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Epigenetic regulation of neutrophil development and function

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ABSTRACT

In addition to performing well-defined effector functions, neutrophils are now recognized as versatile and sophisticated cells with critical immunoregulatory roles. These include the release of a variety of proinflammatory or immunosuppressive cytokines, as well as the expression of genes with regulatory functions. Neutrophils share broad transcriptional features with monocytes, in keeping with the close developmental relation between the two cell types. However, neutrophil-specific gene expression patterns conferring cell type-specific responses to bacterial, viral or fungal components have been identified. Accumulating evidence suggest that these differences reflect the peculiar epigenomic and regulatory landscapes of neutrophils and monocytes, in turn controlled by the specific lineage-determining transcription factors shaping their identity. In this review, we will describe current knowledge on how neutrophil identity and function are controlled at the molecular level, focusing on transcriptional and chromatin regulation of neutrophil development and activation in response to inflammatory stimuli.

Contents

1. Introduction 00
2. Cellular and molecular regulation of neutrophil and monocyte development 00
   2.1. Cellular aspects of neutrophil and monocyte development 00
   2.2. Transcriptional and epigenetic features of myelopoiesis 00
   2.3. Early lineage commitment: qualitative and quantitative regulation of myelopoiesis by LDTFs 00
   2.4. Transcriptional regulation of terminal granulopoiesis: lessons from congenital disorders 00
   2.5. A single-cell view of myelopoiesis 00
3. Epigenetic control of cytokine production in human neutrophils 00
   3.1. Regulation of IFNβ gene expression 00
   3.2. Regulation of IL-10 gene expression 00
   3.3. Regulation of IL-6 gene expression 00
4. DNA methylation 00
5. Conclusion 00
   Conflict of interest 00
   Acknowledgments 00
   References 00

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1. Introduction

Neutrophils function as an essential first line of defense against invading pathogens, such as bacteria, fungi and viruses [1,2]. They utilize several effector mechanisms to defeat pathogens, including phagocytosis, discharge of constitutively stored antimicrobial enzymes or toxic factors, generation of massive amounts of reactive oxygen species (ROS) and active release of nuclear material (DNA, histones and other chromatin proteins) aggregating into neutrophil extracellular traps (NETs) [1]. Accumulating *in vitro* and *in vivo* evidence indicate that, in addition to direct antimicrobial activities, neutrophils play critical functions in the regulation of innate and adaptive immune responses [1]. These activities are exerted largely via the release of cytokines and preformed or newly synthesized mediators in response to pattern recognition receptors (PRR)-mediated sensing of danger signals generated by invading pathogens or tissue damage (Danger- and Pathogen-Associated Molecular Patterns, DAMPs and PAMPs, respectively) [2–4].

Some of the activities described above for neutrophils are broadly shared by monocytes. However, due to their underlying molecular peculiarities these two cell types also show functional differences. Both at steady-state and after activation, neutrophils display a unique transcriptional profile as compared to monocytes stimulated under the same conditions [5–9]. These occurrences likely reflect fundamental differences in neutrophil and monocyte ontogeny, as well as in transcriptional and epigenomic regulation. In this context, our understanding of the epigenomic bases of gene expression in neutrophils is still poor, possibly due to objective difficulties in isolating these cells at high purity [3] and manipulate them in culture, issues that are particularly critical in human samples.

2. Cellular and molecular regulation of neutrophil and monocyte development

In the classical view of hematopoiesis, monocytes and neutrophils are considered highly related cell types originating from a myeloid-committed granulocyte-monocyte common progenitor (GMP) [10,11]. Thus, direct comparison between these two cell populations may effectively uncover shared and distinct mechanisms governing their differentiation. Neutrophils and monocytes can be effectively distinguished from a morphological, phenotypic and functional point of view. A combination of density gradient separation and negative selection based on cell surface markers allows isolating virtually pure population of neutrophils and monocytes. Generally, neutrophils are discriminated from monocytes by the higher expression of Ly6G relative to Ly6C in mouse, and of CD66b relative to CD14 in humans [3].

