Chapter

Introductory Chapter: Development of Neutrophils and Their Role in Hematopoietic Microenvironment Regulation

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1. Development of neutrophils

Neutrophils are the most abundant white blood cells and play a key role in the elimination of pathogens (invading microorganisms). These specialized innate immune cells are a type of polymorphonuclear leukocyte. Humans produce about 10^{10} to 10^{11} neutrophils daily in the bone marrow from myeloid precursors in a process known as granulopoiesis. The initial precursors of neutrophils are hematopoietic stem cells (HSCs) [1]. The majority of adult blood and immune cells are derived from HSCs, which are also capable of generating new HSCs in a process called self-renewal. The interaction of HSCs with their particular microenvironments, known as niches, is important for maintaining the stem cell properties of HSCs, including cell adhesion, survival, and cell division [2].

1.1 The generation of committed proliferative neutrophil precursors

During the development, HSCs lose their self-renewal potential and produce multipotent precursors (MMPs). All blood cell lineages can be developed from MMPs (**Figure 1**). The differentiation of MMPs into erythro-myeloid or lympho-myeloid progenitors is directed by the antagonistic transcription factors GATA-1 and PU.1. High levels of PU.1 are important for the differentiation of MMPs into lympho-myeloid precursors (LMPs), progenitors for granulocyte-monocyte precursors (GMPs). The most important regulator of physiological granulopoiesis are granulocyte colony-stimulating factor (G-CSF) and its receptor whose effects include commitment of progenitor cells to the myeloid lineage, proliferation and differentiation of granulocytic precursors, release of mature neutrophils from the bone marrow, and modulation of their phagocyte function [3]. Neutrophils carry high levels of G-CSF receptor on their surface through their development and also in mature neutrophils. Humans deficient in G-CSF or its receptor have neutropenia. Interleukin (IL)-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3 also stimulate granulopoiesis but are not essential [3]. The transcription factor family of CCAAT enhancer-binding proteins (C/EBPs) is involved in neutrophil development. Granulocytes and macrophages differentiate from the common GMPs [4–6]. Three neutrophil subgroups were identified within the bone marrow by mass cytometry and cell cycle-based analysis [4]. Committed proliferative neutrophil precursors differentiate into nonproliferating immature neutrophils and mature neutrophils.

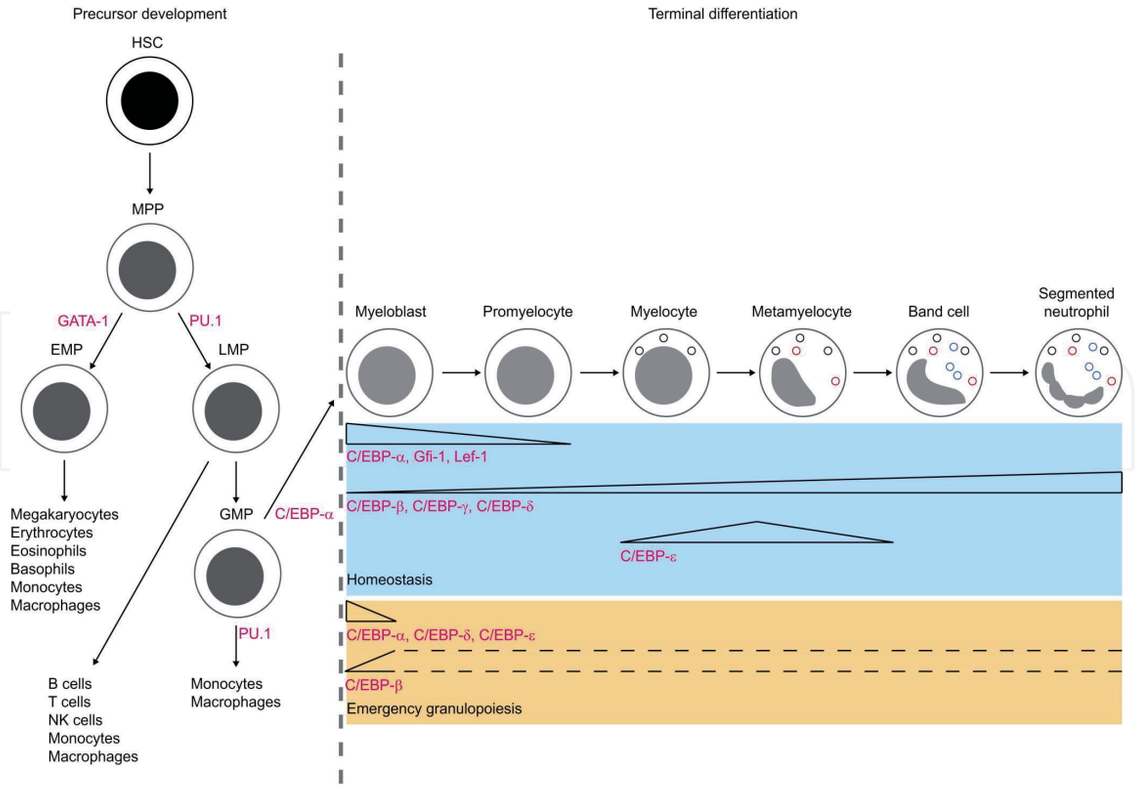

Figure 1.
The maturation process of neutrophils from hematopoietic stem cells. Transcription factors involved at different stages of myeloid cell development are shown. HSC, hematopoietic stem cell; MPP, multipotent precursor; EMP, common erythroid and myeloid precusor; LMP, common lymphoid and myeloid precursor; GMP, common granulocyte and macrophage precursor. Adapted from Refs. [15, 16].

