Chapter

Neutrophil Activation by Antibody Receptors

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Abstract

Neutrophils, the most abundant leukocytes in blood, are relevant cells of both the innate and the adaptive immune system. Immunoglobulin (Ig) G antibody molecules are crucial activators of neutrophils. IgGs identify many types of pathogens via their two Fab portions and are in turn detected through their Fc portion by specific Fcγ receptors (FcγRs) on the membrane of neutrophils. Thus, antibodies bring the specificity of the adaptive immune response to the potent antimicrobial and inflammatory functions of neutrophils. Two types of FcγRs with several polymorphic variants exist on the human neutrophil. These receptors are considered to be redundant in inducing cell responses. Yet, new evidence presented in recent years on how the particular IgG subclass and the glycosylation pattern of the antibody modulate the IgG–FcγR interaction has suggested that a particular effector function may in fact be activated in response to a specific type of FcγR. In this chapter, we describe the main types of FcγRs on neutrophils and our current view on how particular FcγRs activate various signaling pathways to promote unique effector cell functions, including phagocytosis, activation of integrins, nuclear factor activation, and formation of neutrophil extracellular traps (NETs).

Keywords: neutrophil, phagocytosis, degranulation, NETs, antibody, Fc receptors, integrins, NF-κB

1. Introduction

Neutrophils are the most abundant cell type in human blood. They are produced in the bone marrow and then released into the circulation. At sites of infection or inflammation, neutrophils migrate to tissues, where they complete their functions. Finally, neutrophils die by apoptosis and are eliminated by macrophages. Neutrophils are an essential part of the innate immune system [1], with significant antimicrobial functions, including phagocytosis, degranulation, and the formation of neutrophil extracellular traps (NETs). These antimicrobial functions were believed to be the only goal of neutrophils. However, it has recently become clear that neutrophils display many functional responses that go beyond the simple killing of microorganisms. Neutrophils produce cytokines [2] and other inflammatory factors [3] that regulate the whole immune system [4, 5]. Consequently, neutrophils are also key effector cells of the adaptive immune system.

Immunoglobulin (Ig) G antibody molecules are an essential part of the adaptive immune system. IgGs recognize antigens via their two Fab portions and are in turn linked through their Fc portion to specific Fcγ receptors (FcγRs) on the membrane of leukocytes [6, 7]. In this way, antibodies function as a bridge between the specific adaptive immune response and the potent innate immune functions of leukocytes. In the human neutrophil, two types of FcγR exist. Thus, antibodies are important activators of neutrophils. The Fcγ receptors on the neutrophil are considered to be redundant in inducing cell responses [8, 9]. However, recent findings on how a particular IgG subclass and the glycosylation pattern of the antibody regulate the IgG–FcγR interaction suggest that a particular effector function may in fact be activated in response to a specific type of FcγR. It is the purpose of this chapter to describe the FcγRs on human neutrophils and present our current view of how particular FcγRs activate various signaling pathways to promote unique effector cell functions.

2. Neutrophils

Neutrophils are the most abundant leukocytes in blood and because they are the first cells to appear at sites of inflammation and infection; they are regarded as the first line of defense of the innate immune system [10]. Neutrophils can rapidly move from the blood into affected sites through a process known as the leukocyte adhesion cascade. Once in the tissues, they perform important antimicrobial functions, including phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs) [11, 12].

2.1 Leukocyte adhesion cascade

Neutrophils leave the blood circulation at sites of infection or inflammation by binding to the endothelial cells and then transmigrating into the tissues [13]. This process known, as the leukocyte adhesion cascade (**Figure 1**), begins with the activation of endothelial cells at the affected site. Activated endothelial cells upregulate the expression of adhesion receptors such as E- and P-selectins. Neutrophils bind to these selectins via glycoprotein ligands on their membrane. As a consequence, neutrophils can then roll on endothelial cells. Next, neutrophils get activated by chemokines, which induce a high affinity state on integrins, another group of adhesion receptors. Binding of integrins with their corresponding ligands, such as intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on endothelial cells, results in slower neutrophil rolling and then firm adhesion that makes neutrophils stop. Finally, neutrophils transmigrate the endothelium into the tissues. Engagement of endothelial-cell adhesion molecules seems to provoke the opening of endothelial-cell contacts by redistributing junctional molecules in a way that promotes transmigration of neutrophils. Molecules that do not help neutrophil migration, such as vascular endothelial cadherin (VE-cadherin), are moved away from junctional regions. Other endothelial junctional molecules for which neutrophils express ligands concentrate on the endothelial cell luminal surface creating an adhesive environment for the neutrophil. Platelet/endothelial-cell adhesion molecule 1 (PECAM1) and CD99 support homophilic interactions between endothelial cells and neutrophils. While, junctional adhesion molecule (JAM)-1 and JAM-2 on the endothelial cell bind to the β1 integrin VLA4, and the β2 integrins LFA-1 and Mac-1 on the neutrophil, respectively. The endothelial cell-selective adhesion molecule (ESAM) is also involved in transmigration by binding to an unknown

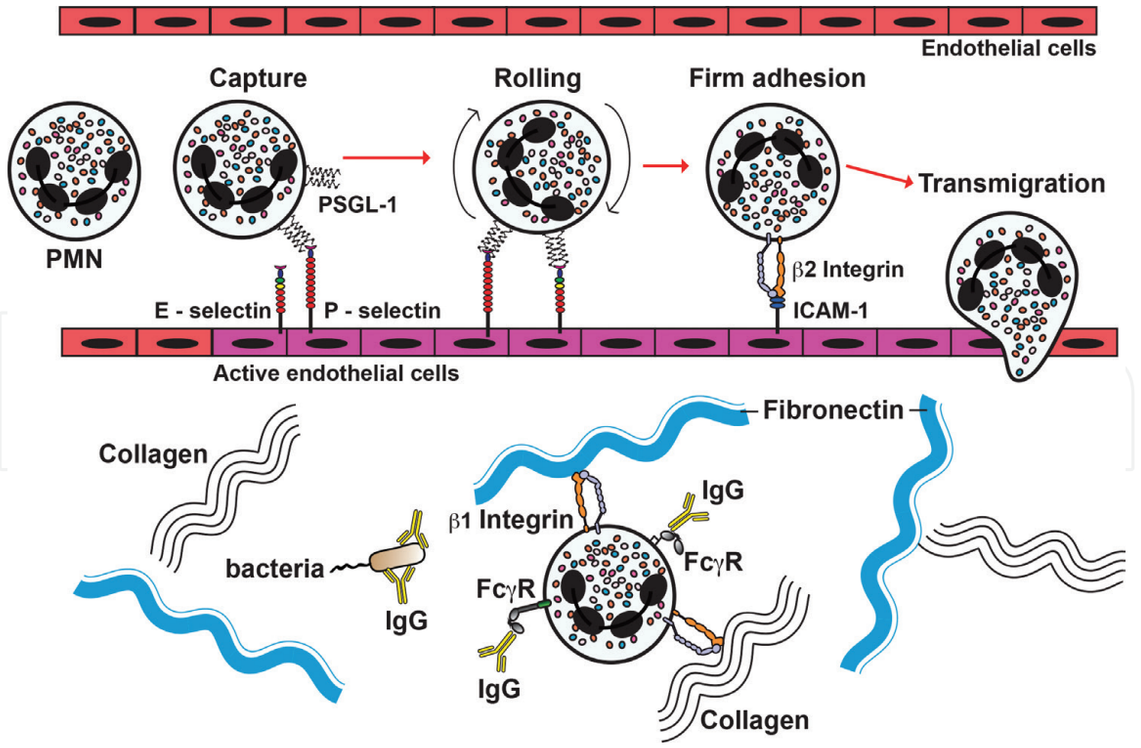

Figure 1.
Leukocyte adhesion cascade. Neutrophils, also known as polymorphonuclear (PMN) cells, move to sites of inflammation via a leukocyte adhesion cascade that includes activation of endothelial cells with upregulation of E- and P-selectins. Neutrophils bind to these selectins via glycoprotein ligands, such as P-selectin glycoprotein ligand-1 (PSGL-1), and begin rolling on endothelial cells. Next, neutrophils get stimulated by chemokines and activate their β2 integrins, which bind to their corresponding ligands, such as intercellular adhesion molecule-1 (ICAM-1). Integrin binding induces firm adhesion and transmigration of neutrophils into tissues. Once in tissues, neutrophils move following chemoattractant gradients to reach affected sites using now adhesion of β1 integrins to proteins of the extracellular matrix, such as collagen and fibronectin. Antibodies (IgG) bind to microorganisms (bacteria) and are in turn recognized by Fcγ receptors (FcγR) on the membrane of neutrophils.

ligand on neutrophils [12, 14]. Once neutrophils move into tissues, they follow chemoattractant gradients to reach affected sites using now adhesion of β1 integrins to proteins of the extracellular matrix, such as collagen and fibronectin [15] (**Figure 1**). Important chemoattractants for neutrophils are activated complement components, such as the anaphylatoxin C5a, bacterial components, such as formyl-methionyl-leucyl-phenylalanine (fMLF) and cytokines, such as interleukin (IL) 8.

