In: Advances in Medicine and Biology. Volume 15 ISBN: 978-1-61122-467-2
Editor: Leon V. Berhardt

Chapter 3

FcγRs on Human Neutrophils: Emerging Patho-Physiological Concerns

Louis Marois, Emmanuelle Rollet-Labelle and Paul H. Naccache
Centre de recherche en rhumatologie et immunologie, Centre de recherche du CHUQ, Department of Microbiology-Infectiology and Immunology, Faculty of Medicine, Laval University, Québec, Canada

Abstract

In the immune system, phagocytes resolve a very large proportion of infections subsequently to the recognition and the destruction of pathogens. This crucial function is enhanced by the opsonisation of pathogens with immunoglobulins (mostly immunoglobulin G (IgG)). The latter are recognized by Fcγ receptors (FcγRs) through the Fc portion of IgGs. FcγRs also interact with IgG-containing immune complexes. Human neutrophils, the most abundant circulating phagocytes, constitutively express two types of FcγRs, FcγRIIa (CD32a) and FcγRIIIb (CD16b). This combination of isoforms is unique to human neutrophils. FcγRIIa possesses an Immunoreceptor Tyrosine-based Activation Motif (ITAM) in its cytoplasmic portion, whereas FcγRIIIb is a membrane-anchored receptor which does not possess any cytoplasmic sequence. Neutrophils are the only human phagocytes which do not express Immunoreceptor Tyrosine-based Inhibition Motif (ITIM)-bearing FcγR, i.e. FcγRIIb, which is thought to downregulate the signal of ITAM-bearing FcγRs. The absence of FcγRIIb on human neutrophils indicates that alternative negative regulatory processes may be at play. A recent study from our laboratory described a novel down-regulation mechanism of the activation of FcγRIIa mediated by a proteasomal degradation process.

Little is known about the biological relevance of the co-expression of two FcγRs with overlapping ligand-binding specificity on human neutrophils. Cholesterol-rich and detergent-resistant plasma membrane micro-domains (DRMs or lipid rafts) appear to be required for the transmission of the appropriate signal leading to the functional

responsiveness of neutrophils. Our previous studies provided evidence for a recruitment of both FcγR isoforms in these micro-domains. FcγRs recruitment in DRMs is a very early event observed following their cross-linking. These observations suggest that cooperation between FcγRIIa and FcγRIIIb, leading to an optimal neutrophil response, may take place in these domains. We are presently investigating this functional cooperation and our results indicate that both isoforms are essential for an optimal phagocytosis.

Beside their roles in innate immune responses, FcγRs could be also involved in patho-physiological responses. The presence of immune complexes (ICs) in auto-immune diseases (e.g., rheumatoid arthritis or systemic lupus erythematosus) may potently activate effector cells (including neutrophils) via cross-linking of FcγRs. Accordingly, FcγR polymorphisms have been identified as risk factors in specific pathologies or infections and an ever enlarging number of studies illustrate the contribution of this family of receptors to the pathogenesis of autoimmune diseases. The potential mechanisms by which the human neutrophil FcγRs may contribute to autoimmune diseases will be discussed and the possibilities for the development of medical strategies targeting FcγRs will also be reviewed.

Introduction

Polymorphonuclear neutrophils play an important role in the innate immune system where one of their major, though not unique, functions is to internalise and degrade pathogens or immune complexes. This phagocytic process is greatly aided by opsonisation of pathogens by complement fragments and immunoglobulins, mostly IgGs. The latter are recognised, through their Fc tails, by Fcγ receptors. Recognition of pathogens or immune complexes by FcγRs initiates intracellular signals that lead to multiple neutrophil physiological responses including degranulation, activation of the respiratory burst and phagocytosis [1-5].

FcγRs are important key activators of the immune system. They play major roles in host resistance linking humoral and cellular responses, in particular in the contexts of phagocytosis, antibody-dependent cell cytotoxicity (ADCC), enhanced antigen presentation and clearance of immune complexes (ICs) [6-8]. These receptors have also been associated with susceptibility to a number of autoimmune diseases [9, 10].

FcγRs derive their names from their capacity to recognize the crystallizable fragment (Fc) region of antibodies. FcRs are classified on the basis of their specificity for the recognition of a particular antibody isotype. FcγRs belong to the large immunoglobulin superfamily and recognize the Fc portion of IgGs [10-12]. The human FcγR system comprises FcγRI (also known as cluster of differentiation 64 (CD64)), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRIIc (CD32c), FcγRIIIa (CD16a) and FcγRIIIb (CD16b). With the exception of FcγRIIIb, these receptors are type I transmembrane glycoproteins. They differ in their affinities for various classes of antibodies due to their different molecular structure [13]. Their structure comprises N-terminal extracellular Ig-like domains, a transmembrane region and a C-terminal cytoplasmic portion. FcγRIa and FcγRIIIa are multimeric receptors with a ligand-binding α-chain and a signal-transducing γ-chain dimer. FcγRIIa and FcγRIIb are single chain receptors. FcγRIIIb is unique in that it is attached to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor and does not possess any cytoplasmic domain [14].

Human FcγRs (with the exception of FcγRIIIb) are associated with an immunoreceptor tyrosine-based activation motif (ITAM)-containing subunit or possess an ITAM or an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains. These tyrosine-containing motifs are crucial for downstream cell signalling. ITAM-containing FcγRs are usually considered in the context of the activation of immune cells while FcγRIIb is the only known "inhibitory" FcγR. The FcγRs family comprising activating and inhibitory receptors provides a prime example of how simultaneous triggering of activating and inhibitory signalling pathways can generate a well-balanced immune response.

The distribution of the different FcγRs is cell- and species-specific (each cell type possessing a distinct combination of FcγRs). Despite similarities between human and mouse FcγRs, there are major differences in the intracellular domains and the expression pattern of these receptors. For example, neither FcγRIIa nor FcγRIIIb are expressed in mice. Although FcγRs are predominantly expressed on hematopoietic cells, they are also present, among others, on endothelial cells [15], osteoclasts [16] and on cardiomyocytes [17].

Expression of FcγRs on Human Neutrophils

Isoform Expression and Structure

Human neutrophils constitutively and specifically express FcγRIIa and FcγRIIIb. The expression of FcγRI on these cells is observed only following incubation with pro-inflammatory cytokines [18-20]. This combination of FcγRs is not found on other human phagocytes or on neutrophils from other species including mice whose neutrophils express instead FcγRIIb and FcγRIIIa. On the other hand, human neutrophils do not constitutively express ITIM-bearing FcγRs (including FcγRIIb ([8] and Figure 1)) that other immune cells (including B cells) rely upon to terminate FcγR-initiated signals.

This unique combination of FcγRs is a signature of the human neutrophil. This particularity highlights the limitations of the extrapolation of data collected in other cell types and species to human neutrophils. Studies on FcγRs performed in mice neutrophils may, in particular, not be directly applicable to human neutrophils. Additionally, efforts to humanize mouse models still do not accurately reproduce the pattern of FcγR expression of human neutrophils [10]. For these reasons, this review will strictly focus on data obtained in human neutrophils.

FcγRs Constitutively Expressed in Human Neutrophils

The structure of FcγRIIa is unique among FcγRs consisting of a single transmembrane polypeptide chain comprising two extracellular immunoglobulin domains, a single transmembrane domain and a short cytoplasmic segment containing three tyrosine residues. Two of them constitute an ITAM in which the signature tyrosines are 12 amino acids apart rather than 8 as in canonical ITAMs [21, 22]. This unique molecular signature is likely to

impact on the specific composition of the signalling complex through which FcγRIIa transmits the appropriate signals to neutrophils.

FcγRIIIb was thought to be exclusively expressed by human neutrophils [23] but a recent publication demonstrated that this receptor is also expressed by a subset of human basophils [24]. As opposed to the other FcγRs whose primary structures include a transmembrane protein, FcγRIIIB is a GPI-linked receptor with no transmembrane or cytoplasmic domains [25]. Its gene (FCGR3B) is very similar to that of FcγRIIIa, but a mutation at nucleotide 733 encodes a stop codon that prevent the translation of the 21 c-terminal amino acids that differentiate these two isoforms [1]. The density of FcγRIIIb is 4-5 times higher than that of FcγRIIa in human neutrophils (135 000 vs 31 000 per cell) and FcγRIIIb is the predominant FcγR in blood [14]. In contrast to FcγRIIa that is exclusively expressed at the plasma membrane, FcγRIIIb is also present in intracellular vesicules [26].

Inducible FcγR Isoform in Human Neutrophils

Little is known about the high-affinity FcγRI in human neutrophils. It is induced after 4 to 24 hours of interferon (IFN)-γ or granulocyte colony-stimulating factor (G-CSF) incubation *in vitro* and *in vivo* [20, 27-34] and a direct effect of these cytokines on FcγRI gene expression at the transcriptional level has been proposed [35]. Cross-linking of β2 integrins also leads to an up-regulation of FcγRI on human neutrophils [36]. This inducible FcγR isoform possesses three Ig-like domains in its extracellular domain that are thought to contribute to its high affinity for IgG. This particular structure allows FcγRI activation by monomeric IgG in contrast to FcγRIIa and FcγRIIIb that require multimerization to be engaged [37]. The level of FcγRI expression is often considered to be a quantitative marker for the activation of the innate immunity response in the case of sepsis or infection [38, 39]. This receptor is upregulated on neutrophils during pregnancy [40-42], in preterm newborns [43] and in formula-fed as opposed to breast-fed infants [44].

The FcγrIIb Question

Most reviews on FcγRs state that neutrophils express FcγRIIb. However, although FcγRIIb was observed on resting mice neutrophils [45-47], its presence in human neutrophils remains controversial [48]. To clarify the situation, we examined the presence of FcγRIIb on different cell types including human neutrophils and in our hands, human neutrophils express little if any FcγRIIb when compared to, e.g., Raji cells (Figure 1). The low expression of FcγRIIb in human neutrophils, or its possible absence, suggests that neutrophils rely on mechanisms of limitations of the responses of FcγRs independent from it (see *Down-regulation signalling* section). It also remains to be examined whether the expression of FcγRIIb may be upregulated in human neutrophils. Relevant to this point is the recent observation that the expression of FcγRIIb on neutrophils can be detected and is increased in patients with rheumatoid arthritis who are treated with Infliximab (anti-TNFα monoclonal antibody) [49] suggesting a possible role of TNFα in the modulation of FcγR expression.

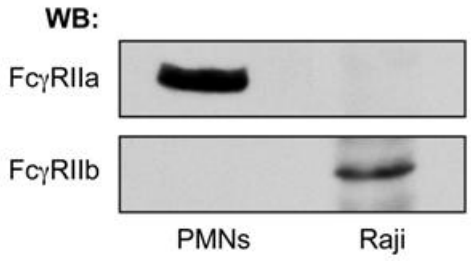

Figure 1. FcγRIIb expression on human neutrophils and Raji cell line

Aliquots of freshly isolated human neutrophils and Raji cells were boiled 7 minutes in Laemmli's sample buffer (equal protein loading). Whole cell lysates were analysed by SDS-PAGE and probed for FcγRIIb (antibody kindly provided by Dr JL Teillaud [173]) and FcγRIIa (CT10 antibody [86]). Whereas FcγRIIb is clearly detected in Raji cells, we find no evidence of its presence in human neutrophils. FcγRIIa blotting serves as a positive control for the integrity of the neutrophil samples.