2.1. Cellular aspects of neutrophil and monocyte development

Granulopoiesis occurs mostly in the bone marrow (BM), and then in peripheral blood, over a time frame of *ca.* 2 weeks in humans. In the BM, granulocyte differentiation occurs progressively in a continuum of developmental stages with partially overlapping morphological features. The first recognizable bi-lineage progenitor of neutrophils and monocytes is the myeloblast, which generally arises from an upstream multi-lineage common myeloid progenitor (CMP). After the myeloblast stage, BM granulopoiesis (i.e. production of neutrophils, basophils, and eosinophils) is thought to proceed separately from monopoiesis via progressive generation of promyelocytes, myelocytes, metamyelocytes and band cells, ultimately leading to polymorphonuclear neutrophils (PMN) with characteristic granules and morphology. After this stage, neutrophils enter the bloodstream, where they undergo terminal maturation [12].

On the other hand, monocytes develop in the BM through a series of incompletely understood committed precursors and are thought to enter the circulation as $Ly6C^{hi}$ cells (in mouse; possibly corresponding to $CD14^{+}CD16^{+}$ intermediate monocytes in humans) in a CCR2-dependent fashion. These monocytes are generally referred to as “inflammatory”, as they represent the main population of monocytes entering inflamed tissues upon infection or stress. Conversely, $Ly6C^{low}$ cells are referred to as “patrolling” monocytes because of their characteristic ability to scan endothelial surfaces where they scavenge debris and also exert trophic functions. Accumulating evidence in mice suggests that circulating monocytes contribute minimally (with some exception, such as in the intestines) to the homeostatic pool of tissue-resident macrophages, which are instead generated through dedicated precursors seeding tissues early in embryogenesis. Circulating monocytes that are recruited to tissues are instead essential to replenish the pool of tissue macrophages in non-homeostatic conditions such as upon infection. We refer the reader to recent excellent reviews describing the very active research focusing on the developmental relationships, functions and contributions in disease of these monocyte subsets with those of tissue-resident macrophages [13–16].

Steady-state production of neutrophils quantitatively represents a major activity that is performed daily in the BM. This is because of the abundance (up to ≈70% of total blood leukocytes) and of the highly regulated cycles of neutrophil elimination and production. Under homeostatic conditions, neutrophil elimination requires their extravasation into peripheral tissues, where they are phagocytosed by resident macrophages; in mice, this process was shown to indirectly reduce the release of granulocyte colony-stimulating factor (G-CSF), thus limiting further neutrophil production in the BM [17]. In addition to this homeostatic negative feedback loop, it was shown in mice that neutrophils remaining in the circulation for prolonged periods without being recruited to tissues (referred to ‘aged neutrophils’) home back to the BM in a CXCR4/CXCL12-dependent manner [18]. Their phagocytosis by resident BM macrophages in turn modulates the hematopoietic niche *via* incompletely understood mechanisms, stimulating egress into the bloodstream of hematopoietic precursor cells (HPC) that produce new neutrophils. The phenotype of aged neutrophils shares some features with that of tumor necrosis factor (TNF) α-activated neutrophils, including activation of proinflammatory signaling pathways, and this was recently shown to be dependent on Toll-like receptor (TLR)- and Myd88-dependent recognition of signals from the microbiota [19]. Whether similar mechanisms occur in humans is completely unexplored; however, these observations further expand the range of functional interactions between the immune system and the microbiota during aging. Dissection of the underlying molecular mechanisms represents undoubtedly one of the big future challenges in the field.

During severe infection (or inflammation), stress-induced granulopoiesis is activated to increase the innate immune responses against the pathogenic insult, as well as to compensate for the increased consumption of activated neutrophils, which die of apoptosis upon recruitment to infected tissues. This adaptation mechanism, ‘emergency granulopoiesis’, underlies a rapid change in the hematopoietic cellular output determined by the massive *de novo* generation of neutrophils [20]. Notably, analogous stress-induced responses are activated in response to non-infectious insults that impair neutrophil homeostasis, such as myeloablative chemotherapy. Whereas the molecular drivers of emergency (or stress-induced) granulopoiesis are still largely unknown, recent studies in mice have shed some light on the cell populations and mechanisms involved. Specifically, it appears that the increased

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