2.2 Antimicrobial mechanisms of neutrophils

Neutrophils recruited from the circulation into infected tissues can eliminate microorganisms by phagocytosis, by releasing antimicrobial substances or by forming NETs [11, 12] (**Figure 2**).

2.2.1 Phagocytosis

Phagocytosis is the process by which particles larger than 5 μm get internalized by the cell into a vacuole called the phagosome. Neutrophils recognize pathogens directly through pattern-recognition receptors (PAMPs), or indirectly through opsonin receptors. Opsonins are host proteins, such as antibody molecules or complement components, that bind to microorganisms and facilitate their detection and destruction by leukocytes [16, 17]. After internalization, the nascent phagosome matures by fusing with lysosomes [18]. During maturation, antimicrobial

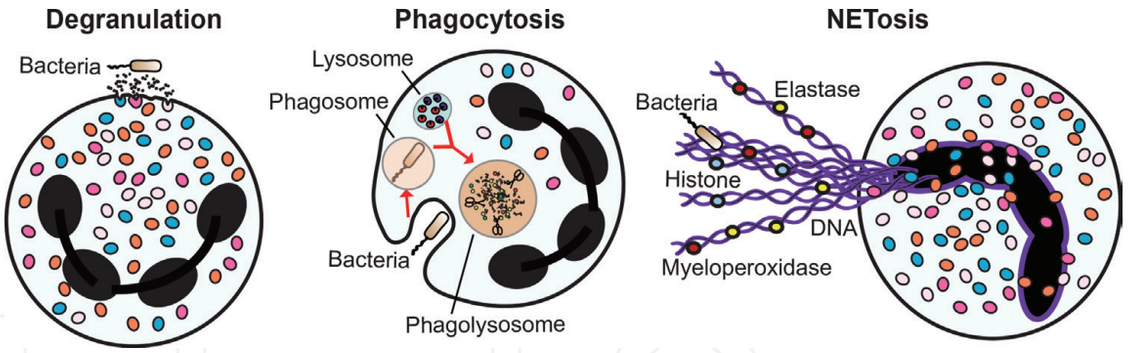

Figure 2.
Antimicrobial mechanisms of neutrophils. Neutrophils can destroy microbial pathogens, such as bacteria by (a) degranulation, (b) phagocytosis, and (c) NETosis. During degranulation, antimicrobial proteins are released outside the neutrophil. In phagocytosis, the pathogen is ingested in a vacuole named phagosome, which then fuses to lysosomes and becomes a phagolysosome, where the pathogen is destroyed. During NETosis, DNA fibers decorated with histones and granular proteins, such as elastase and myeloperoxidase are released in structures known as neutrophil extracellular traps (NETs).

molecules are delivered into the phagosomal lumen, and the vesicle is transformed into a phagolysosome [19]. In the phagolysosome, reactive oxygen species (ROS) are produced by the NADPH oxidase on the phagosomal membrane, and the pH inside drops to 4.5–5. Also, hydrogen peroxide (H_2O_2) is converted to hypochlorous acid (HOCl) in a reaction catalyzed by myeloperoxidase (MPO) [20]. Together, these actions form a toxic environment for the microorganism.

2.2.2 Degranulation

During neutrophil formation in the bone marrow, immature neutrophils synthesize proteins that are sorted into different granules [10]. Granules are classified into three different types based on their content. Azurophilic granules contain mainly myeloperoxidase, elastase, and cathepsin G. Specific granules contain mainly collagenase, lactoferrin, and lysozyme. Gelatinase granules contain mainly gelatinase, lysozyme, and cytochrome b558 [21]. Neutrophils also form secretory vesicles at the last step of their differentiation. These secretory vesicles contain several important receptors on their membrane, including complement receptors (CR1), Fc receptors (CD16), lipopolysaccharide (LPS) receptors (CD-14), and fMLF receptors. Granule heterogeneity is due to the controlled expression of the granule protein genes [22]. Mature neutrophils are released into the circulation and when they reach sites of infection, neutrophils can degranulate in order to deliver their antimicrobial proteins. Secretory vesicles present the greatest predisposition for extracellular release, followed by gelatinase granules, specific granules, and azurophil granules [23]. The hierarchical mobilization of neutrophil granules and secretory vesicles depend on intracellular Ca^{2+}-level [24].

2.2.3 Neutrophil extracellular traps (NETs)

When neutrophils cannot ingest large microorganisms, they can display another antimicrobial strategy [25]. Neutrophils can release long chromatin fibers that are decorated with proteins from their granules. These fibers can trap microorganisms, and therefore, they have been called neutrophil extracellular traps (NETs) [26]. The process of NETs formation is called NETosis [27]. NETosis has been described as a special form of programmed cell death. The complete mechanisms of NETs formation are still unknown; it seems that NETosis requires NADPH oxidase activation, reactive oxygen species (ROS) production, myeloperoxidase (MPO), and neutrophil elastase (NE) release [28, 29] (**Figure 2**).

3. Fcγ receptors

Antibodies produced by the adaptive immune response are mainly of the IgG class. These antibodies present higher affinity and greater specificity for their particular antigen. Thus, IgG antibodies are key for controlling infections from all types of pathogens, including viruses, bacteria, fungi, and protozoa [30]. However, IgG molecules do not directly damage the microorganisms they recognize. It is in fact, the cells of the innate immune system, which are responsible for the antimicrobial functions of these antibodies. Although, some antibodies can activate complement, which is then deposited on microorganisms to promote phagocytosis via complement receptors [17, 31], or to induce bacterial lysis via the formation of the membrane attack complex [32], most IgG antibodies bind to specific receptors on the membrane of leukocytes [7, 8]. These receptors recognize the fragment crystallizable (Fc) portion of IgG molecules and are therefore known as Fcγ receptors (FcγR). Cross-linking of FcγR on the surface of cells activates several antimicrobial functions [6].

3.1 Human Fcγ receptors

Human Fcγ receptors comprise a family of glycoproteins expressed on the membrane of immune cells [7, 8]. These receptors can bind to the various IgG subclasses with different affinities [7], and induce different cellular responses [6]. FcγR can be classified as activating receptors (FcγRI/CD64, FcγRIIa/CD32a, FcγRIIIa/CD16a, and FcγRIIIb/CD16b), and one inhibitory receptor (FcγRIIb/CD32b) [7, 9, 33, 34] (**Figure 3**).

FcγRI is a high affinity receptor, having three Ig-like extracellular domains. It binds mainly monomeric IgG [9]. In contrast, FcγRII and FcγRIII are low-affinity receptors, having two Ig-like extracellular domains. They bind only multimeric immune complexes [9, 35]. FcγRI is associated with a dimer of the common Fc receptor γ chain, which contains an immunoreceptor tyrosine-based activation motif (ITAM) sequence (**Figure 3**). The ITAM sequence is important for receptor signaling [36].