Transcription factor C/EBPε is necessary for the generation of committed proliferative neutrophil precursors from the GMPs [7]. The deficiency of C/EBPε caused phenotypic and functional abnormalities of neutrophils and impaired their chemotaxis and bactericidal action [8–10]. The gene for C/EBPε knockout mice (*CEBPE*) displays a block in terminal granulocytic differentiation and fails to produce functional neutrophils and eosinophils. *CEBPE*-nul mice are susceptible to gram-negative bacterial sepsis and die from systemic infection. Loss-of-function *CEBPE* mutations have been found in patients with defects in neutrophil function and with neutrophil-specific granule deficiency [11–13].

Granulocyte development requires also SMARCD2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 2), also known as BAF60b (BRG1/Brahma-associated factor 60b) [13, 14]. *SMARCD2*-deficient mice were not able to generate mature and functional neutrophils and eosinophils. SMARCD2 interacts with the transcription factor C/EBPε and controls the expression of neutrophil proteins.

1.2 Neutrophil terminal differentiation, granules and secretory vesicles, and release of neutrophils from the bone marrow

Neutrophil precursors differentiate into myeloblasts, promyelocytes, myelocytes, metamyelocytes, band cells, and finally segmented neutrophils (**Figure 1**) [15, 16]. Three types of neutrophil granules are formed continuously starting at the promyelocyte stage during the differentiation process [16, 17]. Primary granules, also known as azurophilic granules, are generated in promyelocytes and contain myeloperoxidase (MPO). Secondary granules, also named specific granules, contain

lactoferrin and are MPO-negative. Tertiary (gelatinase) granules contain matrix metalloproteinase 9, also known as gelatinase B.

Terminal differentiation of neutrophils is regulated by a balance between transcription factors. Runx 1 and c-myb were heavily expressed at the early stages of neutrophil differentiation and are required for the expression of azurophilic granule proteins such as MPO and elastase [18]. Runx1 and c-myb stimulate proliferation, and their downregulation after the myelocyte stage is connected with terminal neutrophil differentiation. C/EBPα is required for granulopoiesis and is found all over the neutrophil differentiation. The selective block in neutrophil differentiation was found in mice with a targeted disruption of the *CEBPA* [19]. On the other hand, an induced expression of the *CEBPA* is necessary for the induction of terminal granulocytic differentiation in 32Dcl3 myeloblasts [20]. The levels of C/EBPβ, C/EBPδ, and C/EBPζ increased substantially at the stage of metamyelocytes where proliferation terminates. However, a high expression of C/EBPγ is connected with proliferation. C/EBPγ is devoid of a transactivating domain and can inhibit other C/EBPs in their transactivating function. Cell cycle arrest and initiation of terminal neutrophil differentiation in metamyelocytes are associated with downregulation of the proliferation-stimulating factors (Runx1, c-myb, C/EBPγ) as it has been mentioned above. PU.1 transcript was found at all stages of neutrophil differentiation [18, 21]. This transcription factor is important for optimal gene expression of both early (MPO, proteinase-3, and elastase) and late (Toll-like receptors 2 and 4 (TLR2, TLR4), CD35, complement receptor 1 (CR1)) neutrophil markers [18, 21].

Neutrophils contain easily mobilized secretory vesicles (intracellular storage granules formed by endocytosis) that can transport their content to plasma membranes of human neutrophils [22–25]. Polymorphonuclear neutrophils contain multiple distinct secretory compartments that are sequentially mobilized during cell activation. Complement receptor type 1 is a marker for a readily mobilizable secretory vesicle compartment, which can undergo exocytic fusion with the plasma membrane independently of the secretion of traditional granule contents.

Release of neutrophils from the bone marrow requires the coordinate action of G-CSF and ELR-type CXC (two N-terminal cysteines separated by one amino acid) chemokines with a specific amino acid sequence (or motif) of glutamic acid-leucine-arginine (or ELR for short) such as CXCL1 and CXCL2 [26]. The interaction between CXC chemokine receptor 4 (CXCR4), a G protein-coupled receptor, and its main ligand stromal-derived factor 1 (SDF-1, also known as CXCL12) retains neutrophils within the microenvironment of the bone marrow. Efficient mobilization of neutrophils requires G-CSF-mediated disruption of the neutrophil retention mechanism and activation of neutrophil migration [26]. Deletion of CXCR4 or CXCR2 has a similar negative effect on neutrophil migration from the bone marrow to circulation. The development from a neutrophil myeloblast to a mature polymorphonuclear neutrophil lasts approximately 14 days [21]. The postmitotic phase lasts 6–7 days [21].