The Neonatal FcR

Another IgG receptor, the neonatal FcR (FcRn), was previously described to be expressed in human neutrophils and to be present in azurophilic and specific granules [50]. It is a major histocompatibility class I (MHC-I) homolog with a structure composed of a unique α-chain coupled to β2-microglobulin [51]. Classically, this isoform is associated with IgG transport across mucosal cells [52] or from mother to foetus (via the placenta and milk) [53, 54]. FcRn of endothelial cells also contributes to the homeostatic regulation of serum IgG levels [55]. FcRn does not bind IgGs at physiologic pH (7.4). A novel role of FcRn in phagocytosis was recently described in human neutrophils where it has been proposed that the acidic conditions of the phagolysosome protonates the Fc fragment of IgGs and permits binding to FcRn [50]. Accordingly, phagocytosis was severely impaired in conditions preventing FcRn ligation (with mutated IgGs) [50]. These data are consistent with a novel mechanism of regulation of IgG-dependent phagocytosis that depends 1/ on a first signal derived from plasma membrane FcγRs which stimulates particle internalisation and the fusion of granules with the phagosome and 2/ on a subsequent acidification of the phagosome which helps generates a second signal dependent on FcRn to complete the phagocytic process.

Polymorphisms

Functionally relevant polymorphisms have been described for FcγRIIa and FcγRIIIb [56]. They are located in the extracellular Fc-binding portion of the FcγR and affect the ability of FcγRs to interact with IgG subclasses. The major FcγRIIa polymorphism is a point mutation affecting amino acid position 131, coding for either arginine (R131) or histidine (H131). These alleles are co-dominantly expressed. FcγRIIa-H131 has higher affinity for human IgG3 and is the only FcγR that interacts with IgG2. Regarding the GPI-anchored FcγRIIIb, the neutrophil antigen (NA) polymorphism results in the expression of two isoforms, termed NA1 and NA2. FcγRIIIb-NA1 has higher affinity for IgG1 and IgG3 than FcγRIIIb-NA2 [56].

Differences in phagocytic capacities of H/H and R/R FcγRIIa were previously reported [57]. Because these polymorphisms affect the affinity of the interaction of IgG with FcγR, they may have an impact on the susceptibility of developing auto-immune diseases [58] (see *Physio-pathological implications* section).

Signalling

In the following, we will limit the present discussion to common and specific signalling pathways associated with the engagement of FcγRIIa and FcγRIIIb as the signalling events associated with FcγRI have not been investigated in details as of yet. We will more specifically focus on presently incompletely understood fundamental signalling concepts associated with the activation and inhibition of these receptors.

DRMs, Common Element of Signalling Through FcγRs

Detergent resistant membrane domains (DRMs) are described as assembly platforms for a multitude of receptors [59-61]. Several immunoreceptors, including Fc receptors, are thought to initiate their signalling cascades in detergent-insoluble glycolipid-enriched domains named DRMs [62]. These lipid domains often called rafts represent signalling platforms where adaptor, anchoring and signalling proteins are regrouped and allowed to interact to generate the appropriate signals inside the cell [63]. A fraction of FcγRIIIb is constitutively present in neutrophil DRMs [64]. In contrast, FcγRIIa is recovered in the soluble region of the plasma membrane in resting neutrophils. Signalling through FcγRIIa involves its translocation to DRMs [65-69]. The transmembrane domain of the receptor plays a key role in this translocation [70]. DRMs may also play a major role in the regulation of IgG binding to FcγRIIa [71].

The specific manner in which FcγRIIIb transmits intracellular signals remains to be adequately elucidated. One view considers that FcγRIIIb, which does not associate with γ chain and has no transmembrane or cytoplasmic tail [25], transduces signals only by interaction with FcγRIIa, by favoring the recognition of immune complexes by the latter [72]. On the other hand, several lines of evidences indicate that, in human neutrophils, FcγRIIIb generates intracellular signals without necessitating the engagement of FcγRIIa. Hence, cross-linking of FcγRIIIb alone initiates multiple functions and signalling events. Lipid-lipid interactions have been postulated to mediate the activation of Src kinases by GPI-linked receptors. However, this hypothesis has yet to be experimentally tested in the context of FcγRIIIb. FcγRIIIb may cooperate with CD11b [73] although this result was not confirmed biochemically [64]. These various hypotheses remain to be rigorously tested and validated. The stimulated translocation of FcγRIIIb to DRMs [64] may in part explain its capacity to signal in the absence of a cytoplasm domain as several signalling proteins such as Src kinases are also present in these domains.

Our previous data indicate that DRMs are heterogeneous in human neutrophils and include subpopulations with low (DRM_L) and high (DRM_H) densities [64]. The functionally

relevant subpopulation of DRMs in FcγR-stimulated human neutrophils appears to be DRM_Hs as both FcγRIIIb and FcγRIIa are present in these fractions and not in DRM_Ls [64-66, 74]. DRM_Hs cannot be isolated using commonly used sucrose gradients (they are recovered in the pellet of these gradients) but require instead Optiprep gradients with their larger density ranges. They have therefore been missed by many investigators. Importantly, the translocation of FcγRIIa to DRM_Hs can be detected subsequent to its engagement in isolated plasma membranes [66] and the rapid degradation of FcγRIIa which follows its engagement in whole neutrophils requires intact DRMs [65]. Disrupting DRMs decreased FcγRIIa-dependent phagocytosis, receptor degradation and the mobilization of intracellular calcium [65, 74]. These observations indicate that DRM_Hs contain functional FcγRs signalling units. An element that has not been investigated as of yet is the potential heterogeneity of the cellular localisation of DRMs. Indeed, some of our preliminary data suggest that FcγRIIIb is present in intracellular DRMs in resting cells and is found in plasma membranes DRMs only after its cross-linking as opposed to FcγRIIa which is exclusively localised at the plasma membrane (unpublished observations). Altogether, these results provide evidence that DRMs are involved in the signalling pathways of both FcγRs in human neutrophils. The specifics of the involvement of DRMs in phagocytosis remain unclear and part of the ambiguity existing in the literature may relate to the details of the phagocytosis assay utilized (nature of the particles, type of opsonisation).

Activating Pathways

Tyrosine phosphorylation pathways play a critical role in FcγRs signalling [13, 75-78]. Cross-linking of FcγRIIa enhances the level of tyrosine phosphorylation in intact human neutrophils [65, 75]. FcγRIIa engagement leads to the tyrosine phosphorylation of the receptor itself [13, 66, 79, 80]. Intracellular Src family kinases are activated upon clustering of FcγRs and phosphorylate tyrosine residues in the ITAM. This early step appears to be common to all FcγRs downstream signalling pathways [81]. This is supported by the inhibitory effects of Src kinase inhibitors on FcγR-dependent functions and phagocytosis in particular [82]. It is believed, based in most part on the data obtained in other FcγR-bearing cell types and in transfected cells as well as on the basis of the observed activation of Src family kinases upon FcγRIIa or FcγRIIIb ligation [83-86] and on the effects of pharmacological inhibitors of the Src family of tyrosine kinases, that Lyn, Hck or Fgr may be involved in the proximal phosphorylation events. However, the specific Src kinases associated with each receptor in human neutrophils and their respective functional relevance have yet to be unambiguously identified.

Data implicating tyrosine kinases other than Src family kinases in the regulation of the responses to the stimulation of FcγRs have also been obtained. These include Syk and Tec family kinases. Activation of the tyrosine kinase Syk in neutrophils in response to stimulation of FcγRs has been reported [64, 87]. Accordingly, piceatannol, a Syk inhibitor, decreases FcγR-dependent phagocytosis and reactive oxygen species (ROS) production in human neutrophils [82, 88]. These data implicating Syk in the responses of human neutrophils to the engagement of FcγRs are in accord with the phagocytic defects observed in neutrophils

derived from Syk knock-out mice [89]. Tec family kinases have been shown to respond to ligation of FcγRIIIb [90]. Some of the physiological consequences of this activation have been described and in particular the involvement of Tec kinases in the regulation of the phosphorylation of the phospholipase Cγ2 ($PLC\gamma_2$) in response to FcγRIIIb. Tec is also activated upon cross-linking of FcγRIIa (unpublished observations).

While the available data clearly establish the prominent role of the tyrosine phosphorylation pathways in the regulation of the responsiveness of human neutrophils to the engagement of FcγRs, several elements remain unexplored or ambiguous. As mentioned above, the exact nature of the Src kinase responsive to FcγRs is still to be defined. Furthermore, the understanding of the relative contributions of the multiple families of tyrosine kinases activated upon the engagement of FcγRs is still in its infancy. Additional studies are clearly required to further map the potential interactions activated (or inhibited) upon the stimulation of these various kinases. Importantly, little, if anything, is known about the specifics of the tyrosine phosphorylation pathways activated upon the joint stimulation of both FcγRIIa and FcγRIIIb, i.e., under conditions likely to exist in a patho-physiological setting (see *Functional cooperation* section below). Finally, our understanding of the significance and control of the tyrosine phosphorylation pathways in the context of the stimulation of FcγRs will not be complete without a detailed examination of the role and regulation of tyrosine phosphatases.

Changes in the concentrations of intracellular free calcium are likely to be involved in multiple steps of the responses to FcγRs, including the control of the actin cytoskeleton the phagocytotic process relies upon [91]. Accordingly, increases in free cytoplasmic calcium levels have been observed in response to cross-linking of FcγRs, to the phagocytosis of IgG-opsonized particles and to the addition of immune complexes ([90, 92] and *Marois, L. et al*., submited manuscript).

An additional layer of complexity concerning the mobilisation of calcium upon engagement of FcγRs becomes evident when the responses to FcγRIIa and FcγRIIIb are compared. While the calcium response to the cross-linking of FcγRIIIb relies on both a mobilisation of intracellular calcium and an influx of extracellular calcium, that to the cross-linking of FcγRIIa leads only to the mobilisation of intracellular calcium (with no calcium influx detectable). The elucidation of the molecular basis of these differences which is not known at present is of topical importance as the integrity of the phagocytosis of IgG-opsonized bacteria by human neutrophils (a function dependent on both FcγRIIa and FcγRIIIb) is profoundly reduced in the absence of calcium in the extracellular medium, i.e., in the absence of calcium influx (*Marois, L. et al*., submitted manuscript). These observations provide mechanistic hints about the complementary (as opposed to redundant) mechanisms employed by these two receptors to allow neutrophils to respond adequately to IgG-mediated threats.

MAP kinases (Erk1/2 and p38) are activated in response to homotypic and heterotypic engagement of FcγRIIa and FcγRIIIb [93, 94] as well as in response to stimulation by opsonised zymosan [94]. These signalling events are dependent on the upstream activation of unidentified tyrosine kinases and of PI 3kinases and may be related to the polymerization of actin and to the activation of the NADPH oxidase [93] and of phospholipase A_2 [94].

Inhibitory Mechanisms

The mechanisms underlying the termination of the signals generated upon the engagement of FcγRs in human neutrophils have been ignored until recently. The classical model in which negative signals are generated upon the activation of FcγRIIb (and the recruitment of lipid phosphatases mediated by its ITIM) such as described in details among others in B cells does not apply to human neutrophils which do not express FcγRIIb (see figure 1). Recent data have however provided hints about possible alternative mechanisms of down-regulation of FcγRs.