FcγRIIa contains its own ITAM within its cytoplasmic tail. In contrast, the inhibitory receptor FcγRIIb contains an immunoreceptor tyrosine-based inhibition

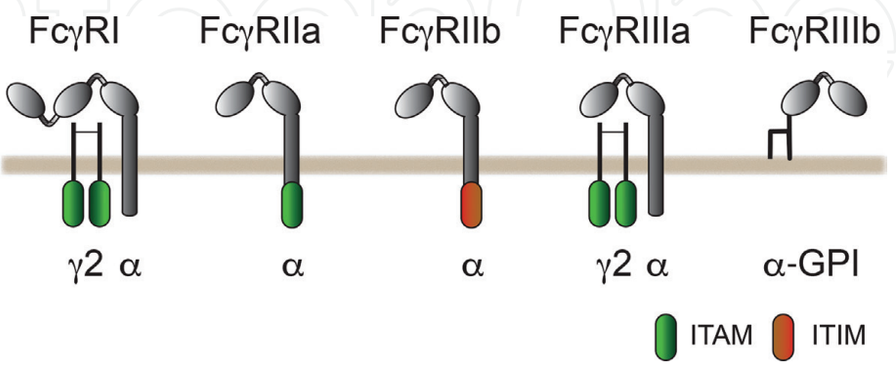

Figure 3.
Human Fcγ receptors. Schematic illustration of human receptors for IgG. Fcγ receptors are shown relative to the cell membrane (brown line). The IgG-binding chain (α) is expressed together with their respective γ2 signaling subunits. FcγRI is a high affinity receptor, having three Ig-like extracellular domains. FcγRII and FcγRIII are low-affinity receptors, having two Ig-like extracellular domains. FcγRIIIb is expressed exclusively on neutrophils, and it is a glycosylphosphatidylinositol (GPI)-linked receptor missing a cytoplasmic tail. ITAM, immunoreceptor tyrosine-based activation motif with consensus sequence YxxI/Lx $_{(6–12)}$YxxI/L [36] (green oval); ITIM, immunoreceptor tyrosine-based inhibitory motif with the consensus sequence I/V/L/SxYxxL/V [39] (red oval).

motif (ITIM) within its cytoplasmic tail (**Figure 3**). The FcγRIIb negatively regulates various cell functions including antibody production by the B cell [37], proliferation, degranulation, and phagocytosis in other leukocytes when it is cross-linked with activating FcγRs [38, 39]. Most leukocytes express both activating and inhibitory FcγRs, hence simultaneous cross-linking establishes a threshold for cell activation [40] that maintains a balanced immune response [41, 42].

FcγRIII has two isoforms: FcγRIIIa is a receptor with a transmembrane domain and a cytoplasmic tail, associated with an ITAM-containing homodimer of Fc receptor γ chains (**Figure 3**). It is expressed mainly on macrophages, natural killer (NK) cells, and dendritic cells [7, 8]. In contrast, FcγRIIIb is expressed exclusively on neutrophils and it is a glycosylphosphatidylinositol (GPI)-linked receptor missing a cytoplasmic tail. Also, no other subunits are known to associate with it (**Figure 3**). It is important to mention that human FcγRIIa and FcγRIIIb are exclusive receptors that are not found in other species [33, 43].

4. IgG binding to Fcγ receptors

As mentioned before, there is one high-affinity Fcγ receptor, FcγRI (CD64), and two groups of low-affinity Fcγ receptors, FcγRII and FcγRIII (**Figure 3**). This causes that a single IgG molecule cannot bind to most Fcγ receptors. However, when IgG molecules form antigen-antibody (immune) complexes, they can have many low affinity interactions with Fcγ receptors. Thus, only immune complexes are able to induce the cross-linking of FcγR required for the activation of various antibody-mediated cell functions. It is clear then that depending on the nature of the immune complex, the interaction with various FcγR will change. Several factors have been identified as having an important influence on the affinity of antibody molecules for particular FcγRs. These factors include the type of IgG subclass [7, 44], the IgG glycosylation pattern [45, 46], and receptor polymorphisms.

4.1 The type of IgG subclass

There are four subclasses of IgG (IgG1, IgG2a, IgG2b, and IgG3 in mice; and IgG1, IgG2, IgG3, and IgG4 in humans) [47]. This leads to the formation of different types of immune complexes. Several *in vivo* studies have indeed suggested that different IgG subclasses can activate particular cell responses. For example, in mice, IgG2b was better than IgG1 at eliminating B cell [48] and T cell lymphomas [49]. Also, antierythrocyte antibodies of IgG2a and IgG2b subclasses were better than antibodies of IgG1 and IgG3 subclasses in mediating phagocytosis of opsonized erythrocytes [50]. In humans, it was shown that most FcγRs bind primarily IgG1 and IgG3 over the other subclasses of IgG [6, 7]. Together, these reports confirm that different IgG subclasses mediate different cellular responses *in vivo,* and suggest that different cellular activities result from cross-linking different FcγRs. However, the mechanism used to generate this IgG-FcγR selectivity is not completely understood. Accordingly, a great interest exists for determining which type of IgG binds to which FcγR and what particular receptor is involved in mediating a certain cellular function.

Obviously, this selectivity depends mainly on the affinities of different IgG subclasses to particular Fcγ receptors. For this reason, detailed studies to measure the affinities of IgG subclasses to the various Fcγ receptors have been conducted both for mice FcγRs [51] and for all human FcγRs [35]. Through these studies, it was found that IgG1 and IgG3 bind to all FcγR. IgG2 binds mainly to FcγRIIa (H_{131} isoform),

and FcγRIIIa (V_{158} isoform), but not to FcγRIIIb [35]. IgG4 binds to many FcγRs [35]. Thus, it is clear that different IgG subclasses engage different Fcγ receptors depending on the relative affinity of these receptors for a particular IgG class [33].

4.2 The IgG glycosylation pattern

All IgG molecules are glycoproteins with an N-glycosylated carbohydrate side chain that is important for antibody function [52]. Deletion of this carbohydrate (sugar) side chain results in poor binding to FcγRs [53]. The N-glycans are heterogeneous in their sugar composition and are attached to asparagine 297 (Asp^{297}) in the Fc portion of the IgG [54]. The carbohydrate side chain may contain sugar residues such as galactose, fucose, and sialic acid in straight or branching patterns [46], and the differences in the glycosylation pattern seem to regulate IgG activity [55].

Many IgG antibodies present a fucose residue linked to an N-acetylglucosamine residue [56]. When this residue is removed, IgG molecules present an increased affinity to the FcγRIIIa [57], and also an increase in antibody-dependent cell cytotoxicity (ADCC) activity against various tumor cells [51, 57, 58]. Based on these findings, recombinant IgG antibodies with low fucose levels have been produced in order to increase their ADCC activity. Several of these antibodies are now in clinical trials to test their therapeutic potential [59].

Many IgG antibodies also present a carbohydrate side chain that terminates with sialic acid residues [60]. Contrary to antibodies without fucose, terminal sialic acid usually correlates with low affinity for FcγRs and also with lower ADCC activity [61, 62]. Interestingly, these sialic acid-rich antibodies seem to preferentially bind other receptors different from FcγRs. The receptor dendritic cell specific ICAM-3 grabbing nonintegrin (DC-SIGN) was identified as a receptor for sialic acid-rich IgG [63]. Therefore, terminal sialic acid can modify IgG activity by promoting less binding to FcγRs and more binding to other receptors [45].

4.3 Polymorphisms of receptors

Another factor influencing the affinity of antibody molecules is the existence of several polymorphisms for the unique FcγRIIa and FcγRIIIb present on human neutrophils [64]. There are two isoforms for FcγRIIa with different amino acids at position 131. These are identified as low-responder (H_{131}) and high-responder (R_{131}) [65]. Similarly, for FcγRIIIb two isoforms exist differing at four positions, NA1 (R36 N65 D82 V106) and NA2 (S36 S65 N82 I106) [66], and with different glycosylation patterns [67]. In addition, another FcγRIIIb isoform named SH is generated by a point mutation (A78D) in the NA2 allele [68]. These multiple FcγR isoforms display diverse binding affinity for different IgG classes [35], creating variable cell responses to different antibodies.

