Seminars in Immunology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

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

Review

Immunoreceptors on neutrophils

Dieke J. van Rees[a], Katka Szilagyi[a], Taco W. Kuijpers[a,b], Hanke L. Matlung[a], Timo K. van den Berg[a,*]

[a] Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[b] Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form 24 February 2016
Accepted 26 February 2016
Available online xxx

Keywords:
Neutrophils
Immunoreceptors
ITAM
ITIM
SHP
SHIP
Syk

ABSTRACT

Neutrophils play a critical role in the host defense against infection, and they are able to perform a variety of effector mechanisms for this purpose. However, there are also a number of pathological conditions, including autoimmunity and cancer, in which the activities of neutrophils can be harmful to the host. Thus the activities of neutrophils need to be tightly controlled. As in the case of other immune cells, many of the neutrophil effector functions are regulated by a series of immunoreceptors on the plasma membrane. Here, we review what is currently known about the functions of the various individual immunoreceptors and their signaling in neutrophils. While these immunoreceptors allow for the recognition of a diverse range of extracellular ligands, such as cell surface structures (like proteins, glycans and lipids) and extracellular matrix components, they commonly signal via conserved ITAM or ITIM motifs and their associated downstream pathways that depend on the phosphorylation of tyrosine residues in proteins and/or inositol lipids. This allows for a balanced homeostatic regulation of neutrophil effector functions. Given the number of available immunoreceptors and their fundamental importance for neutrophil behavior, it is perhaps not surprising that pathogens have evolved means to evade immune responses through some of these pathways. Inversely, some of these receptors evolved to specifically recognize these pathogens. Finally, some interactions mediated by immunoreceptors in neutrophils have been identified as promising targets for therapeutic intervention.

1. The dynamic and versatile nature of the neutrophil: opportunities for regulation

Neutrophils (also called polymorphonuclear leukocytes: PMNs) are the most abundant type of white blood cell in the human body. Their primary task is to protect the body from harmful microbial infections, particularly those exerted by bacterial and fungal pathogens [1,2]. The importance of neutrophils in host defense is well illustrated by the enhanced susceptibility to such opportunistic infections often observed in patients with acquired or inherited defects in neutrophil formation or function [3–5]. To fulfill their obligations, PMNs can perform a variety of effector functions, such as migration to an infectious site, phagocytosis of pathogens, the intracellular killing in phagosomes with the help of reactive oxygen species (ROS) generated via the phagocyte NADPH oxidase, and with anti-microbial components from the granules [6]. While much of the intracellular production of these mediators ensures that microbial killing occurs in a contained fashion, with minimal collateral damage to the surrounding healthy tissue, there is definitely also extracellular production and this inevitably gives rise to some level of inflammation. In fact, some neutrophil activities are directed into the extracellular milieu. For

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ANCAs, anti-neutrophil autoantibodies; Btk, Bruton's tyrosine kinase; DAG, diacyl-glycerol; DAP12, DNAX activation protein of 12 kDa; ERK, extracellular signal-regulated kinase; fMLP, *N*-Formylmethionine-leucyl-phenylalanine; GBS, group B *Streptococci*; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; GPI, glycophosphatidylinositol; Grb2, growth factor receptor-bound protein 2; IgSF, Immunoglobulin superfamily; IP_3, inositol triphosphate; IRAK, interleukin-1 receptor-associated kinase; ITAM, immunoreceptor tyrosine-based activating motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; IVIg, intravenous immunoglobulins; MAPK, mitogen-activated protein kinase; MLCK, myosin light-chain kinase; MPO, myeloperoxidase; MRP8, myeloid-related protein 8; NADPH, nicotinamide adenine dinucleotide phosphate; NETs, neutrophil extracellular traps; ORF, open reading frame; PE, phosphatidylethanolamine; PH domain, pleckstrin homology domain; $PI(3,4)P_2$, phosphatidylinositol (3,4)-bisphosphate; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PLC, phospholipase C; PMN, polymorphonuclear leukocytes; PS, phosphatidylserine; PTPN6, tyrosine-protein phosphatase non-receptor type 6; RA, rheumatoid arthritis; ROS, reactive oxygen species; SH2, Src homology 2; SHIP, SH2-domain containing inositol phosphatase; SHP, SH2-domain containing phosphatase; SLE, systemic lupus erythematosus; SLP-76, SH2 domain containing leukocyte protein of 76 kDa; SNP, single-nucleotide polymorphism; SOCS, suppressor of cytokine signaling proteins; Syk, spleen tyrosine kinase.