Our recent results suggest that c-Cbl and src homology 2 domain-containing inositide phosphatase 1 (SHIP-1) play important roles in the regulation of the activation of FcγRIIa in neutrophils. The E3 ubiquitin ligase c-Cbl is responsible, to a major extent, for the rapid degradation of the receptor which accompanies its engagement [74]. SHIP-1, on the other hand, appears to negatively regulate neutrophil functions ([95] and unpublished data). These observations indicate that these two proteins play significant, albeit different, roles in the attenuation of the responses of human neutrophils to stimulation through FcγRIIa.

Phosphatidyl inositol 3-kinases (PI3K) are activated following FcγRIIa ligation [95]. Consistently with this observation, wortmanin, a pan PI3K inhibitor, inhibits FcγRIIa-dependent phagocytosis [13]. The clustering of the p85 PI3K subunit with FcγRIIa has been reported [96]. Our previous data [95] provided evidence for the involvement of SHIP-1 in the responses of human neutrophils to FcγRIIa ligation. The modulation of the levels of $PI(3,4,5)P_3$ in neutrophils plays an important role in the regulation of multiple signalling events and neutrophil functions including calcium mobilisation [97], chemotaxis [98] and phagocytosis [99]. They are tightly regulated and controlled by the activities of lipid kinases (PI 3-kinases) (responsible for the generation of $PI(3,4,5)P_3$) and the opposing effects of lipid phosphatases (PTEN and SHIP-1/2) (which eliminate $PI(3,4,5)P_3$). The inositol phosphatase SHIP-1 translocates to the plasma membrane and is tyrosine phosphorylated in response to FcγRIIa [95] and FcγRIIIb (unpublished observations) cross-linking. It is likely that the activation of SHIP-1 serves to attenuate the responses to the ligation of FcγRs as its silencing in differentiated PLB-985 cells results in an enhancement of the mobilisation of calcium in response to cross-linking of FcγRIIa (unpublished observations). On the other hand, silencing SHIP-1 had no effect on the stimulated degradation of FcγRIIa (unpublished observations). The mechanisms regulating the recruitment and activation of SHIP-1 in response to the ligation of individual FcγRs have not been investigated as of yet. Additionally, the fate of SHIP-1 following the simultaneous engagement of both FcγRIIa and FcγRIIIb has also not been studied so far.

The stimulated degradation of FcγRIIa depends on the ubiquitin ligase c-Cbl [74]. In human neutrophils, we have previously shown that c-Cbl is rapidly tyrosine phosphorylated following the cross-linking of FcγRIIa and accounted for a major proportion of the band of 116 kDa that appears in the tyrosine phosphorylation pattern observed in response to ligation of this receptor [78]. The tyrosine phosphorylation of c-Cbl plays a critical role in the stimulation of its ubiquitin-ligase activity [100-102]. Stimulation of human neutrophils with heat-aggregated IgGs (HA-IgGs) and cross-linking of FcγRIIa both induce (i) the Src kinase-dependent ubiquitination and degradation of FcγRIIa and (ii) the tyrosine phosphorylation and

the translocation of a fraction of c-Cbl from the cytosol to plasma membranes where it co-fractionates with FcγRIIa in DRM_H (*Marois L. et al*, submitted manuscript). Silencing c-Cbl inhibits FcγRIIa ubiquitination and degradation. c-Cbl-dependent degradation of FcγRIIa plays an important role in the regulation of the duration and intensity of the human neutrophil responses elicited by FcγRIIa engagement [74].

CD300a (also known as IRp60, CMRF-35H) is a receptor with three ITIM motifs within its cytoplasmic tail that belongs to a multigene family of activating/ inhibitory receptors that are clustered in human chromosome 17 (17q25.1). The ligand for CD300a, as well as for the other members of the CD300 family, is unknown [103]. CD300a is expressed on a variety of immune cells. Cross-linking of CD300a with a specific monoclonal antibody exerts an inhibitory effect on natural killer (NK) cell activity through recruitment of src homology 2 domain-containing tyrosine phosphatases 1 and 2 (SHP-1/2) [104]. In human eosinophils, cross-linking of CD300a suppresses the effects of eotaxin, IL-5 and GM-CSF also by recruiting SHP-1 [105]. CD300a inhibits IgE-dependent, but not IgE-independent, activities in mast cells by recruiting SHP-1 and SHIP [106]. This receptor appears to modulate FcγRIIa mediated signalling in response to inflammatory stimuli on human neutrophils. Its expression is up-regulated following LPS or GM-CSF incubation by the translocation of an intracellular pool to the plasma membrane. Its cross-linking inhibits ROS production and calcium mobilisation in response to FcγRIIa ligation. These observations suggest that, in the absence of FcγRIIb, FcγRs-mediated downregulation could be mediated in human neutrophils by other ITIM-bearing receptors, amongst which CD300a is an attractive candidate [107].

In contrast to FcγRIIa the responsiveness of which is controlled by its stimulated proteasomal-dependent degradation [74], that of FcγRIIIb appears to be dependent on the modulation of its expression levels at the plasma membrane. The maintenance of the surface levels of FcγRIIIb is regulated by a balance between the rates of shedding to the extracellular medium (generating a soluble form) and the mobilisation of preformed receptor in intracellular stores in response to neutrophil stimulation [26, 108]. Shedding of the GPI-anchored receptor is observed in response to neutrophil apoptosis and stimulation by PMA [109-111] or fMLP [109] and by jasplakinolide, an actin-polymerizing peptide [112]. The mechanism of shedding remains unclear. Metalloproteases were proposed to be involved in this process, but the shedding is also inhibited by serine protease inhibitors [113, 114]. The physiological significance of the shedding of FcγRIIIb is not well understood. Apoptotic neutrophils could be marked for clearance by phagocytic cells by this mechanism. The surface levels of FcγRIIIb may also be down-regulated by internalisation following its engagement (Figure 2). Shedding and/or internalisation of FcγRIIIb is likely to play a role in limiting the responses to IgG-dependent processes. Lower levels of FcγRIIIb on neutrophils impair the ability of neutrophils to respond to immune complexes [115, 116]. Furthermore, soluble FcγRIIIb binds IgG [117] and has been proposed to inhibit FcγRs engagement by IgG-containing immune complexes [118]. Other functions for soluble FcγRIIIb have been proposed such as inhibitory effects on B cell proliferation and IgG/IgM production [119].

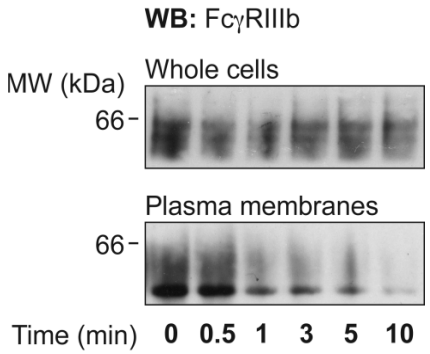

Figure 2. Stimulated internalisation of FcγRIIIb

Neutrophils (40×10^6 cells/ml) were preincubated with 1 mM DFP for 10 minutes at 37ºC before cross-linking of FcγRIIIb with PeliCluster antibody (5 µg/ml, from Sanquin) and a goat anti-mouse F(ab')2 anti-F(ab')2 (125µg/ml, from Jackson Laboratories) for the indicated times. Stimulations were stopped on ice and aliquots of whole cells were collected, washed and boiled in Laemmli's sample buffer. Plasma membranes were prepared as previously described [74]. Whole cell lysates and plasma membranes samples were analysed by SDS-PAGE and probed for FcγRIIIb (DJ130c antibody, Dako). The same blots were reprobed for flotillin-1 (from BD Biosciences) as protein loading control (data not shown). The diffuse pattern of FcγRIIIb blotting is due to is extensive glycosylation. Engagement of FcγRIIIb modifies little, if at all, its levels in whole cell samples (upper panel) but drastically decreases the levels detected in plasma membrane samples (lower panel). These data suggest that engagement of FcγRIIIb leads to its internalisation.

Taken together, these observations indicate that human neutrophils rely on a unique and non-standard combination of mechanisms, some of which are beginning to be characterized, to down-regulate and prevent overactivation of FcγRs (Figure 3).

Functional Cooperation

The biological significance of co-expressing two FcγRs with overlapping ligand-binding specificity (but distinct membrane anchors and signalling capacities) is not fully understood. Several studies indicate that FcγRIIa is directly involved in the phagocytic process [13, 120-122], whereas FcγRIIIb participates only indirectly and probably in a non-redundant manner in the phagocytic function of the neutrophil. The results of multiple phagocytosis assays indicate that the expression of FcγRIIa, but not of FcγRIIIb is sufficient to confer phagocytic ability to transfected fibroblasts [3, 123, 124] However, a synergistic enhancement of phagocytosis is observed when these two receptors are triggered [124]. Moreover, recent publications report a decreased phagocytic activity in neutrophils from FcγRIIIb deficient donors despite the presence of functional FcγRIIa [125, 126]. These data illustrate the complexity of the roles of FcγRIIa and FcγRIIIb in FcγR-dependent phagocytosis in human neutrophils. Previous data obtained using TNFα-primed human neutrophils indicate that, under those conditions, FcγRIIIb plays a minor role in phagocytosis [127] while it is preponderantly involved in the activation of the oxidative burst [128].

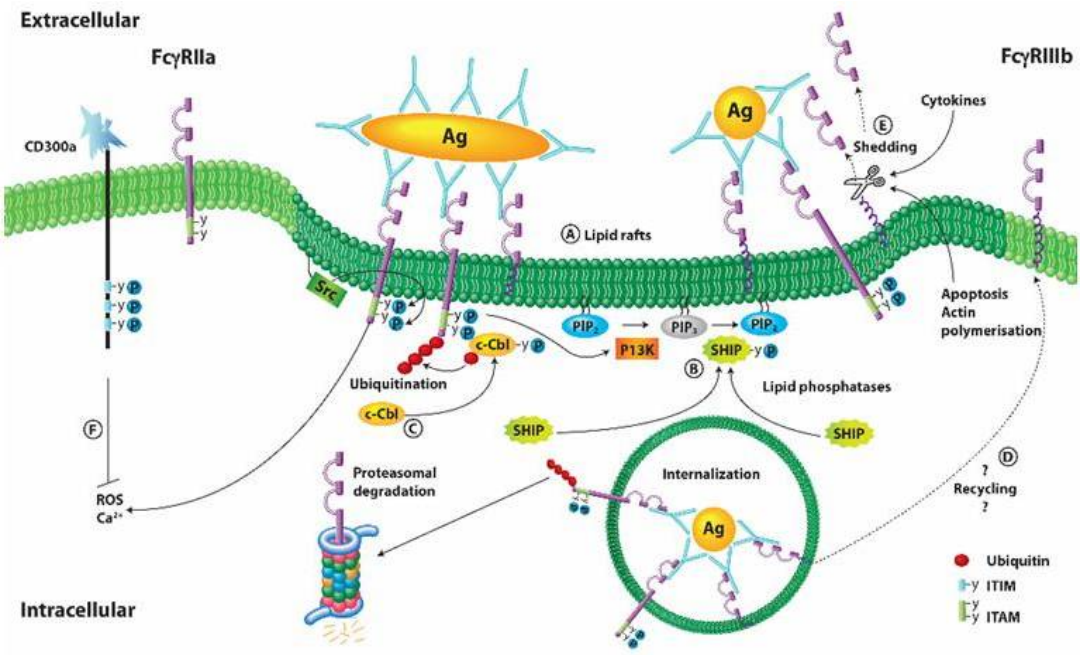

Figure 3. Mechanisms implicated in the downregulation of the activation of the human neutrophil's FcγRs

Ligation of FcγRIIa and FcγRIIIb on human neutrophils leads to a rapid recruitment of these receptors in the detergent-resistant membrane microdomains named lipid rafts (A). In this signalling platform different inhibitory events are stimulated such as the recruitment of the lipid phosphatase SHIP-1 (B) that hydrolyzes PIP_3 and thereby inhibits FcγR responses (unpublished data). The ubiquitin-ligase c-Cbl is also recruited from the cytosol to lipid rafts and affixes ubiquitin molecules on the Fc tail of FcγRIIa. This ubiquitination event targets FcγRIIa to a proteasomal degradation following its internalisation (C). FcγRIIIb is also internalised in response to its ligation but the details and outcome of this event remained poorly characterized (D). FcγRIIIb also appears to be shed from the cell surface (E) though the specific enzyme(s) involved is not identified. As FcγRIIb is not constitutively expressed on human neutrophils, an alternative ITIM-dependent mechanism is proposed to downregulate FcγRIIa-dependent functions involving the orphan receptor CD300a (F).