5. Fcγ receptor signaling

The human neutrophil expresses two unique activating Fc receptors: FcγRIIa and FcγRIIIb. FcγRIIa is a receptor containing ITAM sequences [36, 69], and it signals similarly to other typical immunoreceptors, such as the antigen receptor of T lymphocytes (TCR) and the antigen receptor of B lymphocytes (BCR) [70]. The initial signaling steps for all immunoreceptors are alike and involve first cross-linking of the receptors on the membrane of the cell, followed by the activation of Src family tyrosine kinases (**Figure 4**). These kinases lead to activation of spleen

tyrosine kinase (Syk), which in turn phosphorylates tyrosines within the ITAM sequence. Phosphorylated ITAM then becomes a binding site for Syk. After binding to the receptor, Syk phosphorylates multiple substrates leading to different cell responses [6, 31, 71] (**Figure 4**). Syk can phosphorylate and activate phospholipase Cγ (PLCγ), which in turn generates diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG also activates protein kinase C (PKC), an important serine/threonine kinase that can lead to the activation of MAP kinases extracellular signal-regulated kinase (ERK) and p38 (**Figure 4**). IP_3 induces release of intracellular calcium from the endoplasmic reticulum. Calcium regulates several proteins such as calmodulin and calcineurin. Syk can also induce activation of phosphatidylinositol-3 kinase (PI3K), which produces phosphatidylinositol 3,4,5-trisphosphate (PIP_3). This phospholipid is relevant to the activation of small GTPases, such as Rho and Rac, which are involved in cytoskeleton remodeling for phagocytosis. Rac also leads to activation of the MAPK/ERK kinase (MEK)—ERK pathway, and to activation of c-Jun N-terminal kinases (JNK). These kinases are important for activation of nuclear factors, such as Elk-1, AP-1, and nuclear factor of activated T cells (NFAT) (**Figure 4**). These nuclear factors induce the expression of cytokines important for inflammation and immune regulation, such as IL-2, IL-6, IL-8, IL-10, tumor necrosis factor α (TNF-α), and IFN-γ [72–74] (**Figure 4**).

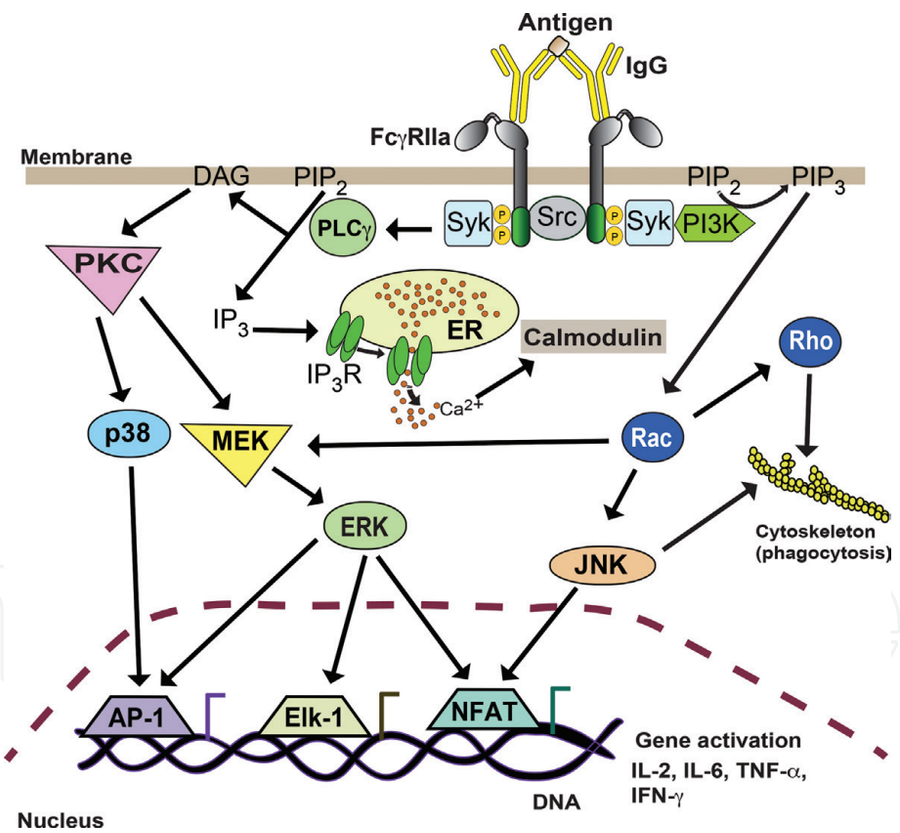

Figure 4.
Signaling transduction pathway of the neutrophil FcγRIIa. Engagement of activating FcγRIIa by IgG-antigen immune complexes induces receptor cross-linking and phosphorylation of tyrosine residues in the immunoreceptor tyrosine-based activation motif domains (green oval) by Src family kinases. Phosphorylated tyrosines then become docking sites for Syk, which in turn phosphorylates multiple substrates leading to different signaling pathways that ultimately activate various cell responses. See text for details. P represents a phosphate group; Syk, spleen tyrosine kinase; PI3K, phosphatidylinositol-3 kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; JNK, c-Jun N-terminal kinase; NFAT, nuclear factor of activated T cells; PLCγ, phospholipase Cγ; DAG, diacylglycerol; IP_3, inositol triphosphate; IP_3R, receptor for IP_3; ER, endoplasmic reticulum; PKC, protein kinase C; MEK, MAPK/ERK kinase; ERK, extracellular signal-regulated kinase; p38, p38 MAP kinase; AP-1, activator protein 1; Elk-1, Ets LiKe gene1 (ETS) transcription factor; IL-2, interleukin-2; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; and IFN-γ, interferon-γ.

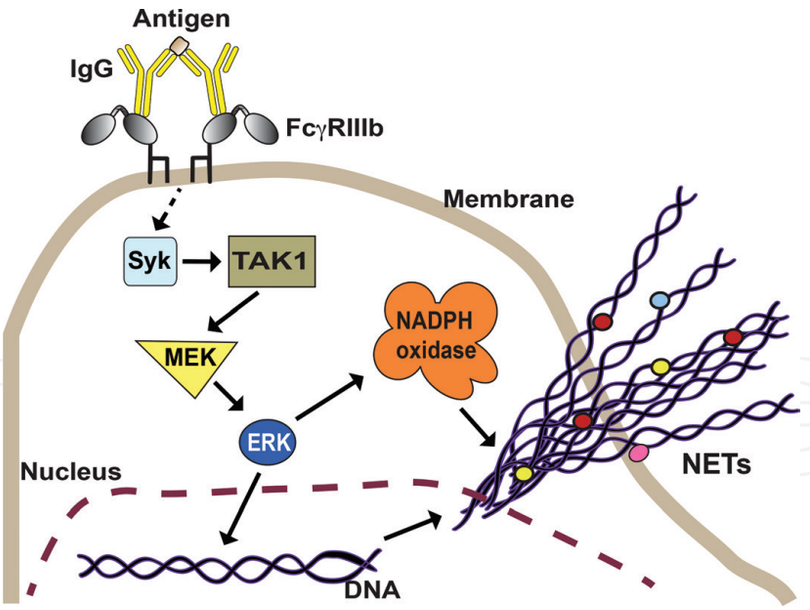

Figure 5.
Signaling transduction pathway of the neutrophil FcγRIIIb. Cross-linking of the human FcγRIIIb by IgG-antigen immune complexes induces activation of spleen tyrosine kinase (Syk) by a mechanism not yet described. Syk then activates transforming growth factor-β-activated kinase 1 (TAK1). TAK1 is in turn required for activation of ERK kinase (MEK) and extracellular signal regulated kinase (ERK). Activated ERK signals to the nucleus and contributes to activation of NADPH oxidase, which together lead to formation of neutrophil extracellular traps (NETs).