2. Regulation of hematopoietic stem cells in the bone marrow microenvironment by neutrophils

2.1 Various cells influence the bone marrow microenvironment

Stem cell niches are local tissue microenvironments that promote the maintenance of stem cells and regulate their function by producing factors that act directly on stem cells [27]. The determination of niche cells can be based on the

identification of cells which synthesize HSC regulators (CXCL12, CXCL4, stem cell factor (SCF), thrombopoietin, osteopontin, transforming growth factor-β, vascular cell adhesion molecule 1 (VCAM1), glycoprotein 130, Notch ligands such as Jagged-1, fibroblast growth factor 1, angiopoietin-1, and pleiotrophin). Many further factors produced by other tissues can also affect stem cells and their microenvironment. The main site of hematopoiesis in adults is bone marrow. However, in response to severe hematopoietic stresses, extramedullary hematopoiesis was found in other niches of the liver and the spleen HSCs.

In the steady state, 90% of neutrophils reside in the bone marrow, and only 1–2% of neutrophils are present in the circulation [28]. Two neutrophil-derived proteases, cathepsin G and elastase, cleave receptors and cytokines essential for HSC retention in the bone marrow (CXCR4, CXCL1, and VCAM1) and change the HSC-supportive properties of the bone marrow [28]. Further experiments showed that neutrophil-derived proteases are not necessary for HSC mobilization. Macrophages, megakaryocytes, regulatory T cells (Tregs), and neutrophils influence HSC homeostasis and fate.

A majority of HSCs in the bone marrow localize near the sinusoidal blood vessels. Endothelial cells supply HSCs by CXCL12 and SCF. Both these factors are important for HSCs maintaining within the microenvironment of the bone marrow [29, 30]. Nestin-positive (Nes^+) cells are important hematopoiesis-supporting constituents in an adult bone marrow. Studies using green fluorescent protein under the direction of nestin promoter/enhancer (*Nes*-GFP) revealed two groups of mesenchymal progenitor cells, one associated with arterioles (bright cells) and one associated with sinusoids (dim cells). The sympathetic nervous system also regulates hematopoietic stem cells in the bone marrow microenvironment [31].

2.2 Tumor necrosis factor α (TNF α) synthesized by activated bone marrow neutrophils and its role in the regeneration of the damaged hematopoietic stem cell microenvironment

Gr1/Ly6G (lymphocyte antigen 6 complex locus G6D) is a 21–25 kD glycosylphosphatidylinositol (GPI)-linked differentiation antigen that is expressed by myeloid-derived cells in a tightly developmentally regulated manner in the bone marrow. CD115 (M-CSF receptor) has been used to define monocytes. Bowers et al. showed that bone marrow $Gr1^+CD115^-$ neutrophils support the regeneration of the damaged vascular hematopoietic microenvironment in mice after transplantation [32]. Bone marrow $Gr1^+CD115^-$ neutrophils are a heterogenous population which contains proliferating neutrophil progenitors and immature and mature neutrophils with different transcriptional signatures in comparison with circulating neutrophils [4]. Bone marrow neutrophils are selectively recruited to the damaged sinusoidal vasculature, where they secrete TNFα. This cytokine is a potent inducer of new blood vessel growth (angiogenesis) [32]. The treatments used before transplantation for abolishment of the host hematopoietic cells destroy the bone marrow vascular microenvironment. Donor HSCs increase their proliferation and neutrophils together with other myeloid cell production. Therefore, the hematopoietic progenitor engraftment is facilitated by neutrophils [33, 34].

3. Various phenotypes and functions displayed by neutrophils

Neutrophils are innate immune cells engaged in protection against bacterial, viral, parasitic, and fungal pathogens and in tissue repair [35]. Infected tissues and tissues with sterile stress can be also damaged by the toxic activity of neutrophils [35].

Neutrophils have very efficient migratory capabilities. Neutrophils are released from the bone marrow and circulate in the blood for about 12 h and disappear with circadian frequency [35]. As neutrophils disappear from circulation, they infiltrate the vast majority of naive tissues, mainly the spleen, lungs, and liver, and have nonimmune regulatory functions together with supporting B-cell maturation and immunoglobulin production. These infiltrated neutrophils affected cell survival, migration, and susceptibility to cancer.

In addition to direct effects during injury and infections, neutrophils are also able to regulate immunity through modulation of antigen-presenting dendritic cells [36]. The cross talk between neutrophils and lymphocytes takes place at the interface between innate and adaptive immunity. Neutrophils can become involved in positive or negative regulation of interactions with various T cells such as T helper type 1 (Th1), Th2, Th17, Treg, CD8, and γδ T cells [37, 38].

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