Most of the studies examining the specific roles and signaling pathways of the neutrophil's FcγRs were performed using stimulation with monoclonal antibodies or were restricted to myeloid/neutrophil-like cells which do not express the same combination of FcγRs as human neutrophils. We recently examined the phagocytosis of IgG-opsonised zymosan by human neutrophils and observed that it is greatly diminished by antibodies directed against either FcγRIIa or FcγRIIIb indicating that both receptors cooperate in this function. Furthermore, antibodies directed against either FcγRIIa or FcγRIIIb also inhibited the tyrosine phosphorylation response as well as the stimulated degradation of FcγRIIa elicited by HA-IgGs which simultaneously engaged both receptors. Phagocytosis of IgG-opsonised zymosan by human neutrophils requires an extracellular influx of calcium which is strictly dependent on the engagement of FcγRIIIb. This step could represent the specific contribution of FcγRIIIb to the phagocytic signal. We also observed that the functional regulation of phagocytosis is dependent of the integrity of detergent-resistant membrane microdomains where both FcγRs are recruited following heat-aggregated IgGs stimulation

(*Marois, L. et al.,* submitted manuscript). Previous studies also suggest that immune complexes primarily engage FcγRIIIb and this ligation converts FcγRIIa to a high-affinity state that permits FcγRIIa-dependent signalling [125]. Functional interactions in human neutrophils may not be limited to the two FcγRs but may also involve complement receptors (i.e., CR3). Evidence for this is the level of the production of reactive oxygen species [84, 129, 130]. It is also of interest that a positive role of reactive oxygen intermediates in the phagocytic capacity and signalling of FcγRs on human neutrophils has also been described [131] thereby suggesting potential positive feed-back loops.

On a technical level, it is worthwhile to note that a commonly used antibody directed against FcγRIIIb, 3G8, has been shown to interact through its Fc chain with FcγRIIa on neutrophils [97]. Previously reported effects observed in response to stimulation of human neutrophils by the complete 3G8 antibody may in fact correspond to a joint stimulation of both FcγRs.

Taken together, these data illustrate the complexity of the functional cooperation and regulation that exists between the two constitutively expressed FcγRs on human neutrophils and that is likely to occur under patho-physiological conditions.

Patho-Physiological Implications

Neutrophil's FcγRs are implicated in the clearance of naturally occurring ICs. Intravascular ICs trigger transient accumulation of neutrophils which internalize the immune deposits. Immune complexes are generated in all antibody responses but they can become pathogenic depending on their size, their amounts and their affinities. Insoluble ICs are cleared primarily by the phagocyte system but small soluble ICs have a propensity for tissue deposition [132]. Neutrophils are also challenged by IgG-containing autoimmune complexes and represent the most important quantitative pool of potential FcγRs implicated in different autoimmune pathologies [10]. In both type II and type III hypersensitivity responses, neutrophils are the first immune cells to be recruited and the resulting inflammation depends on the relative accessibility of the immune complexes to circulating leukocytes. Type II hypersensitivity reactions arise when antibodies are produced against cell- or matrix-associated antigen whereas type III hypersensitivity arises when antibodies are produced against soluble self antigens. In the following, we present a selected list of examples of the involvement of the FcγRs expressed in neutrophils in hypersensitivity responses.

ANCA-Associated Diseases

Anti-neutrophil cytoplasmic antibody (ANCA)-associated diseases, including Wegener's granulomatosis, implicate neutrophils' FcγRs. The primary antigenic targets of ANCA are the granule enzymes, myeloperoxidase (MPO) and proteinase 3 (PR3) that are translocated to the plasma membrane following a still poorly understood priming mechanism in which TNFα might play a key role [133]. *In vivo*, priming may be initiated by concurrent inflammatory events such as infection. Dissection of the intracellular signalling indicates that simultaneous engagement of the $F(ab')_2$ portion of ANCA with ANCA antigens on the cell surface and

interaction of the Fc part of the antibody with FcγRs triggers the signalling cascades that leads to neutrophil activation [134, 135]. A number of models, all involving neutrophil's FcγRs, can explain how ANCA activate neutrophils. Several ANCA may bind target antigens on one neutrophil and present oligomerized Fc portion to surrounding neutrophils. Alternatively ANCA may heterodimerize FcγRs and target antigens on the same neutrophil. Although ANCA-associated diseases are not firmly characterized by the presence of circulating IC, a third possibility is that degranulated PR3 and MPO bind ANCA in solution, forming immune complexes that bind and activate FcγRs. An *in vitro* study showed that the intracellular signals that mediate neutrophil activation in response to ANCA does not depend on the inducible presence of the high affinity receptor FcγRI [134, 136] thereby emphasizing the prominent role of the low affinity and constitutively expressed FcγRs on neutrophils (FcγRIIa and FcγRIIIb). ANCA also increase neutrophil adherence to endothelial cells leading to endothelium damage and vasculitis [137-139].

Goodpasture Syndrome

Goodpasture syndrome is another autoimmune disease involving the neutrophils' FcγRs. In this disease that leads to glomerulonephritis, antibodies against glomerular basement membrane (GBM) are produced. Resident cells (macrophages) sense ICs through FcγRs and complement receptors and secrete chemokines which activate endothelial cells and cause neutrophil extravasation. Neutrophils will bind ICs via their FcγRs. The consequent activation of the respiratory burst and degranulation results in vascular damage [140]. Alternatively or concomitantly, when anti-GBM are accessible to circulating neutrophils or deposited within the vasculature, neutrophils can be recuited via their own FcγRs without previous chemoattractant release [141]. Progressive nephrotoxic serum nephritis in mice is a prototypic type II-hypersensitivity response that resembles Goodpasture syndrome. Using this model, a recent study showed that neutrophil-selective transgenic expression of FcγRIIa and FcγRIIIb (in mice in which the common γ chain of FcγRs was deleted) restored susceptibility to glomerulonephritis. Both human neutrophil receptors promoted glomerular neutrophil accumulation, however, FcγRIIa alone was responsible for glomerular injury [10].

Idiopathic Thrombocytopenic Purpura

The etiological basis of idiopathic thrombocytopenic purpura (ITP) is the targeting of platelets by membrane antigen antibodies, followed by FcγR-mediated phagocytosis [142]. Although recent studies indicate the involvement of Fc-independent pathways [143], it is currently accepted that antibody-mediated platelet destruction is Fc dependent. FcγRIIa and FcγRIIIb have been speculated to play key roles. Their engagement initiates cytosolic signalling pathways which culminate in the uptake and destruction of platelets [144] and does not produce an inflammatory response. High doses of intravenous Ig (IVIg) is highly effective in the treatment of ITP. Despite years of use, the mechanism of immunomodulation involved is still unclear. However, the beneficial effects of high-dose IVIg therapy might, at least in

part, be due to the blockade of activating FcγRs by monomeric IgGs. This would prevent the binding of opsonized platelets and the induction of phagocytosis [145].

Rheumatoid Arthritis and Systemic Lupus Erythematosus

Type III hypersensitivity diseases are caused by the deposition of antigen-antibody aggregates or immune complexes in vessels and tissues. FcγRs play a role in the effector arm of the autoimmune process, inducing inflammatory lesions. Typical of these pathologies is rheumatoid arthritis (RA), an auto-immune disease characterized by a sustained recruitment of leucocytes in joints leading to synovium inflammation and bone erosion. Neutrophils are the prominent leukocyte infiltrating the joint [146]. RA is a systemic inflammatory disease as high levels of auto-antibodies form immune complexes with their cognate antigens and represent potent activators of effector cells (including neutrophils) *via* cross-linking of FcγRs [147].

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the presence of autoantibodies principally against nuclear components. These antibodies are not *per se* pathogenic but the deposition of immune complexes in vessels initiate an inappropriate and excessive inflammatory response [9].

In RA, as well as in SLE, small IC deposition depends on systemic factors, physiochemical properties of the IC and tissue-specific hemodynamic characteristics which may explain why inflammatory lesions often take place in joints and glomerules [132]. Circulating ICs first deposit in vasculature and then translocate to the extravascular tissue [140, 148]. FcγRIIIb plays an important role in slowing down neutrophils [149] leading to adhesion to endothelial cells and transmigration. Neutrophils can then signal cytotoxic functions through, among others, FcγRs and promote tissue damage. Neutrophils are the first immune cells to be recruited and it is a consistent feature of IC-mediated diseases.

Animal models for SLE [150] and RA [151] have been described. They contributed to the elaboration of the hypothesis that the FcγRs of the neutrophils play a role in the development of autoimmune diseases. However, as FcγRs expression on mouse and human differs, it is difficult to directly extrapolate results observed in mouse models to human diseases. Mice deficient in the common γ-signalling chain of FcγRs are not susceptible to arthritis induction; in addition the lack of the inhibitory receptor FcγRIIb was found to exacerbate collagen-induced arthritis in susceptible mice [152]. FcγRIIa has been implicated in RA [153], and human FcγRIIa transgenic mice become susceptible to collagen-induced arthritis.

The contribution of human FcγRs to neutrophil recruitment in response to soluble ICs was examined in a neutrophil-selective transgenic murine model expressing specifically FcγRIIa and FcγRIIIb. On the basis of this study, the authors concluded that these two receptors play non-redundant roles and that human neutrophils may play a primary role in initiating IC-mediated diseases [10].