In contrast, the human FcγRIIIb is a GPI-linked receptor that lacks an intracellular portion. Thus, it is not clear how it can connect to intracellular signaling molecules. However, there is no doubt that FcγRIIIb is an activating receptor inducing several neutrophil responses such as increase in calcium concentration [75], activation of the respiratory burst [76], activation of integrins [77], and induction of NETosis [78, 79]. Despite, the initial signaling mechanism for FcγRIIIb remains unknown, the signaling pathway for this receptor engages Syk and then transforming growth factor-β-activated kinase 1 (TAK1), as well as the MEK/ERK cascade [80] (**Figure 5**). One possibility to connect FcγRIIIb with Syk is that the receptor could link with signaling molecules such as Src family tyrosine kinases on the plane of the cell membrane. Because GPI-linked proteins, like the FcγRIIIb, concentrate in lipid rafts on the cell membrane together with Src kinases [81, 82], we can imagine that after cross-linking FcγRIIIb, it associates somehow with these kinases and activates Syk. A possible connection is the binding of the receptor, within the lipid rafts, to a putative ITAM-containing molecule [83]. Many steps are still unknown and future research will help in completely elucidate this signaling pathway.

6. Each FcγR leads to unique cellular responses

The signaling pathways activated by immune complexes binding to Fcγ receptors stimulate different neutrophil responses including phagocytosis, respiratory burst, cytokine and chemokine production, and antibody-dependent cellular cytotoxicity (ADCC) [7, 8, 33]. However, our understanding of what particular function is activated in a cell responding to an individual type of FcγR is still very limited. This lack of knowledge is due, in part, to the fact that each cell expresses several types of FcγRs and all receptors can bind to more than one type of IgG. Thus, it is not clear whether each receptor leads to a particular response or the average signaling from various receptors activates a predetermined cell response. Traditionally, it

has been thought that each cell is set to activate a particular cell function after FcγR cross-linking. More recently, however, another interpretation has been considered: each FcγR activates a particular signaling pathway leading to a unique cell response. In the traditional view, each cell is already programmed to perform a particular cell function after FcγR cross-linking, independently of the receptor used. This idea is not really supported by experimental evidence. As indicated above, different IgG subclasses bind particular Fcγ receptors with different affinity, leading to unique cell functions *in vivo* [42]. In the most recent view, each FcγR activates a distinctive signaling pathway leading to an individual cell function. This view is supported by recent reports, where individual FcγRs on human neutrophils initiate particular cell responses [77, 78, 84–86].

The idea that particular Fcγ receptors could activate unique cell functions was initially published more than 20 years ago. It was found that the neutrophil FcγRIIIb induced actin polymerization in a Ca^{2+}-dependent manner, while FcγRIIa did not [87]. This initial report was not followed by similar reports and the idea of one receptor one response was forgotten. However, with time, other reports have provided new evidence that supports this idea. Some years later, it was reported that FcγRIIa, but not FcγRIIIb caused shedding of L-selectin expression [88] (**Figure 6**). Consequently, it was proposed that binding of antibodies to FcγRIIIb could induce a proadhesive phenotype of neutrophils [88]. More recently, new evidence supporting this idea was found. When each receptor was selectively activated with specific monoclonal antibodies, FcγRIIIb but not FcγRIIa, was able to activate β1 integrins [77] (**Figure 7**). This activation resulted from an increase in binding affinity to fibronectin [77]. Thus, after neutrophils leave the circulation, engagement of FcγRIIIb could lead to activation β1 integrins, allowing the cells to adhere to extracellular matrix proteins and migrate into tissues [89] (**Figure 1**). In contrast, for antibody-mediated phagocytosis [17], FcγRIIa was the main Fcγ receptor mediating this response, while FcγRIIIb contribution to phagocytosis was minimal [86]. Therefore, at least in human neutrophils, each Fcγ receptor initiates particular cell functions. FcγRIIa induces phagocytosis (**Figure 6**), while FcγRIIIb promotes an adhesive phenotype via activation of β1 integrins (**Figure 7**).

In addition, it was also reported that FcγRIIIb signals to the neutrophil nucleus more efficiently than FcγRIIa. FcγRIIIb, but not FcγRIIa, induced a large increase

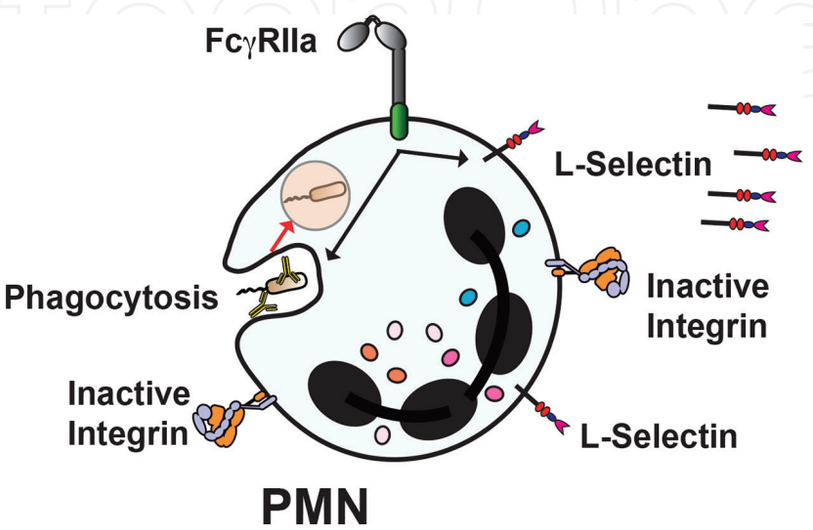

Figure 6.
Neutrophil functions activated by FcγRIIa. In human neutrophils, FcγRIIa signaling induces L-selectin shedding from the cell membrane, and also activates efficient phagocytosis. The oval represents IgG-opsonized bacteria.

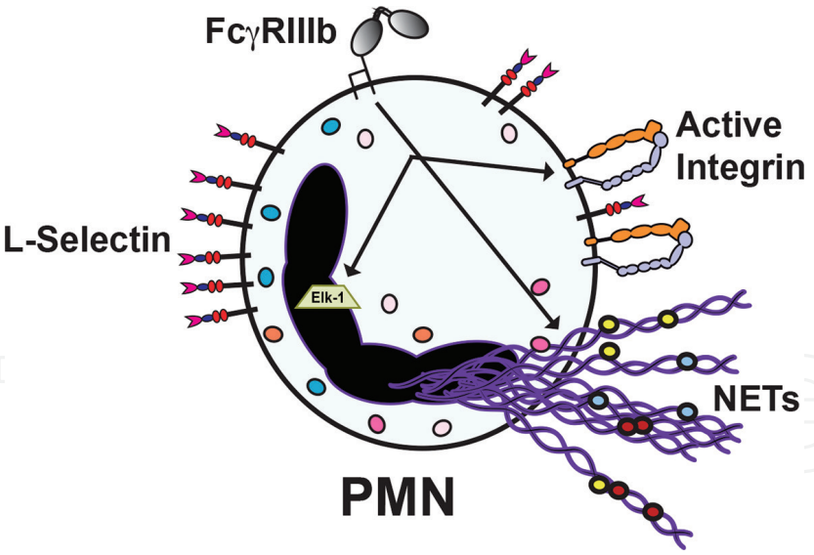

Figure 7.
Neutrophil functions activated by FcγRIIIb. Cross-linking of the human FcγRIIIb stimulates activation of β1 integrins promoting in this way a proadhesive neutrophil phenotype. FcγRIIIb also induces activation of the nuclear factor Elk-1 and formation of neutrophil extracellular traps (NETs).

in phosphorylated ERK in the nucleus, and also efficient phosphorylation of the nuclear factor Elk-1 [84] (**Figure 7**). Interestingly, FcγRIIa also induced phosphorylation of ERK in the cytosol [84, 90], but this active ERK seems to function mainly in enhancing phagocytosis and not in nuclear signaling [91] (**Figure 4**).

