Seminars in Immunology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Neutrophil-derived chemokines on the road to immunity

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ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 4 April 2016
Accepted 5 April 2016
Available online xxx

Keywords:
Neutrophils
Chemokines
Innate immunity
Adaptive immunity
Infections
Tumors
Immune-mediated diseases

ABSTRACT

During recent years, it has become clear that polymorphonuclear neutrophils are remarkably versatile cells, whose functions go far beyond phagocytosis and killing. In fact, besides being involved in primary defense against infections–mainly through phagocytosis, generation of toxic molecules, release of toxic enzymes and formation of extracellular traps–neutrophils have been shown to play a role in finely regulating the development and the evolution of inflammatory and immune responses. These latter neutrophil-mediated functions occur by a variety of mechanisms, including the production of newly manufactured cytokines.

Herein, we provide a general overview of the chemotactic cytokines/chemokines that neutrophils can potentially produce, either under inflammatory/immune reactions or during their activation in more prolonged processes, such as in tumors. We highlight recent observations generated from studying human or rodent neutrophils *in vitro* and *in vivo* models. We also discuss the biological significance of neutrophil-derived chemokines in the context of infectious, neoplastic and immune-mediated diseases. The picture that is emerging is that, given their capacity to produce and release chemokines, neutrophils exert essential functions in recruiting, activating and modulating the activities of different leukocyte populations.

Contents

1. Introduction

Chemokines are 8- to 12-kDa polypeptides, sharing 20–70% homology in amino acid sequence, that are classified into four families (XC, CC, CXC and CX3C families) based on the positioning of their initial cysteine residues [1]. CXC and CC chemokines

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http://dx.doi.org/10.1016/j.smim.2016.04.003
1044-5323/

represent the two major and most studied groups, being the CXC chemokines further divided into two subfamilies, depending on the presence of the glutamate-leucine-arginine (ELR) motif preceding the first two cysteins [2]. CXC ELR-expressing chemokines are mostly chemotactic for neutrophils and include, among other members, CXCL8/IL-8, CXCL1/growth-related gene product-α (GRO-α), CXCL2/macrophage inflammatory protein 2-alpha (MIP-2α)/GROβ, CXCL3/MIP-2β/GROγ and CXCL5/epithelial cell-derived and neutrophil-activating 78-amino acid peptide (ENA-78) [1,3,4]. By contrast, CXCL members lacking the ELR motif, such as CXCL10/interferon (IFN)γ-inducible protein of 10 kDa (IP-10), CXCL9/monokine induced by IFNγ (MIG) and CXCL11/IFNγ-inducible T-cell α chemoattractant (I-TAC), act instead on natural killer (NK) and activated T cells [1,5,6]. CXC chemokines containing the ELR motif also display a potent angiogenic activity, while CXC chemokines lacking the ELR motif are angiostatic [2]. The CC family includes chemokines such as CCL2/monocyte chemotactic protein/MCP-1, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, CCL5/Regulated on Activation, Normal T cells Expressed and Activated (RANTES), CCL7/MCP-3, CCL17/Thymus and activation regulated chemokine (TARC), CCL18/Pulmonary and activation regulated chemokine (PARC), CCL19/MIP-3β and CCL20/MIP-3α, that are mostly chemotactic and stimulatory for monocytes, macrophages, dendritic cells (DCs), T cells, NK cells, eosinophils and basophils [1,3,4,7]. Chemokines are mostly secreted into the extracellular space as soluble factors or bound to the extracellular matrix, thus forming transient or stable concentration gradients, respectively [1]. They promote increased cell motility and directional migration upon binding to their corresponding cell-surface, seven transmembrane-spanning receptors (*e.g.*, CXCRs and CCRs), that signal through G protein-mediated cascades [8,9]. Based on the pattern of receptors expressed, discrete cell populations are specifically recruited by different chemokines [1,10]. Usually a given leukocyte population has receptors for, and responds to, different chemokines [11]. Besides regulating leukocyte trafficking, and therefore coordinating immune responses, chemokines play an important role also in regulating T and B cell-development [12], modulating angiogenesis [13,14] and influencing tumor growth [15]. Although virtually all cell types may release chemokines, innate and adaptive immunity cells, including polymorphonuclear neutrophils, represent the major source of them [1,16], especially in inflammatory (infectious and/or non-infectious) [17,18] or tumor settings [17,19].