In RA, as well as in SLE [154], the efficiency of anti-TNFα therapy [155] or methotrexate treatment [156] is related to a loss of function of FcγRIIa, underlying the pivotal role of this receptor in these diseases. The FcγRs of neutrophils are challenged in blood circulation where inflammatory modulators that can prime neutrophils are present. While the neutrophils' FcγRs display low-affinity for IgGs under resting conditions, *in vitro* pre-

incubation of neutrophils with TNFα [128] or the chemotactic peptide fMLP [125] enhances subsequent FcγRs-dependent functions [128]. These data suggest that the neutrophils' FcγRs are likely to be regulated by cytokines or inflammatory conditions as found in RA [157]. By increasing FcγRs activity, TNFα (and other pro-inflammatory cytokines) can enhance IC-dependent activation of neutrophils leading to exacerbation of inflammation.

Rheumatoid factors (RF) are prominent autoantibodies present non only in RA but also in SLE and other diseases. These antibodies recognize the Fc portion of IgGs and can form RF-containing immune complexes. High levels of RF in the serum of RA patients are correlated with the severity of the disease. RF-containing IC may engage FcγRs on circulating neutrophils, thus contributing to systemic inflammation. In accord with this hypothesis, we obtained preliminary results indicating that circulating neutrophils may be activated by RF or RF-containing IC. These data were obtained using anti-human IgGs to reproduce the effect of RF (unpublished observations).

Incidence of FcγR Genes Polymorphisms

In humans, several polymorphisms of FcγR genes which alter IgG binding affinities have been associated with autoimmune diseases. Low copy number of FcγIIIb gene has been associated with SLE and predisposes SLE patients to glomerulonephritis [158]. Reduced FcγIIIb expression is thus likely to contribute to the impaired clearance of ICs which is a feature of SLE. In contrast, ANCA-associated vasculitis which is not strictly associated with immune complex deposition is associated with high FcγRIIIb gene copy number [159, 160].

A polymorphism in FcγRIIb gene that alters isoleucine to threonine at position 232 in the transmembrane region was proposed to be associated with the higher severity and incidence of SLE in some racial groups. This mutation alters FcγRIIb partitioning to lipid raft [161] and interestingly several signaling proteins, some of them being involved in neutrophils FcγRs signaling [162] also are differentially recruited to lipid rafts in SLE patients. These data suggest that these signaling platforms play important, though presently undefined, roles in the molecular mechanisms leading to the development of the disease. As lipid rafts are thought to control the signalling events downstream of FcγRs in human neutrophils [64, 66], the comparison of these membrane domains in neutrophils isolated from SLE and healthy donors, might shed relevant hints about the involvement of FcγRIIa and FcγRIIIb in the development of the inflammatory damage associated with this disease.

FcγRIIa polymorphisms have been proposed to influence the development or susceptibility to several infectious diseases as well as to autoimmune diseases. The FcγRIIa isoform FcγRIIa-H131 is the only human FcγR capable of efficiently binding IgG2 opsonised pathogens. IgG2-deficiency predisposes to streptococcal infection and an association between FcγRIIa-R/R131 homozygosity and susceptibility to invasive pneumococcal diseases has been observed [163, 164]. Furthermore, as IgG2 poorly activates complement, binding to FcγRIIa is likely to be important in the immune response to streptococcal infection. *In vitro*, neutrophils from homozygous FcγRIIa-H/H131 donors have a higher phagocytic capacity for IgG-opsonized *Streptococcus pneumoniae* than neutrophils from FcγRIIa-R/R131 donors [165]. Individuals who have the H/H131 allotype are less susceptible to severe

meningococcal disease than are individuals with the R/R131 or R/H131 genotype and an in vitro study showed that neutrophils with the FcγRIIa-R/R131 allotype phagocytized Neissaria meningitides opsonized with polyclonal IgG2 antibodies less effectively than did FcγRIIa-H/H131 neutrophils [166, 167]. Finally, more periodontal destruction occurs in the H/H131 genotype of FcγRIIa than in the H/R131 or R/R131 genotype. This may be due to hyper-reactivity of H/H131-neutrophils upon interaction with bacteria [168].

FcγRIIa-H131 and FcγRIIIb-NA1 genotypes are also associated with susceptibility and severity of myasthenia gravis [169] whereas numerous inflammatory neutropathies have been shown to be associated with FcγRIIa-R131 and FcγRIIIb-NA2 alleles.

Role of Neutrophil FcγRs in Antibody Treatment

Antibody treatment utilizes chimeric murine-human antibodies, humanised antibodies and Fc-fusion proteins [7]. Rituximab is an anti-CD20 antibody that depletes B cells and is used to treat lymphoma as well as antibody-mediated autoimmune diseases including ITP and SLE [170, 171]. A recent study using a nonfucosylated variant of rituximab having strong FcγRIII-binding capacity indicates that neutrophils can serve as physiological cross-linkers that augment anti-CD20-mediated apoptosis. Furthermore, this mechanism depends on FcγRIIIb but not on FcγRIIa [172].

Conclusion

This review presents a selected and non-exhaustive list of key roles of neutrophils in immunity, autoimmunity and chronic inflammation. Accordingly, numerous pathologies have been related to defective or aberrant neutrophil function. Controlling neutrophil recruitment and activation at the molecular level is important for the development of strategies to modulate their involvement in host defence or chronic inflammation.

References

[1] Huizinga, T. W., van Kemenade, F., Koenderman, L., Dolman, K. M., von dem Borne, A. E., Tetteroo, P. A. & Roos, D. (1989). The 40-kDa Fc gamma receptor (FcRII) on human neutrophils is essential for the IgG-induced respiratory burst and IgG-induced phagocytosis. *J Immunol*, *142*, 2365-2369.

[2] Anderson, C. L., Shen, L., Eicher, D. M., Wewers, M. D. & Gill, J. K. (1990). Phagocytosis mediated by three distinct Fc gamma receptor classes on human leukocytes. *J Exp Med*, *171*, 1333-1345.

[3] Indik, Z., Kelly, C., Chien, P., Levinson, A. I. & Schreiber, A. D. (1991). Human Fc gamma RII, in the absence of other Fc gamma receptors, mediates a phagocytic signal. *J Clin Invest*, *88*, 1766-1771.

[4] Rosales, C. & Brown, E. J. (1991). Two mechanisms for IgG Fc-receptor-mediated phagocytosis by human neutrophils. *J Immunol*, *146*, 3937-3944.

[5] Hundt, M. & Schmidt, R. E. (1992). The glycosylphosphatidylinositol-linked Fc gamma receptor III represents the dominant receptor structure for immune complex activation of neutrophils. *Eur J Immunol*, *22*, 811-816.

[6] Schmidt, R. E. & Gessner, J. E. (2005). Fc receptors and their interaction with complement in autoimmunity. *Immunol Lett*, *100*, 56-67.

[7] Willcocks, L. C., Smith, K. G. & Clatworthy, M. R. (2009). Low-affinity Fcgamma receptors, autoimmunity and infection. *Expert Rev Mol Med*, *11*, e24.

[8] Selvaraj, P., Fifadara, N., Nagarajan, S., Cimino, A. & Wang, G. (2004). Functional regulation of human neutrophil Fc gamma receptors. *Immunol Res 29*,*219-230*.

[9] Niederer, H. A., Clatworthy, M. R., Willcocks, L. C. & Smith, K. G. FcgammaRIIB, FcgammaRIIIB, and systemic lupus erythematosus. *Ann N Y Acad Sci*., *1183*, 69-88.

[10] Tsuboi, N., Asano, K., Lauterbach, M. & Mayadas, T. N. (2008). Human neutrophil Fcgamma receptors initiate and play specialized nonredundant roles in antibody-mediated inflammatory diseases. *Immunity*, *28*, 833-846.

[11] Takai, T. (2005). Fc receptors and their role in immune regulation and autoimmunity. *J Clin Immunol*, *25*, 1-18.

[12] Nimmerjahn, F. & Ravetch, J. V. (2008). Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol*, *8*, 34-47.

[13] Indik, Z. K., Park, J. G., Hunter, S. & Schreiber, A. D. (1995). The molecular dissection of Fc gamma receptor mediated phagocytosis. *Blood*, *86*, *4389-4399*.

[14] Selvaraj, P., Rosse, W. F., Silber, R. & Springer, T. A. (1988). The major Fc receptor in blood has a phosphatidylinositol anchor and is deficient in paroxysmal nocturnal haemoglobinuria. *Nature*, *333*, 565-567.

[15] Mousavi, S. A., Sporstol, M., Fladeby, C., Kjeken, R., Barois, N. & Berg, T. (2007). Receptor-mediated endocytosis of immune complexes in rat liver sinusoidal endothelial cells is mediated by FcgammaRIIb2. *Hepatology*, *46*, 871-884.

[16] Hentunen, T. A., Jackson, S. H., Chung, H., Reddy, S. V. , Lorenzo, J., Choi, S. J. & Roodman, G. D. (1999). Characterization of immortalized osteoclast precursors developed from mice transgenic for both bcl-X(L) and simian virus 40 large T antigen. *Endocrinology*, *140*, 2954-2961.

[17] Staudt, A., Eichler, P., Trimpert, C., Felix, S. B. & Greinacher, A. (2007). Fc(gamma) receptors IIa on cardiomyocytes and their potential functional relevance in dilated cardiomyopathy. *J Am Coll Cardiol*, *49*, 1684-1692.

[18] Petroni, K. C., Shen, L. & Guyre, P. M. (1988). Modulation of human polymorphonuclear leukocyte IgG Fc receptors and Fc receptor-mediated functions by IFN-gamma and glucocorticoids. *J Immunol*, *140*, 3467-3472.

[19] Guyre, P. M., Campbell, A. S., Kniffin, W. D. & Fanger, M. W. (1990). Monocytes and polymorphonuclear neutrophils of patients with streptococcal pharyngitis express increased numbers of type I IgG Fc receptors. *J Clin Invest*, *86*, 1892-1896.

[20] Repp, R., Valerius, T., Sendler, A., Gramatzki, M., Iro, H., Kalden, J. R. & Platzer, E. (1991). Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. *Blood*, *78*, 885-889.

[21] Cambier, J. C. (1995). New nomenclature for the Reth motif (or ARH1/TAM/ARAM/YXXL). *Immunol Today*, *16*, 110.

[22] Reth, M. (1989). Antigen receptor tail clue. *Nature*, *338*, 383-384.

[23] Li, M., Wirthmueller, U. & Ravetch, J. V. (1996). Reconstitution of human Fc gamma RIII cell type specificity in transgenic mice. *J Exp Med*, *183*, 1259-1263.

[24] Meknache, N., Jonsson, F., Laurent, J., Guinnepain, M. T. & Daeron, M. (2009). Human basophils express the glycosylphosphatidylinositol-anchored low-affinity IgG receptor FcgammaRIIIB (CD16B). *J Immunol*, *182*, 2542-2550.

[25] Lanier, L. L., Cwirla, S., Yu, G., Testi, R. & Phillips, J. H. (1989). Membrane anchoring of a human IgG Fc receptor (CD16) determined by a single amino acid. *Science*, *246*, 1611-1613.

[26] Tosi, M. F. & Zakem, H. (1992). Surface expression of Fc gamma receptor III (CD16) on chemoattractant-stimulated neutrophils is determined by both surface shedding and translocation from intracellular storage compartments. *J Clin Invest*, *90*, 462-470.

[27] Buckle, A. M. & Hogg, N. (1989). The effect of IFN-gamma and colony-stimulating factors on the expression of neutrophil cell membrane receptors. *J Immunol*, *143*, 2295-2301.