A recently discovered antimicrobial function of neutrophils is the formation of neutrophil extracellular traps (NETs) [92, 93]. NETs are induced by several pathogens, including virus, bacteria, fungi, and parasites [94]. Also, pro-inflammatory stimuli such as IL-8, TNF-α, and phorbol-12-myristate-13-acetate (PMA) are efficient inducers of NETs [95]. Because, antigen-antibody complexes are also capable of inducing NET formation [96]; it was clear that FcγRs were involved in NET formation. Recently, it was found that FcγRIIIb, but not FcγRIIa, is the receptor responsible for NET formation [78–80] (**Figure 8**).

Together, all these reports strongly reinforce the modern view that each FcγR induces a particular signaling pathway that activates a single cellular function. Elucidating the conditions that engage a single type of FcγR to activate a particular cellular response would be very helpful in the future for controlling some of cellular

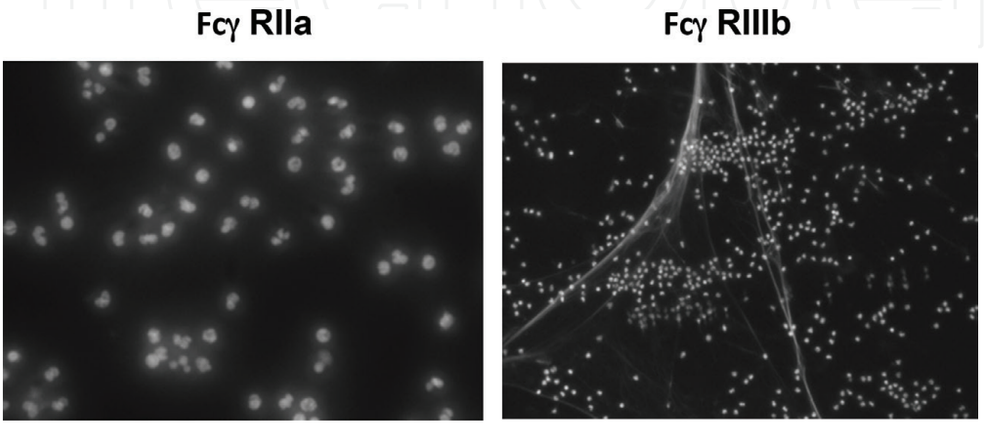

Figure 8.
Neutrophil FcγRIIIb, but not FcγRIIa, induces neutrophil extracellular traps (NETs) formation. Human neutrophils were stimulated by cross-linking FcγRIIa with the specific monoclonal antibody IV.3, or by cross-linking FcγRIIIb with the specific monoclonal antibody 3G8. After 4 hours, neutrophils were fixed and stained for DNA.

functions in clinical settings. For example, in intense infections, it may be important to activate phagocytosis. Because IgG2 binds better to FcγRIIa than to FcγRIIIb [33, 35], it is likely that IgG2 antibodies would activate phagocytosis by neutrophils much better than other IgG subclass antibodies. In consequence, promoting IgG2 antibodies against certain pathogens would result in better phagocytosis against them.

7. Conclusion

Fcγ receptors expressed in different immune cells are capable of activating different cellular responses important not only for controlling microbial infections but also for regulating immunity [71, 97]. Different subclasses of IgG antibodies bind the various Fcγ receptors with different affinities [33, 35] and can activate various cellular functions of great importance for host defense and for immune regulation. In the human neutrophil, it is clear that a specific Fcγ receptor activates particular cellular responses. FcγRIIa induces efficient phagocytosis [86], while FcγRIIIb signals to the nucleus for nuclear factor activation [84] and for NETs formation [78]. Therefore, in principle, a particular cell response could be induced or inhibited by engaging or blocking the corresponding FcγR. Information similar to the one described for neutrophil Fcγ receptors on other immune cells, such as monocytes or dendritic cells, is not available. Future research is needed in this area.

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References

[1] Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annual Review of Pathology. 2014;**9**:181-218. DOI: 10.1146/annurev-pathol-020712-164023

[2] Tecchio C, Cassatella MA. Neutrophil-derived chemokines on the road to immunity. Seminars in Immunology. 2016;**28**:119-128. DOI: 10.1016/j.smim.2016.04.003

[3] Greenlee-Wacker MC. Clearance of apoptotic neutrophils and resolution of inflammation. Immunological Reviews. 2016;**273**:357-370. DOI: 10.1111/imr.12453

[4] Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. Blood. 2014;**124**:710-719. DOI: 10.1182/blood-2014-03-453217

[5] Nauseef WM, Borregaard N. Neutrophils at work. Nature Immunology. 2014;**15**:602-611. DOI: 10.1038/ni.2921

[6] Rosales C, Uribe-Querol E. Antibody—Fc receptor interactions in antimicrobial functions. Current Immunology Reviews. 2013;**9**:44-55. DOI: 10.2174/15733955113090 10006

[7] Rosales C, Uribe-Querol E. Fc receptors: Cell activators of antibody functions. Advances in Bioscience and Biotechnology. 2013;**4**:21-33. DOI: 10.4236/abb.2013.44A004

[8] Nimmerjahn F, Ravetch JV. FcγRs in health and disease. Current Topics in Microbiology and Immunology. 2011;**350**:105-125. DOI: 10.1007/82_2010_86

[9] Ravetch JV. Fc receptors. In: Paul WE, editor. Fundamental Immunology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2003. pp. 631-684

[10] Borregaard N. Neutrophils, from marrow to microbes. Immunity. 2010;**33**:657-670. DOI: 10.1016/j.immuni.2010.11.011

[11] Deniset JF, Kubes P. Recent advances in understanding neutrophils. F1000Res. 2016;**5**:2912. DOI: 10.12688/f1000research.9691.1

[12] Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nature Reviews. Immunology. 2013;**13**:159-175. DOI: 10.1038/nri3399

[13] Ley K, Laudanna C, Cybulsky M, Nourshargh S. Getting to the site of inflammation: The leukocyte adhesion cascade updated. Nature Reviews. Immunology. 2007;**7**:678-689. DOI: 10.1038/nri2156

[14] Arnaout MA. Biology and structure of leukocyte β2 integrins and their role in inflammation. F1000Res. 2016;**5** (F1000 Faculty Rev):2433. DOI: 10.12688/f1000research.9415.1

[15] Kourtzelis I, Mitroulis I, von Renesse J, Hajishengallis G, Chavakis T. From leukocyte recruitment to resolution of inflammation: The cardinal role of integrins. Journal of Leukocyte Biology. 2017;**102**:677-683. DOI: 10.1189/jlb.3MR0117-024R

[16] Gordon S. Phagocytosis: An immunobiologic process. Immunity. 2016;**44**:463-475. DOI: 10.1016/j.immuni.2016.02.026

[17] Rosales C, Uribe-Querol E. Phagocytosis: A fundamental process in immunity. BioMed Research International. 2017;**2017**:9042851. DOI: 10.1155/2017/9042851

[18] Jaumouillé V, Grinstein S. Molecular mechanisms of phagosome formation. Microbiology Spectrum. 2016;**4**: MCHD-0013-2015. DOI: 10.1128/microbiolspec.MCHD-0013-2015

[19] Levin R, Grinstein S, Canton J. The life cycle of phagosomes: Formation, maturation, and resolution. Immunological Reviews. 2016;**273**: 156-179. DOI: 10.1111/imr.12439

[20] Pham C. Neutrophil serine proteases: Specific regulators of inflammation. Nature Reviews. Immunology. 2006;**6**:541-550. DOI: 10.1038/nri1841

[21] Häger M, Cowland JB, Borregaard N. Neutrophil granules in health and disease. Journal of Internal Medicine. 2010;**268**:25-34. DOI: 10.1111/j. 1365-2796.2010. 02237.x

[22] Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. Immunological Reviews. 2016;**273**:11-28. DOI: 10.1111/imr.12440

[23] Sengeløv H, Follin P, Kjeldsen L, Lollike K, Dahlgren C, Borregaard N. Mobilization of granules and secretory vesicles during *in vivo* exudation of human neutrophils. Journal of Immunology. 1995;**154**:4157-4165

[24] Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. Microbes and Infection. 2003;**5**:1317-1327. DOI: 10.1016/j.micinf.2003.09.008

[25] Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nature Immunology. 2014;**15**:1017-1025. DOI: 10.1038/ni.2987