* Corresponding author at: Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.
E-mail address: t.k.vandenberg@sanquin.nl (T.K. van den Berg).

http://dx.doi.org/10.1016/j.smim.2016.02.004
1044-5323/

instance, neutrophils are apparently also capable of releasing their DNA content and to form so-called neutrophil extracellular traps (NETs) with presumed anti-microbial activity *in vivo* [7]. In addition, neutrophils communicate with other immune cells through the secretion of cytokines and chemokines [1,8]. However, inadequate neutrophil recruitment and functioning can contribute to serious disease. For example, the chronic inflammatory response in autoimmune diseases, such as *e.g.* autoimmune rheumatoid arthritis (RA), leads to the recruitment of neutrophils that subsequently contribute substantially to tissue damage, ultimately resulting in irreversible processes like cartilage destruction [9]. In cancer, neutrophils, often designated as tumor-associated neutrophils (TANs), can confer either pro- or anti-tumor effects, depending on the conditions. Whereas neutrophils involved in associated inflammatory processes may actually support tumor progression, *e.g.* by releasing tissue-degrading proteins from granules, cytokine production and even ROS production [10–12], the presence of therapeutic antibodies directed against the tumor, may change the situation radically and turn the cancer cells into targets for neutrophil-mediated antibody-dependent cellular cytotoxicity (ADCC) [13,14]. It thus seems clear that, while maintaining the capacity to mobilize these highly efficient mechanisms when required, it is also very important to tightly control the effector functions of neutrophils to avoid undesirable collateral damage. One of the most striking examples of lack of inherent neutrophil control is that observed in neutrophil-specific deficiency of the tyrosine phosphatase SHP-1 in mice, which, even in the absence of any deliberate pathogenic challenge, causes an obvious cutaneous inflammatory phenotype [15]. SHP-1 and other inhibitory signaling molecules act downstream of a variety of immune inhibitory receptors that counterbalance the activities of activating immunoreceptors. The interplay between these activating and inhibitory receptors is a major determinant of the behavior of the neutrophil.

In this review, we consider the different immunoreceptors that are expressed on neutrophils. For this purpose we define an immunoreceptor as a transmembrane structure containing extracellular immunoglobulin (Ig)-like domains and intracellular signaling via conserved immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs). We will describe in detail the immunoreceptors known to be expressed on either human or murine neutrophils. We will also explain whether and how these receptors modulate the functions of neutrophils and discuss their roles in different pathological conditions.

2. Immunoreceptors

Several classes of cell surface receptors on neutrophils are involved in cellular activation and intracellular signal transduction. These include G protein-coupled receptors (GPCRs), cytokine and chemokine receptors, adhesion receptors (*e.g.* integrins or selectins) and pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or C-type lectin receptors (CLRs) [16–18]. Additionally, modulation of immune responses by neutrophils is regulated through activating and inhibitory immunoreceptors, that we defined in Section 1 as structures containing Ig-like binding domains that mediate signaling via intracellular ITAM or ITIM motifs.