[28] Buckle, A. M., Jayaram, Y. & Hogg, N. (1990). Colony-stimulating factors and interferon-gamma differentially affect cell surface molecules shared by monocytes and neutrophils. *Clin Exp Immunol*, *81*, 339-345.

[29] Gericke, G. H., Ericson, S. G., Pan, L., Mills, L. E., Guyre, P. M. & Ely, P. (1995). Mature polymorphonuclear leukocytes express high-affinity receptors for IgG (Fc gamma RI) after stimulation with granulocyte colony-stimulating factor (G-CSF). *J Leukoc Biol.*, *57*, 455-461.

[30] Bovolenta, C., Gasperini, S. & Cassatella, M. A. (1996). Granulocyte colony-stimulating factor induces the binding of STAT1 and STAT3 to the IFNgamma response region within the promoter of the Fc(gamma)RI/CD64 gene in human neutrophils. *FEBS Lett*, *386*, 239-242.

[31] Pan, L. Y., Mendel, D. B., Zurlo, J. & Guyre, P. M. (1990). Regulation of the steady state level of Fc gamma RI mRNA by IFN-gamma and dexamethasone in human monocytes, neutrophils, and U-937 cells. *J Immunol*, *145*, 267-275.

[32] Klebanoff, S. J., Olszowski, S., Van Voorhis, W. C., Ledbetter, J. A., Waltersdorph, A. M. & Schlechte, K. G. (1992). Effects of gamma-interferon on human neutrophils: protection from deterioration on storage. *Blood*, *80*, 225-234.

[33] Simms, H. H. & D'Amico, R. (1994). Regulation of intracellular polymorphonuclear leukocyte Fc receptors by lipopolysaccharide. *Cell Immunol*, *157*, 525-541.

[34] Repp, R., Valerius, T., Wieland, G., Becker, W., Steininger, H., Deo, Y., Helm, G., Gramatzki, M., Van de Winkel, J. G., Lang, N. & et al. (1995). G-CSF-stimulated PMN in immunotherapy of breast cancer with a bispecific antibody to Fc gamma RI and to HER-2/neu (MDX-210). *J Hematother*, *4*, 415-421.

[35] Cassatella, M. A., Flynn, R. M., Amezaga, M. A., Bazzoni, F., Vicentini, F. & Trinchieri, G. (1990). Interferon gamma induces in human neutrophils and macrophages expression of the mRNA for the high affinity receptor for monomeric IgG (Fc gamma R-I or CD64). *Biochem Biophys Res Commun*, *170*, 582-588.

[36] Takano, K., Kaganoi, J., Yamamoto, K., Takahashi, A., Kido, T. & Sasada, M. (2000). Rapid and prominent up-regulation of high-affinity receptor for immunoglobulin G (Fc

gamma RI) by cross-linking of beta 2 integrins on polymorphonuclear leukocytes. *Int J Hematol*, *72*, 48-54.

[37] Fridman, W. H. (1991). Fc receptors and immunoglobulin binding factors. *Faseb J*, *5*, 2684-2690.

[38] Hoffmann, J. J. (2009). Neutrophil CD64: a diagnostic marker for infection and sepsis. *Clin Chem Lab Med*, *47*, 903-916.

[39] van der Meer, W., Pickkers, P., Scott, C. S., van der Hoeven, J. G. & Gunnewiek, J. K. (2007). Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia. *J Endotoxin Res*, *13*, 94-100.

[40] Sacks, G. P., Studena, K., Sargent, K. & Redman, C. W. (1998). Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol*, *179*, 80-86.

[41] Naccasha, N., Gervasi, M. T., Chaiworapongsa, T., Berman, S., Yoon, B. H., Maymon, E. & Romero, R. (2001). Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. *Am J Obstet Gynecol*, *185*, 1118-1123.

[42] Luppi, P., Haluszczak, C., Betters, D., Richard, C. A., Trucco, M. & DeLoia, J. A. (2002). Monocytes are progressively activated in the circulation of pregnant women. *J Leukoc Biol*, *72*, 874-884.

[43] Fjaertoft, G., Hakansson, L., Foucard, T., Ewald, U. & Venge, P. (2005). CD64 (Fcgamma receptor I) cell surface expression on maturing neutrophils from preterm and term newborn infants. *Acta Paediatr*, *94*, 295-302.

[44] Gronlund, M. M., Nuutila, J., Pelto, L., Lilius, E. M., Isolauri, E., Salminen, S., Kero, P. & Lehtonen, O. P. (1999). Mode of delivery directs the phagocyte functions of infants for the first 6 months of life. *Clin Exp Immunol*, *116*, 521-526.

[45] Salmon, J. E. & Pricop, L. (2001). Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. *Arthritis Rheum*, *44*, 739-750.

[46] Kimberly, R. P., Salmon, J. E. & Edberg, J. C. (1995). Receptors for immunoglobulin G. Molecular diversity and implications for disease. *Arthritis Rheum*, *38*, 306-314.

[47] Dijstelbloem, H. M., van de Winkel, J. G. & Kallenberg, C. G. (2001). Inflammation in autoimmunity: receptors for IgG revisited. *Trends Immunol*, *22*, 510-516.

[48] Su, K., Yang, H., Li, X., Gibson, A. W., Cafardi, J. M., Zhou, T., Edberg, J. C. & Kimberly, R. P. (2007). Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. *J Immunol*, *178*, 3272-3280.

[49] Belostocki, K., Pricop, L., Redecha, P. B., Aydin, A., Leff, L., Harrison, M. J. & Salmon, J. E. (2008). Infliximab treatment shifts the balance between stimulatory and inhibitory Fcgamma receptor type II isoforms on neutrophils in patients with rheumatoid arthritis. *Arthritis Rheum*, *58*, 384-388.

[50] Vidarsson, G., Stemerding, A. M., Stapleton, N. M., Spliethoff, S. E., Janssen, H., Rebers, F. E., de Haas, M. & van de Winkel, J. G. (2006). FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood*, *108*, 3573-3579.

[51] Simister, N. E. & Mostov, K. E. (1989). An Fc receptor structurally related to MHC class I antigens. *Nature*, *337*, 184-187.

[52] Yoshida, M., Claypool, S. M., Wagner, J. S., Mizoguchi, E., Mizoguchi, A., Roopenian, D. C., Lencer, W. I. & Blumberg, R. S. (2004). Human neonatal Fc receptor mediates

transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity*, *20*, 769-783.

[53] Simister, N. E., Jacobowitz Israel, E., Ahouse, J. C. & Story, C. M. (1997). New functions of the MHC class I-related Fc receptor, FcRn. *Biochem Soc Trans*., *25*, 481-486.

[54] Firan, M., Bawdon, R., Radu, C., Ober, R. J. Eaken, D., Antohe, F., Ghetie, V. & Ward, E. S. (2001). The MHC class I-related receptor, FcRn, plays an essential role in the maternofetal transfer of gamma-globulin in humans. *Int Immunol*, *13*, 993-1002.

[55] Roopenian, D. C., Christianson, G. J., Sproule, T. J., Brown, A. C., Akilesh, S., Jung, N., Petkova, S., Avanessian, L., Choi, E. Y., Shaffer, D. J., Eden, P. A. & Anderson, C. L. (2003). The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. *J Immunol*, *170*, 3528-3533.

[56] van der Pol, W. & van de Winkel, J. G. (1998). IgG receptor polymorphisms: risk factors for disease. *Immunogenetics*, *48*, 222-232.

[57] Sanders, L. A., Feldman, R. G., Voorhorst-Ogink, M. M., de Haas, M., Rijkers, G. T., Capel, P. J., Zegers, B. J. & van de Winkel, J. G. (1995). Human immunoglobulin G (IgG) Fc receptor IIA (CD32) polymorphism and IgG2-mediated bacterial phagocytosis by neutrophils. *Infect Immun*, *63*, 73-81.

[58] Binstadt, B. A., Geha, R. S. & Bonilla, F. A. (2003). IgG Fc receptor polymorphisms in human disease: implications for intravenous immunoglobulin therapy. *J Allergy Clin Immunol*, *111*, 697-703.

[59] Danielsen, E. M. & Hansen, G. H. (2006). Lipid raft organization and function in brush borders of epithelial cells. *Mol Membr Biol*., *23*, 71-79.

[60] Manes, S. & Viola, A. (2006). Lipid rafts in lymphocyte activation and migration. *Mol Membr Biol*., *23*, 59-69.

[61] Kabouridis, P. S. (2006). Lipid rafts in T cell receptor signalling. *Mol Membr Biol*., *23*, 49-57.

[62] Van Laethem, F. & Leo, O. (2002). Membrane lipid rafts: new targets for immunoregulation. *Curr Mol Med*, *2*, 557-570.

[63] Nebl, T., Pestonjamasp, K. N., Leszyk, J. D., Crowley, J. L., Oh, S. W. & Luna, E. J. (2002). Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. *J Biol Chem*., *277*, 43399-43409.

[64] Fernandes, M. J., Rollet-Labelle, E., Pare, G., Marois, S., Tremblay, M. L., Teillaud, J. L. & Naccache, P. H. (2006). CD16b associates with high-density, detergent-resistant membranes in human neutrophils. *Biochem J*, *393*, 351-359.

[65] Barabe, F., Rollet-Labelle, E., Gilbert, C., Fernandes, M. J., Naccache, S. N. & Naccache, P. H. (2002). Early events in the activation of Fc gamma RIIA in human neutrophils: stimulated insolubilization, translocation to detergent-resistant domains, and degradation of Fc gamma RIIA. *J Immunol*, *168*, 4042-4049.

[66] Rollet-Labelle, E., Marois, S., Barbeau, K., Malawista, S. E. & Naccache, P. H. (2004). Recruitment of the cross-linked opsonic receptor CD32A (FcgammaRIIA) to high-density detergent-resistant membrane domains in human neutrophils. *Biochem J*, *381*, 919-928.

[67] Kwiatkowska, K. & Sobota, A. (2001). The clustered Fcgamma receptor II is recruited to Lyn-containing membrane domains and undergoes phosphorylation in a cholesterol-dependent manner. *Eur J Immunol*, *31*, 989-998.

[68] Strzelecka-Kiliszek, A., Korzeniowski, M., Kwiatkowska, K., Mrozinska, K. & Sobota, A. (2004). Activated FcgammaRII and signalling molecules revealed in rafts by ultra-structural observations of plasma-membrane sheets. *Mol Membr Biol*., *21*, 101-108.

[69] Katsumata, O., Hara-Yokoyama, M., Sautes-Fridman, C., Nagatsuka, Y., Katada, T., Hirabayashi, Y., Shimizu, K., Fujita-Yoshigaki, J., Sugiya, H. & Furuyama, S. (2001). Association of FcgammaRII with low-density detergent-resistant membranes is important for cross-linking-dependent initiation of the tyrosine phosphorylation pathway and superoxide generation. *J Immunol*, *167*, 5814-5823.

[70] Garcia-Garcia, E., Brown, E. J. & Rosales, C. (2007). Transmembrane mutations to FcgammaRIIA alter its association with lipid rafts: implications for receptor signaling. *J Immunol*, *178*, 3048-3058.