[26] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;**303**:1532-1535. DOI: 10.1126/science.1092385

[27] Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. The Journal of Cell Biology. 2007;**176**: 231-241. DOI: 10.1083/jcb.200606027

[28] Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. The Journal of Cell Biology. 2010;**191**:677-691. DOI: 10.1083/jcb.201006052

[29] Björnsdottir H, Welin A, Michaëlsson E, Osla V, Berg S, Christenson K, et al. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. Free Radical Biology & Medicine. 2015;**89**:1024-1035. DOI: 10.1016/j.freeradbiomed.2015.10.398

[30] Ballow M. Historical perspectives in the diagnosis and treatment of primary immune deficiencies. Clinical Reviews in Allergy and Immunology. 2014;**46**:101-103. DOI: 10.1007/s12016-013-8384-9

[31] Rosales C. Fc receptor and integrin signaling in phagocytes. Signal Transduction. 2007;7:386-401. DOI: 10.1002/sita.200700141

[32] Heyman B. Regulation of antibody responses via antibodies, complement, and Fc receptors. Annual Review of Immunology. 2000;**18**:709-737. DOI: 10.1146/annurev.immunol.18.1.709

[33] Bruhns P, Jönsson F. Mouse and human FcR effector functions.

Immunological Reviews. 2015;**268**: 25-51. DOI: 10.1111/imr.12350

[34] Daëron M. Fc receptor biology. Annual Review of Immunology. 1997;**15**:203-234. DOI: 10.1146/annurev.immunol.15.1.203

[35] Bruhns P, Iannascoli B, England P, Mancardi D, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. Blood. 2009;**113**:3716-3725. DOI: 10.1182/blood-2008-09-179754

[36] Underhill DM, Goodridge HS. The many faces of ITAMs. Trends in Immunology. 2007;**28**:66-73. DOI: 10.1016/j.it.2006.12.004

[37] Stefanescu RN, Olferiev M, Liu Y, Pricop L. Inhibitory Fcγ receptors: From gene to disease. Journal of Clinical Immunology. 2004;**24**:315-326. DOI: 10.1023/B:JOCI.0000029105.47772.04

[38] Baerenwaldt A, Lux A, Danzer H, Spriewald BM, Ullrich E, Heidkamp G, et al. Fcγ receptor IIB (FcγRIIB) maintains humoral tolerance in the human immune system *in vivo*. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**:18772-18777. DOI: 10.1073/pnas.1111810108

[39] Daëron M, Lesourne R. Negative signaling in Fc receptor complexes. Advances in Immunology. 2006;**89**:39-86. DOI: 10.1016/S0065-2776(05)89002-9

[40] Nimmerjahn F, Ravetch J. Fcγ receptors as regulators of immune responses. Nature Reviews. Immunology. 2008;**8**:34-47. DOI: 10.1038/nri2206

[41] Lehmann B, Schwab I, Böhm S, Lux A, Biburger M, Nimmerjahn F. FcγRIIB: A modulator of cell activation and humoral tolerance. Expert Review of Clinical Immunology. 2012;**8**:243-254. DOI: 10.1586/eci.12.5

[42] Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. Immunological Reviews. 2010;**236**:265-275. DOI: 10.1111/j.1600-065X.2010.00910.x

[43] Willcocks LC, Smith KG, Clatworthy MR. Low-affinity Fcγ receptors, autoimmunity and infection. Expert Reviews in Molecular Medicine. 2009;**11**:e24. DOI: 10.1017/S1462399409001161

[44] Nimmerjahn F, Ravetch J. Fcγ receptors: Old friends and new family members. Immunity. 2006;**24**:19-28. DOI: 10.1016/j.immuni.2005.11.010

[45] Anthony RM, Wermeling F, Ravetch JV. Novel roles of the IgG Fc glycan. Annals of the New York Academy of Sciences. 2012;**1253**:170-180. DOI: 10.1111/j.1749-6632.2011.06305.x

[46] Raju T. Terminal sugars of Fc glycans influence antibody effector functions of IgGs. Current Opinion in Immunology. 2008;**20**:471-478. DOI: 10.1016/j.coi.2008.06.007

[47] Schroeder HWJ, Cavacini L. Structure and function of immunoglobulins. The Journal of Allergy and Clinical Immunology. 2010;**125**:S41-S52. DOI: 10.1016/j.jaci.2009.09.046

[48] Uchida J, Hamaguchi Y, Oliver J, Ravetch J, Poe J, Haas K, et al. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. The Journal of Experimental Medicine. 2004;**199**:1659-1669. DOI: 10.1084/jem.20040119

[49] Lambert S, Okada C, Levy R. TCR vaccines against a murine T cell lymphoma: A primary role for

antibodies of the IgG2c class in tumor protection. Journal of Immunology. 2004;**172**:929-936. DOI: 10.4049/jimmunol.172.2.929

[50] Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch J. FcγRIV: A novel FcR with distinct IgG subclass specificity. Immunity. 2005;**23**:41-51. DOI: 10.1016/j.immuni.2005.05.010

[51] Nimmerjahn F, Ravetch J. Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science. 2005;**310**:1510-1512. DOI: 10.1126/science.1118948

[52] Wright A, Morrison SL. Effect of glycosylation on antibody function: Implications for genetic engineering. Trends in Biotechnology. 1997;**15**:26-32. DOI: 10.1016/S0167-7799(96)10062-7

[53] Shields R, Namenuk A, Hong K, Meng Y, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. The Journal of Biological Chemistry. 2001;**276**:6591-6604. DOI: 10.1074/jbc.M009483200

[54] Arnold J, Wormald M, Sim R, Rudd P, Dwek R. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annual Review of Immunology. 2007;**25**:21-50. DOI: 10.1146/annurev.immunol.25.022106.141702

[55] Jefferis R. Isotype and glycoform selection for antibody therapeutics. Archives of Biochemistry and Biophysics. 2012;**526**:159-166. DOI: 10.1016/j.abb.2012.03.021

[56] Mizuochi T, Taniguchi T, Shimitzu A, Kobata A. Structural and numerical variations of the carbohydrate moiety of immunoglobulin G. Journal of Immunology. 1982;**129**:2016-2020

[57] Shields R, Lai J, Keck R, O'Connell L, Hong K, Meng Y, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. The Journal of Biological Chemistry. 2002;**277**:26733-26740. DOI: 10.1074/jbc.M202069200

[58] Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, et al. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. The Journal of Biological Chemistry. 2003;**278**:3466-3473. DOI: 10.1074/jbc.M210665200

[59] Kubota T, Niwa R, Satoh M, Akinaga S, Shitara K, Hanai N. Engineered therapeutic antibodies with improved effector functions. Cancer Science. 2009;**100**:1566-1572. DOI: 10.1111/j.1349-7006.2009.01222.x

[60] Varki A. "Unusual" modifications and variations of vertebrate oligosaccharides: Are we missing the flowers from the trees? Glycobiology. 1996;**6**:707-710

[61] Kaneko Y, Nimmerjahn F, Ravetch J. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science. 2006;**313**:670-673. DOI: 10.1126/science.1129594

[62] Scallon B, Tam S, McCarthy S, Cai A, Raju T. Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Molecular Immunology. 2007;**44**:1524-1534. DOI: 10.1016/j.molimm.2006.09.005

[63] Anthony R, Wermeling F, Karlsson M, Ravetch J. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proceedings of the National Academy of Sciences

of the United States of America. 2008;**105**:19571-19578. DOI: 10.1073/pnas.0810163105

[64] van Sorge NM, van der Pol WL, van de Winkel JG. FcγR polymorphisms: Implications for function, disease susceptibility and immunotherapy. Tissue Antigens. 2003;**61**:189-202. DOI: 10.1034/j.1399-0039.2003.00037.x

[65] Warmerdam PAM, van de Winkel JG, Gosselin EJ, Capel PAJ. Molecular basis for a polymorphism of human Fcγ receptor II (CD32). The Journal of Experimental Medicine. 1990;**172**:19-25. DOI: 10.1084/jem.172.1.19