Historically, receptors containing ITAM motifs became acknowledged first [19], and somewhat later FcγRIIb was described as the first inhibitory immunoreceptor containing ITIM motifs [20]. In the late 1990s a theory arose, proposing the existence of structurally closely related paired receptors that trigger opposing cellular responses in immune cells, to help shaping a fragile balance between host responses to pathogens and tolerance [21]. A whole repertoire of paired receptors has since been described for innate immune cells, of which many are also expressed on neutrophils [22]. The net response of these immunological "yin and yang" forces is determined by the strength of the ligand binding as the extracellular part of both siblings is very similar, if not identical [23]. Analysis of genes encoding for paired receptor families showed that these have evolved rapidly [24], suggesting a strong evolutionary pressure coming most likely from exposure of the host to various pathogens. The ability of pathogens to develop evasion strategies can include for instance the hijacking of the inhibitory receptor, thereby inhibiting immune responses. As a consequence of this never-ending battle between host and pathogen, the immune system created a counterbalancing mechanism of activating receptors that overcome the inhibitory signal. Until now, a number of bacterial and viral pathogens have been identified to interact with immunoreceptors expressed on leukocytes [25], including neutrophils, as will be discussed in more detail below.

A number of activating and inhibitory receptors use conserved signaling motifs situated in the cytoplasmic tail; these are the ITAMs or ITIMs. The ITAM is defined by the consensus sequence $Y_{XX}L_{X6-8}Y_{XX}L/I$ and the inhibitory motif by $I/V/L/S_XY_{XX}L/V/I$ (where X represents any amino acid). However, some activating receptors lack intrinsic signaling motifs and instead associate with ITAM-containing signaling modules such as DNAX activation protein of 12 kDa (DAP12) or the Ig-FcR γ-chain (FcRγ) [27,28]. The ITAM domain is situated in the cytoplasmic domain of these transmembrane adaptor molecules and facilitates downstream signaling upon receptor assembly [26,28–31]. The structural arrangement of the transmembrane domain of the immunoreceptor determines the association with either DAP12 or FcRγ: a transmembrane basic arginine in the receptor pairs with FcRγ, while a lysine in the receptor interacts with DAP12 [32]. The importance of DAP12 and FcRγ signaling modules has been shown in double knockout mice, leading to neutrophils that were strongly impaired in their respiratory burst and degranulation activated through integrin signaling [33]; the mice were furthermore defective in osteoclast development [34]. In humans, loss-of-function mutations or deletions in the genes encoding for DAP12 or one of its recruiting receptors TREM-2, results in Nasu-Hakola disease, which presents with recurrent bone fractures and early-onset dementia [35,36]. Although TREM-2 and DAP12 are essential for osteoclastogenesis and bone-remodeling in these patients [35,36], they do not suffer from apparent immunodeficiencies, possibly due to functional redundancy of FcRγ and DAP12 [33]. In addition to ITAM and ITIM domains, some immunoreceptors contain other signaling motifs as well, which include docking sites for *e.g.* PI3K ($Y_{XX}M$, found for instance in DAP10), Grb2 (Y_XN_X), Fyn or SOCS. While these motifs are vital for functional signaling in immune cells, these will not be discussed in detail here, where we will focus on signaling via the ITAM and ITIM motifs of the various receptors in neutrophils.

2.1. Signaling via immunoreceptor ITAM and ITIM motifs

Upon receptor ligation and aggregation, ITAM phosphorylation of the tyrosine (Y) residues by Src-family kinases, of which mainly Hck, Lyn, Fgr and to a lesser extent also Src are expressed by neutrophils, recruits and activates spleen tyrosine kinase (Syk). Src-family kinases are bound to the inner face of the plasma membrane by their N-terminal acetylation sites, and provide, after initiating phosphorylation of the ITAM adapters, docking sites for the pivotal kinase Syk [37]. Syk binds the dual phosphorylated tyrosines within the ITAM with its SH2 domains and subsequently initiates an activating signaling cascade by recruiting and phosphorylating additional signal molecules (Fig. 1). Both Src-family kinases and Syk have been described to be indispensable for typical ITAM signaling. Src-family kinases are involved in many

Please cite this article in press as: D.J. van Rees, et al., Immunoreceptors on neutrophils, Semin Immunol (2016), http://dx.doi.org/10.1016/j.smim.2016.02.004