[71] Bournazos, S., Hart, S. P., Chamberlain, L. H., Glennie, M. J. & Dransfield, I. (2009). Association of FcgammaRIIa (CD32a) with lipid rafts regulates ligand binding activity. *J Immunol*, *182*, 8026-8036.

[72] Chuang, F. Y., Sassaroli, M. & Unkeless, J. C. (2000). Convergence of Fc gamma receptor IIA and Fc gamma receptor IIIB signaling pathways in human neutrophils. *J Immunol*, *164*, 350-360.

[73] Petty, H. R. & Todd, R. F. 3rd. (1993). Receptor-receptor interactions of complement receptor type 3 in neutrophil membranes. *J Leukoc Biol*., *54*, 492-494.

[74] Marois, L., Vaillancourt, M., Marois, S., Proulx, S., Pare, G., Rollet-Labelle, E. & Naccache, P. H. (2009). The ubiquitin ligase c-Cbl down-regulates FcgammaRIIa activation in human neutrophils. *J Immunol*, *182*, 2374-2384.

[75] Richard, S., Shaw, A. S., Showell, H. J. & Connelly, P. A. (1994). The role of individual Fc gamma receptors in aggregated IgG-stimulated protein tyrosine phosphorylation in the human neutrophil. *Biochem Biophys Res Commun*, *199*, 653-661.

[76] Kobayashi, K., Takahashi, K. & Nagasawa, S. (1995). The role of tyrosine phosphorylation and Ca2+ accumulation in Fc gamma-receptor-mediated phagocytosis of human neutrophils. *J Biochem*, *117*, 1156-1161.

[77] Liang, L. & Huang, C. K. (1995). Tyrosine phosphorylation induced by cross-linking of Fc gamma-receptor type II in human neutrophils. *Biochem J*, *306*, *(Pt 2)*, 489-495.

[78] Naccache, P. H., Gilbert, C., Barabe, F., Al-Shami, A., Mahana, W. & Bourgoin, S. G. (1997). Agonist-specific tyrosine phosphorylation of Cbl in human neutrophils. *J Leukoc Biol*, *62*, 901-910.

[79] Dusi, S., Donini, M., Della Bianca, V., Gandini, G. & Rossi, F. (1994). In human neutrophils the binding to immunocomplexes induces the tyrosine phosphorylation of Fc gamma RII but this phosphorylation is not an essential signal for Fc-mediated phagocytosis. *Biochem Biophys Res Commun*, *201*, 30-37.

[80] Mitchell, M. A., Huang, M. M., Chien, P., Indik, Z. K., Pan, X. Q. & Schreiber, A. D. (1994). Substitutions and deletions in the cytoplasmic domain of the phagocytic receptor Fc gamma RIIA: effect on receptor tyrosine phosphorylation and phagocytosis. *Blood*, *84*, 1753-1759.

[81] Nimmerjahn, F. & Ravetch, J. V. (2007). Fc-receptors as regulators of immunity. *Adv Immunol*, *96*, 179-204.

[82] Rivas-Fuentes, S., Garcia-Garcia, E., Nieto-Castaneda, G., & Rosales, C. Fcgamma receptors exhibit different phagocytosis potential in human neutrophils. *Cell Immunol*.

[83] Hamada, F., Aoki, M., Akiyama, T. & Toyoshima, K. (1993). Association of immunoglobulin G Fc receptor II with Src-like protein-tyrosine kinase Fgr in neutrophils. *Proc Natl Acad Sci U S A*, *90*, 6305-6309.

[84] Zhou, M. J., Lublin, D. M., Link, D. C. & Brown, E. J. (1995). Distinct tyrosine kinase activation and Triton X-100 insolubility upon Fc gamma RII or Fc gamma RIIIB ligation in human polymorphonuclear leukocytes. Implications for immune complex activation of the respiratory burst. *J Biol Chem.*, *270*, 13553-13560.

[85] Gaudry, M., Gilbert, C., Barabe, F., Poubelle, P. E. & Naccache, P. H. (1995). Activation of Lyn is a common element of the stimulation of human neutrophils by soluble and particulate agonists. *Blood*, *86*, 3567-3574.

[86] Ibarrola, I., Vossebeld, P. J., Homburg, C. H., Thelen, M., Roos, D. & Verhoeven, A. J. (1997). Influence of tyrosine phosphorylation on protein interaction with FcgammaRIIa. *Biochim Biophys Acta*, *1357*, 348-358.

[87] Desaulniers, P., Fernandes, M., Gilbert, C., Bourgoin, S. G. & Naccache, P. H. (2001). Crystal-induced neutrophil activation. VII. Involvement of Syk in the responses to monosodium urate crystals. *J Leukoc Biol.*, *70*, 659-668.

[88] Watson, F. & Edwards, S. W. (1998). Stimulation of primed neutrophils by soluble immune complexes: priming leads to enhanced intracellular Ca2+ elevations, activation of phospholipase D, and activation of the NADPH oxidase. *Biochem Biophys Res Commun*, *247*, 819-826.

[89] Kiefer, F., Brumell, J., Al-Alawi, N., Latour, S., Cheng, A., Veillette, A., Grinstein, S. & Pawson, T. (1998). The Syk protein tyrosine kinase is essential for Fcgamma receptor signaling in macrophages and neutrophils. *Mol Cell Biol.*, *18*, 4209-4220.

[90] Fernandes, M. J., Lachance, G., Pare, G., Rollet-Labelle, E. & Naccache, P. H. (2005). Signaling through CD16b in human neutrophils involves the Tec family of tyrosine kinases. *J Leukoc Biol.*, *78*, 524-532.

[91] Howard, T. H. & Watts, R. G. (1994). Actin polymerization and leukocyte function. *Curr Opin Hematol*, *1*, 61-68.

[92] Barabe, F., Pare, G., Fernandes, M. J., Bourgoin, S. G. & Naccache, P. H. (2002). Cholesterol-modulating agents selectively inhibit calcium influx induced by chemoattractants in human neutrophils. *J Biol Chem.*, *277*, 13473-13478.

[93] Coxon, P. Y., Rane, M. J., Powell, D. W., Klein, J. B. & McLeish, K. R. (2000). Differential mitogen-activated protein kinase stimulation by Fc gamma receptor IIa and Fc gamma receptor IIIb determines the activation phenotype of human neutrophils. *J Immunol*, *164*, 6530-6537.

[94] Hazan-Halevy, I., Seger, R. & Levy, R. (2000). The requirement of both extracellular regulated kinase and p38 mitogen-activated protein kinase for stimulation of cytosolic phospholipase A(2) activity by either FcgammaRIIA or FcgammaRIIIB in human neutrophils. A possible role for Pyk2 but not for the Grb2-Sos-Shc complex. *J Biol Chem.*, *275*, 12416-12423.

[95] Vaillancourt, M., Levasseur, S., Tremblay, M. L., Marois, L., Rollet-Labelle, E. & Naccache, P. H. (2006). The Src homology 2-containing inositol 5-phosphatase 1 (SHIP1) is involved in CD32a signaling in human neutrophils. *Cell Signal*, *18*, 2022-2032.

[96] Cooney, D. S., Phee, H., Jacob, A. & Coggeshall, K. M. (2001). Signal transduction by human-restricted Fc gamma RIIa involves three distinct cytoplasmic kinase families leading to phagocytosis. *J Immunol*, *167*, 844-854.

[97] Vossebeld, P. J., Homburg, C. H., Roos, D. & Verhoeven, A. J. (1997). The anti-Fc gamma RIII mAb 3G8 induces neutrophil activation via a cooperative actin of Fc gamma RIIIb and Fc gamma RIIa. *Int J Biochem Cell Biol*., *29*, 465-473.

[98] Hannigan, M. O., Huang, C. K. & Wu, D. Q. (2004). Roles of PI3K in neutrophil function. *Curr Top Microbiol Immunol*, *282*, 165-175.

[99] Scott, C. C., Dobson, W., Botelho, R. J., Coady-Osberg, N., Chavrier, P., Knecht, D. A., Heath, C., Stahl, P. & Grinstein, S. (2005). Phosphatidylinositol-4,5-bisphosphate hydrolysis directs actin remodeling during phagocytosis. *J Cell Biol*., *169*, 139-149.

[100] Levkowitz, G., Waterman, H., Ettenberg, S. A., Katz, M., Tsygankov, A. Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Ciechanover, A., Lipkowitz, S. & Yarden, Y. (1999). Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell*, *4*, 1029-1040.

[101] Kassenbrock, C. K. & Anderson, S. M. (2004). Regulation of ubiquitin protein ligase activity in c-Cbl by phosphorylation-induced conformational change and constitutive activation by tyrosine to glutamate point mutations. *J Biol Chem*., *279*, 28017-28027.

[102] Melander, F., Andersson, T. & Dib, K. (2004). Engagement of beta2 integrins recruits 14-3-3 proteins to c-Cbl in human neutrophils. *Biochem Biophys Res Commun*, *317*, 1000-1005.

[103] Speckman, R. A., Wright Daw, J. A., Helms, C., Duan, S., Cao, L., Taillon-Miller, P., Kwok, P. Y., Menter, A. & Bowcock, A. M. (2003). Novel immunoglobulin superfamily gene cluster, mapping to a region of human chromosome 17q25, linked to psoriasis susceptibility. *Hum Genet*, *112*, 34-41.

[104] Cantoni, C., Bottino, C., Augugliaro, R., Morelli, L., Marcenaro, E., Castriconi, R., Vitale, M., Pende, D., Sivori, S., Millo, R., Biassoni, R., Moretta, L. & Moretta, A. (1999). Molecular and functional characterization of IRp60, a member of the immunoglobulin superfamily that functions as an inhibitory receptor in human NK cells. *Eur J Immunol*, *29*, 3148-3159.

[105] Munitz, A., Bachelet, I., Eliashar, R., Moretta, A., Moretta, L. & Levi-Schaffer, F. (2006). The inhibitory receptor IRp60 (CD300a) suppresses the effects of IL-5, GM-CSF, and eotaxin on human peripheral blood eosinophils. *Blood*, *107*, 1996-2003.

[106] Bachelet, I., Munitz, A., Moretta, A., Moretta, L. & Levi-Schaffer, F. (2005). The inhibitory receptor IRp60 (CD300a) is expressed and functional on human mast cells. *J Immunol*, *175*, 7989-7995.

[107] Alvarez, Y., Tang, X., Coligan, J. E. & Borrego, F. (2008). The CD300a (IRp60) inhibitory receptor is rapidly up-regulated on human neutrophils in response to inflammatory stimuli and modulates CD32a (FcgammaRIIa) mediated signaling. *Mol Immunol*, *45*, 253-258.

[108] Moldovan, I., Galon, J., Maridonneau-Parini, I., Roman Roman, S., Mathiot, C., Fridman, W. H. & Sautes-Fridman, C. (1999). Regulation of production of soluble Fc gamma receptors type III in normal and pathological conditions. *Immunol Lett*, *68*, 125-134.

[109] Huizinga, T. W., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, A. E., Roos, D. & Tetteroo, P. A. (1988). The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature*, *333*, 667-669.

[110] Kuijpers, T. W., Hoogerwerf, M. & Roos, D. (1992). Neutrophil migration across monolayers of resting or cytokine-activated endothelial cells. Role of intracellular calcium changes and fusion of specific granules with the plasma membrane. *J Immunol*, *148*, 72-77.