[66] Huizinga TWJ, Kleijer M, Tetteroo PAT, Roos D, von dem Borne AEGK. Biallelic neutrophil Na-antigen system is associated with a polymorphism on the phospho-inositol-linked Fcγ receptor III (CD16). Blood. 1990;**75**:213-217

[67] Ory PA, Clark MR, Kwoh EE, Clarkson SB, Goldstein IM. Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on human neutrophils. The Journal of Clinical Investigation. 1989;**84**: 1688-1691. DOI: 10.1172/JCI114350

[68] Bux J, Stein E-L, Bierling P, Fromont P, Clay M, Stroncek D, et al. Characterization of a new alloantigen (SH) on the human neutrophil Fcγ receptor IIIB. Blood. 1997;**89**: 1027-1034

[69] Fodor S, Jakus Z, Mócsai A. ITAM-based signaling beyond the adaptive immune response. Immunology Letters. 2006;**104**:29-37. DOI: 10.1016/j.imlet.2005.11.001

[70] Getahun A, Cambier JC. Of ITIMs, ITAMs, and ITAMis: Revisiting immunoglobulin Fc receptor signaling. Immunological Reviews. 2015;**268**: 66-73. DOI: 10.1111/imr.12336

[71] Bournazos S, Ravetch JV. Fcγ receptor pathways during active and passive immunization. Immunological Reviews. 2015;**268**:88-103. DOI: 10.1111/imr.12343

[72] Sánchez-Mejorada G, Rosales C. Signal transduction by immunoglobulin Fc receptors. Journal of Leukocyte Biology. 1998;**63**:521-533. DOI: 10.1002/jlb.63.5.521

[73] Mócsai A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. The Journal of Experimental Medicine. 2013;**210**:1283-1299. DOI: 10.1084/jem.20122220

[74] Uribe-Querol E, Rosales C. Neutrophils in cancer: Two sides of the same coin. Journal of Immunology Research. 2015;**2015**:983698. DOI: 10.1155/2015/983698

[75] Rosales C, Brown EJ. Signal transduction by neutrophil IgG Fc receptors: Dissociation of [Ca^{+2}] rise from IP_3. The Journal of Biological Chemistry. 1992;**267**:5265-5271

[76] Löfgren R, Serrander L, Forsberg M, Wilsson A, Wasteson A, Stendahl O. CR3, FcγRIIA and FcγRIIIB induce activation of the respiratory burst in human neutrophils: The role of intracellular $Ca^{(2+)}$, phospholipase D and tyrosine phosphorylation. Biochimica et Biophysica Acta. 1999;**1452**:46-59. DOI: 10.1016/S0167-4889(99)00112-3

[77] Ortiz-Stern A, Rosales C. FcγRIIIB stimulation promotes β1 integrin activation in human neutrophils. Journal of Leukocyte Biology. 2005;**77**:787-799. DOI: 10.1189/jlb.0504310

[78] Alemán OR, Mora N, Cortes-Vieyra R, Uribe-Querol E, Rosales C. Differential use of human neutrophil Fcγ receptors for inducing

neutrophil extracellular trap formation. Journal of Immunology Research. 2016;**2016**:142643. DOI: 10.1155/2016/2908034

[79] Behnen M, Leschczyk C, Möller S, Batel T, Klinger M, Solbach W, et al. Immobilized immune complexes induce neutrophil extracellular trap release by human neutrophil granulocytes via FcγRIIIB and Mac-1. Journal of Immunology. 2014;**193**:1954-1965. DOI: 10.4049/jimmunol.1400478

[80] Alemán OR, Mora N, Cortes-Vieyra R, Uribe-Querol E, Rosales C. Transforming growth factor-β-activated kinase 1 is required for human FcγRIIIb-induced neutrophil extracellular trap formation. Frontiers in Immunology. 2016;**7**:277. DOI: 10.3389/fimmu.2016.00277

[81] Reeves VL, Thomas CM, Smart EJ. Lipid rafts, caveolae and GPI-linked proteins. Adv. Exp. Med. Biol. 2012;**729**:3-13. DOI: 10.1007/978-1-4614-1222-9_1

[82] Paladino S, Lebreton S, Zurzolo C. Trafficking and membrane organization of GPI-anchored proteins in health and diseases. Current Topics in Membranes. 2015;**75**:269-303. DOI: 10.1016/bs.ctm.2015.03.006

[83] García-García E, Rosales C. Fc receptor signaling in leukocytes: Role in host defense and immune regulation. Current Immunology Reviews. 2009;**5**:227-242. DOI: 10.2174/157339509788921229

[84] García-García E, Nieto-Castañeda G, Ruiz-Saldaña M, Mora N, Rosales C. FcγRIIA and FcγRIIIB mediate nuclear factor activation through separate signaling pathways in human neuthophils. Journal of Immunology. 2009;**182**:4547-4556. DOI: 10.4049/jimmunol.0801468

[85] García-García E, Uribe-Querol E, Rosales C. A simple and efficient method to detect nuclear factor activation in human neutrophils by flow cytometry. Journal of Visualized Experiments. 2013;**74**:e50410. DOI: 10.3791/50410

[86] Rivas-Fuentes S, García-García E, Nieto-Castañeda G, Rosales C. Fcγ receptors exhibit different phagocytosis potential in human neutrophils. Cellular Immunology. 2010;**263**:114-121. DOI: 10.1016/j.cellimm.2010.03.006

[87] Salmon JE, Browle NL, Edberg JC, Kimberly RP. Fcγ receptor III induces actin polymerization in human neutrophils and primes phagocytosis mediated by Fcγ receptor II. Journal of Immunology. 1991;**146**:997-1004

[88] Kocher M, Siegel ME, Edberg JC, Kimberly RP. Cross-linking of Fcγ receptor IIa and Fcγ receptor IIIb induces different proadhesive phenotypes on human neutrophils. Journal of Immunology. 1997;**159**:3940-3948

[89] Ortiz-Stern A, Rosales C. Cross-talk between Fc receptors and integrins. Immunology Letters. 2003;**90**:137-143. DOI: 10.1016/j.imlet.2003. 08.004

[90] Coxon PY, Rane MJ, Powell DW, Klein JB, McLeish KR. Differential mitogen-activated protein kinase stimulation by Fcγ receptor IIa and Fcγ receptor IIIb determines the activation phenotype of human neutrophils. Journal of Immunology. 2000;**164**:6530-6537. DOI: 10.4049/jimmunol.164.12.6530

[91] Garcia-Garcia E, Rosales C. Adding complexity to phagocytic signaling: Phagocytosis-associated cell responses and phagocytic efficiency. In: Rosales C, editor. Molecular Mechanisms of Phagocytosis. Georgetown, Texas: Landes Bioscience/Springer Science; 2005. pp. 58-71

[92] Brinkmann V, Zychlinsky A. Beneficial suicide: Why neutrophils die to make NETs. Nature Reviews. Microbiology. 2007;**5**:577-582. DOI: 10.1038/nrmicro1710

[93] Yipp BG, Kubes P. NETosis: How vital is it? Blood. 2013;**122**:2784-2794. DOI: 10.1182/blood-2013-04-457671

[94] Branzk N, Papayannopoulos V. Molecular mechanisms regulating NETosis in infection and disease. Seminars in Immunopathology. 2013;**35**:513-530. DOI: 10.1007/s00281-013-0384-6

[95] Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: Is immunity the second function of chromatin? The Journal of Cell Biology. 2012;**198**:773-783. DOI: 10.1083/jcb.201203170

[96] Short KR, von Köckritz-Blickwede M, Langereis JD, Chew KY, Job ER, Armitage CW, et al. Antibodies mediate formation of neutrophil extracellular traps in the middle ear and facilitate secondary pneumococcal otitis media. Infection and Immunity. 2014;**82**:364-370. DOI: 10.1128/IAI.01104-13

[97] Yu X, Lazarus AH. Targeting FcγRs to treat antibody-dependent autoimmunity. Autoimmunity Reviews. 2016;**15**:510-512. DOI: 10.1016/j.autrev.2016.02.006