Neutrophils are not anymore viewed as simple "suicide" killer cells at the bottom of the hierarchy of immune response [20,21]. The last two decades have, in fact, witnessed a new wave of exciting studies about the capacity of neutrophils to express a number of genes whose products lie at the core of crucial biological processes, including innate immune responses [21,22]. In such regard, neutrophils have been shown to express and produce a variety of chemokines (Table 1) upon activation by microenvironmental stimuli such as microbial agents or their products, including ligands for Toll-like receptors (TLRs) or other pathogen-associated molecular patterns (PAMPs), even in a timely- and specific stimulus-dependent manner [16,22,23]. In addition to amplify their production of chemokines by autocrine loops [23–25], neutrophils have been also shown to positively/negatively modulate the effects of the chemokines present in the microenvironment by releasing enzymes with proteolytic activities, either in association with extracellular traps [26] or not [27]. Therefore, in the context of an inflammatory reaction that has the ultimate goal to kill and remove the invading pathogens, neutrophils may recruit, *via* chemokine release, discrete innate and adaptive immunity cells to optimally orchestrate the most efficacious immune response. Neutrophils themselves have been shown to migrate into the inflammatory site in tightly regulated waves, which are mediated by chemoattractant/chemokine cascades released by activated resident tissue cells and/or previously activated neutrophils or macrophages [23,28,29].

In the following sections, we will highlight recent literature concerning the role of chemokines derived by human neutrophils in shaping the innate and adaptive immune response, either towards infections, or in the context of other pathological conditions such as cancer or immune-mediated diseases. We will also describe findings generated in *in vivo* mouse models, which either extend, or uncover, differences between species [10,22]. For a more broad comprehension of the knowledge existing in the field, the readers may refer to previously published, very exhaustive, reviews [16,28,30].

2. Neutrophil-derived chemokines in immune responses and infections

Most studies indicate that neutrophils upregulate chemokine-encoding genes and/or release chemokines when appropriately stimulated [30]. Hence, the pattern of chemokines released by neutrophils is strictly dependent on the type of stimulus and/or associated to a specific inflammatory/immunological context, *in vitro* and *in vivo*. A large but non-exhaustive list of the stimuli able to induce the production of chemokines by neutrophils has been previously reported [30], yet agonists triggering neutrophil-derived chemokines are continuously identified, for instance: granulocyte colony-stimulating factor (G-CSF), shown to induce CXCL5 [31] and CXCL2/MIP-2α [32]; Wnt5a, a ligand that activates the non-canonical Wnt signaling pathways (β-catenin-independent pathways), shown to trigger the release of CXCL8 and CCL2 [33]; organic dust, shown to trigger the release of CXCL8 and CCL3 [25]; and an increasing number of microbial-derived products, as described in the following paragraphs.

2.1. Human neutrophils

In vitro studies have demonstrated that, upon stimulation with microbial agents or their derivatives, neutrophils release chemokines potentially able to recruit neutrophils themselves, monocytes, macrophages, DCs and NK cells, as well as T cell subsets (Fig. 1), suggesting that, by this function, they may amplify both the innate and the adaptive immune response [16]. For example, neutrophils cultured with either *Mycobacterium tuberculosis* or lipoarabinomann (its major cell wall component) have been shown to release CXCL1 and CXCL8 [34], two chemokines involved in neutrophil recruitment. Similarly, neutrophils release CXCL8 when exposed *in vitro* to *Candida albicans* [35], *Helicobacter pylori* water soluble surface protein [36] and *H. pylori* neutrophil-activating protein (HP-NAP) [37]. By contrast, phagocytosed *Staphylococcus aureus* has been shown to reduce the production of CXCL8 by neutrophils, concomitantly with suppressing phosphorylation of nuclear factor-κB and accelerating cell death, in this manner favoring its own survival and promoting disease [38].

Further evidence of the role of neutrophil-derived chemokines in amplifying local innate responses has been provided by the capacity of neutrophils to upregulate the expression of CXCL1, CXCL2 and, mostly, CXCL3 when *in vitro* exposed to *Fusobacterium nucleatum* [39]. In other studies, neutrophils incubated with LPS- or tumor necrosis factor-α (TNFα) [40], as well as in the presence of Gram-positive or Gram-negative bacteria [41], were shown to sequentially express and release biologically active CCL20 and CCL19 [40], as revealed by experiments in which neutrophil-derived supernatants induced chemotaxis of immature and mature DCs, respectively. These data have been further supported by subsequent findings showing that both IFNγ and the

Please cite this article in press as: C. Tecchio, M.A. Cassatella, Neutrophil-derived chemokines on the road to immunity, Semin Immunol (2016), http://dx.doi.org/10.1016/j.smim.2016.04.003