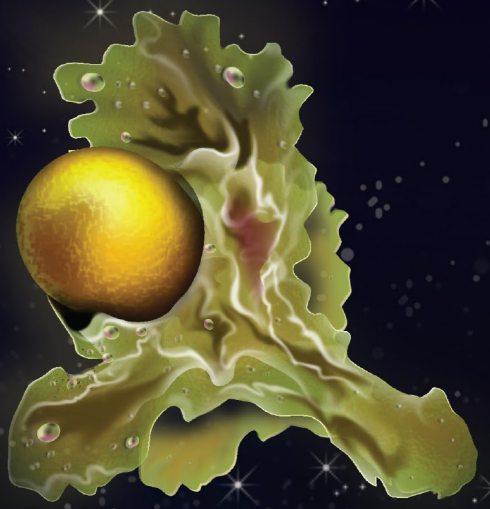
15–20 µm in diameter, the youngest myeloid precursor, large nucleus with finely granular chromatin, absent granules, and scant cytoplasm.

Postmitotic Phase
5–7 days
(Maturation phase)

Klebsiella pneumoniae bacterium (pink) snared in a neutrophil extracellular trap (green)

The neutrophil develops in the bone marrow

Neutrophils are remarkably short-lived with a life span of 6–8 hours once they enter circulation and, consequently, continuously generated in the bone marrow from myeloid precursor cells at the astronomical rate of up to 200 billion cells per day.[1, 9] Under the influence of growth factors and cytokines, pluripotent hematopoietic cells differentiate, proliferate, and mature undergoing a mitotic phase including the myeloblast, promyelocyte, and myelocyte, and a post-mitotic phase where cells mature into metamyelocyte, band cell and, finally, segmented cells.[6] The mitotic phase lasts from 5 to 7 days and the maturation phase takes another 5 to 7 days.[39] This means it takes up to 14 days for a neutrophil to be developed that lives for only 6 to 8 hours.[21]



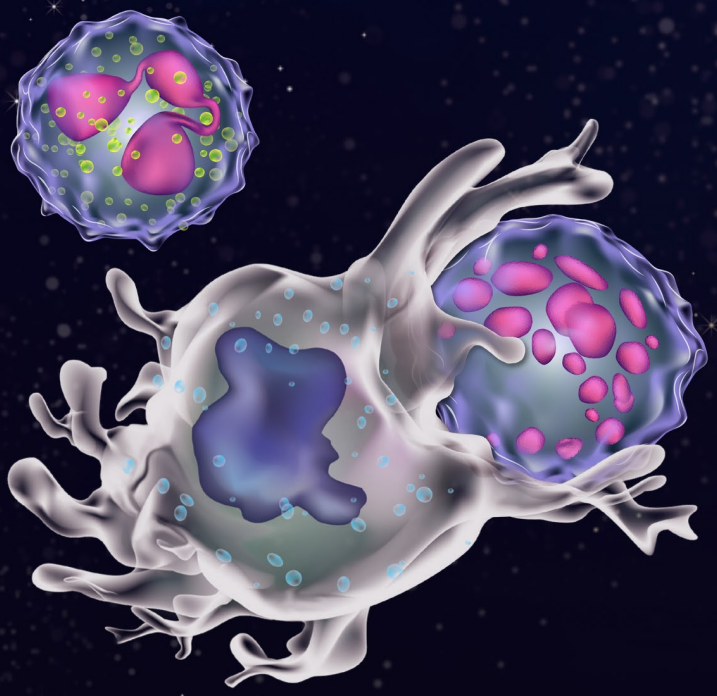
Neutrophil white blood cell engulfing and destroying a hypha (yellow) from a thrush (Candida albicans) fungus

Neutrophil formation is stimulated by growth factor

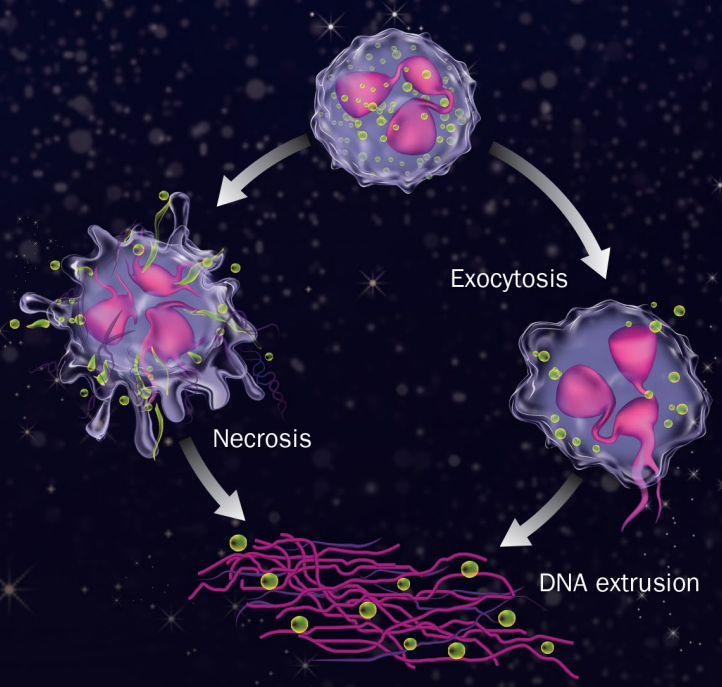
The principal regulator of physiological granulopoiesis is endogenous granulocyte colony stimulating factor (G-CSF) whose effects include commitment of progenitor cells to the myeloid lineage, proliferation of granulocytic precursors, reduction of transit time through the granulocytic compartment, and release of mature cells from the bone marrow.[40] G-CSF exerts its effects through the G-CSF receptor, which is a member of the class I cytokine receptor family.[14]

Death and clearance of neutrophils

In health or disease, a high number of old neutrophils needs to be removed from circulation continuously. Neutrophils die via apoptosis and elimination of apoptotic cells is facilitated through the release of chemokine signals at early stages of cell death, which attract phagocytes.[1] Apoptotic neutrophils are phagocytized mainly in the liver, spleen and bone marrow.[41] Increased CXC-chemokine receptor 4 (CXCR4) expression is seen in aged neutrophils, and this is hypothesized to help direct them back to the bone marrow, where they are then eliminated.[14] Neutrophils can also die in the vasculature and be removed by Kupffer cells (liver-resident macrophages) that live immobilized in the liver vasculature; this applies to both senescent neutrophils and neutrophils that die after fighting infection.[42] The clearance of apoptotic neutrophils by either Kupffer cells or dendritic cells (DCs) was shown to be mainly, but not exclusively, regulated by an interleukin 23 (IL 23)–IL 17–granulocyte colony-stimulating factor (G-CSF) cytokine axis.[41] Another new aspect of neutrophil death is the recent discovery that neutrophils can break down their nuclear contents and release them as neutrophil extracellular traps (NETs). However, how the remnants of such neutrophils and NETs are themselves cleared is not well understood.[27, 43]



Phagocytosis of apoptotic neutrophil



The intriguingly short life of a neutrophil

Interestingly, neutrophils circulate for only approximately 6–8 hours and are among the shortest-lived cells in the human body.[1] In comparison, a human red blood cell remains in circulation for about 4 months.[44] From an evolutionary point of view, the reason for this quick elimination is not well understood, as the astronomical turnover comes at the high biological cost of neutrophil production.[14] One theory is that the short life span may ensure neutrophil integrity and thereby protect the host; this hypothesis is supported by observations that neutrophil apoptosis and subsequent phagocytosis prevent the release of toxic molecules during neutrophil death.[1, 45] Still, the question as to why evolution opted to eliminate neutrophils quickly, as opposed to further stabilizing their dangerous cargo, remains an intriguing mystery.[1]

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