[111] Homburg, C. H., de Haas, M., von dem Borne, A. E., Verhoeven, A. J., Reutelingsperger, C. P. & Roos, D. (1995). Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. *Blood*, *85*, 532-540.

[112] Middelhoven, P. J., van Buul, J. D., Kleijer, M., Roos, D. & Hordijk, P. L. (1999). Actin polymerization induces shedding of FcgammaRIIIb (CD16) from human neutrophils. *Biochem Biophys Res Commun*, *255*, 568-574.

[113] Middelhoven, P. J., Ager, A., Roos, D. & Verhoeven, J. A. (1997). Involvement of a metalloprotease in the shedding of human neutrophil Fc gammaRIIIB. *FEBS Lett*, *414*, 14-18.

[114] Bazil, V. & Strominger, J. L. (1994). Metalloprotease and serine protease are involved in cleavage of CD43, CD44, and CD16 from stimulated human granulocytes. Induction of cleavage of L-selectin via CD16. *J Immunol*, *152*, 1314-1322.

[115] Watson, F., Gasmi, L. & Edwards, S. W. (1997). Stimulation of intracellular Ca2+ levels in human neutrophils by soluble immune complexes. Functional activation of FcgammaRIIIb during priming. *J Biol Chem*., *272*, 17944-17951.

[116] Robinson, J. J., Watson, F., Bucknall, R. C. & Edwards, S. W. (1994). Role of Fc gamma receptors in the activation of neutrophils by soluble and insoluble immunoglobulin aggregates isolated from the synovial fluid of patients with rheumatoid arthritis. *Ann Rheum Dis*., *53*, 515-520.

[117] Huizinga, T. W., de Haas, M., Kleijer, M., Nuijens, J. H., Roos, D. & von dem Borne, A. E. (1990). Soluble Fc gamma receptor III in human plasma originates from release by neutrophils. *J Clin Invest*, *86*, 416-423.

[118] Moulding, D. A., Hart, C. A. & Edwards, S. W. (1999). Regulation of neutrophil FcgammaRIIIb (CD16) surface expression following delayed apoptosis in response to GM-CSF and sodium butyrate. *J Leukoc Biol*., *65*, 875-882.

[119] Teillaud, C., Galon, J., Zilber, M. T., Mazieres, N., Spagnoli, R., Kurrle, R., Fridman, W. H. & Sautes, C. (1993). Soluble CD16 binds peripheral blood mononuclear cells and inhibits pokeweed-mitogen-induced responses. *Blood*, *82*, 3081-3090.

[120] Hunter, S., Kamoun, M. & Schreiber, A. D. (1994). Transfection of an Fc gamma receptor cDNA induces T cells to become phagocytic. *Proc Natl Acad Sci U S A*, *91*, 10232-10236.

[121] Lowry, M. B., Duchemin, A. M., Robinson, J. M. & Anderson, C. L. (1998). Functional separation of pseudopod extension and particle internalization during Fc gamma receptor-mediated phagocytosis. *J Exp Med*, *187*, 161-176.

[122] Odin, J. A., Edberg, J. C., Painter, C. J., Kimberly, R. P. & Unkeless, J. C. (1991). Regulation of phagocytosis and [Ca2+]i flux by distinct regions of an Fc receptor. *Science*, *254*, 1785-1788.

[123] Nagarajan, S., Chesla, S., Cobern, L., Anderson, P., Zhu, C. & Selvaraj, P. (1995). Ligand binding and phagocytosis by CD16 (Fc gamma receptor III) isoforms. Phagocytic signaling by associated zeta and gamma subunits in Chinese hamster ovary cells. *J Biol Chem*., *270*, 25762-25770.

[124] Edberg, J. C. & Kimberly, R. P. (1994). Modulation of Fc gamma and complement receptor function by the glycosyl-phosphatidylinositol-anchored form of Fc gamma RIII. *J Immunol*, *152*, 5826-5835.

[125] Nagarajan, S., Venkiteswaran, K., Anderson, M., Sayed, U., Zhu, C. & Selvaraj, P. (2000). Cell-specific, activation-dependent regulation of neutrophil CD32A ligand-binding function. *Blood*, *95*, 1069-1077.

[126] Nagarajan, S., Fifadara, N. H. & Selvaraj, P. (2005). Signal-specific activation and regulation of human neutrophil Fc gamma receptors. *J Immunol*, *174*, 5423-5432.

[127] Fossati, G., Moots, R. J., Bucknall, R. C. & Edwards, S. W. (2002). Differential role of neutrophil Fcgamma receptor IIIB (CD16) in phagocytosis, bacterial killing, and responses to immune complexes. *Arthritis Rheum*, *46*, 1351-1361.

[128] Fossati, G., Bucknall, R. C. & Edwards, S. W. (2002). Insoluble and soluble immune complexes activate neutrophils by distinct activation mechanisms: changes in functional responses induced by priming with cytokines. *Ann Rheum Dis*, *61*, 13-19.

[129] Zhou, M. J. & Brown, E. J. (1994). CR3 (Mac-1, alpha M beta 2, CD11b/CD18) and Fc gamma RIII cooperate in generation of a neutrophil respiratory burst: requirement for Fc gamma RIII and tyrosine phosphorylation. *J Cell Biol*, *125*, 1407-1416.

[130] Lofgren, R., Serrander, L., Forsberg, M., Wilsson, A., Wasteson, A. & Stendahl, O. (1999). CR3, FcgammaRIIA and FcgammaRIIIB induce activation of the respiratory burst in human neutrophils: the role of intracellular Ca(2+), phospholipase D and tyrosine phosphorylation. *Biochim Biophys Acta*, *1452*, 46-59.

[131] Pricop, L., Gokhale, J., Redecha, P., Ng, S. C. & Salmon, J. E. (1999). Reactive oxygen intermediates enhance Fc gamma receptor signaling and amplify phagocytic capacity. *J Immunol*, *162*, 7041-7048.

[132] Mayadas, T. N., Tsokos, G. C. & Tsuboi, N. (2009). Mechanisms of immune complex-mediated neutrophil recruitment and tissue injury. *Circulation*, *120*, 2012-2024.

[133] Heeringa, P., Huugen, D. & Tervaert, J. W. (2005). Anti-neutrophil cytoplasmic autoantibodies and leukocyte-endothelial interactions: a sticky connection? *Trends Immunol*, *26*, 561-564.

[134] Hewins, P., Williams, J. M., Wakelam, M. J. & Savage, C. O. (2004). Activation of Syk in neutrophils by antineutrophil cytoplasm antibodies occurs via Fcgamma receptors and CD18. *J Am Soc Nephrol*, *15*, 796-808.

[135] Williams, J. M., Ben-Smith, A., Hewins, P., Dove, S. K., Hughes, P., McEwan, R., Wakelam, M. J. & Savage, C. O. (2003). Activation of the G(i) heterotrimeric G protein by ANCA IgG F(ab')2 fragments is necessary but not sufficient to stimulate the recruitment of those downstream mediators used by intact ANCA IgG. *J Am Soc Nephrol*, *14*, 661-669.

[136] Hussain, A., Pankhurst, T., Goodall, M., Colman, R., Jefferis, R., Savage, C. O. & Williams, J. M. (2009). Chimeric IgG4 PR3-ANCA induces selective inflammatory responses from neutrophils through engagement of Fcgamma receptors. *Immunology*, *128*, 236-244.

[137] Radford, D. J., Luu, N. T., Hewins, P., Nash, G. B. & Savage, C. O. (2001). Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum*, *44*, 2851-2861.

[138] Radford, D. J., Savage, C. O. & Nash, G. B. (2000). Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum*, *43*, 1337-1345.

[139] Savage, C. O., Pottinger, B. E., Gaskin, G., Pusey, C. D. & Pearson, J. D. (1992). Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol*, *141*, 335-342.

[140] Norman, M. U., Van De Velde, N. C., Timoshanko, J. R., Issekutz, A. & Hickey, M. J. (2003). Overlapping roles of endothelial selectins and vascular cell adhesion molecule-1 in immune complex-induced leukocyte recruitment in the cremasteric microvasculature. *Am J Pathol*, *163*, 1491-1503.

[141] Suzuki, Y., Gomez-Guerrero, C., Shirato, I., Lopez-Franco, O., Gallego-Delgado, J., Sanjuan, G., Lazaro, A., Hernandez-Vargas, P., Okumura, K., Tomino, Y., Ra, C. & Egido, J. (2003). Pre-existing glomerular immune complexes induce polymorphonuclear cell recruitment through an Fc receptor-dependent respiratory burst: potential role in the perpetuation of immune nephritis. *J Immunol*, *170*, 3243-3253.

[142] Crow, A. R. & Lazarus, A. H. (2008). The mechanisms of action of intravenous immunoglobulin and polyclonal anti-d immunoglobulin in the amelioration of immune thrombocytopenic purpura: what do we really know? *Transfus Med Rev*, *22*, 103-116.

[143] Nieswandt, B., Bergmeier, W., Rackebrandt, K., Gessner, J. E. & Zirngibl, H. (2000). Identification of critical antigen-specific mechanisms in the development of immune thrombocytopenic purpura in mice. *Blood*, *96*, 2520-2527.

[144] Aslam, R., Kim, M., Speck, E. R., Seetanah, A. C., Molinski, S., Freedman, J. & Semple, J. W. (2007). Platelet and red blood cell phagocytosis kinetics are differentially controlled by phosphatase activity within mononuclear cells. *Transfusion*, *47*, 2161-2168.

[145] Tha-In, T., Bayry, J., Metselaar, H. J., Kaveri, S. V. & Kwekkeboom, J. (2008). Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immunol*, *29*, 608-615.

[146] Wright, H. L., Moots, R. J., Bucknall, R. C. & Edwards, S. W. Neutrophil function in inflammation and inflammatory diseases. *Rheumatology (Oxford)*.

[147] Firestein, G. S. (2003). Evolving concepts of rheumatoid arthritis. *Nature*, *423*, 356-361.

[148] Stokol, T., O'Donnell, P., Xiao, L., Knight, S., Stavrakis, G., Botto, M., von Andrian, U. H. & Mayadas, T. N. (2004). C1q governs deposition of circulating immune complexes and leukocyte Fcgamma receptors mediate subsequent neutrophil recruitment. *J Exp Med*, *200*, 835-846.

[149] Skilbeck, C. A., Lu, X., Sheikh, S., Savage, C. O. & Nash, G. B. (2006). Capture of flowing human neutrophils by immobilised immunoglobulin: roles of Fc-receptors CD16 and CD32. *Cell Immunol*, *241*, 26-31.

[150] Theofilopoulos, A. N. & Dixon, F. J. (1985). Murine models of systemic lupus erythematosus. *Adv Immunol*, *37*, 269-390.

[151] Boross, P. & Verbeek, J. S. (2006). The complex role of Fcgamma receptors in the pathology of arthritis. *Springer Semin Immunopathol*, *28*, 339-350.

[152] Kleinau, S., Martinsson, P. & Heyman, B. (2000). Induction and suppression of collagen-induced arthritis is dependent on distinct fcgamma receptors. *J Exp Med*, *191*, 1611-1616.

[153] Hogarth, P. M. (2002). Fc receptors are major mediators of antibody based inflammation in autoimmunity. *Curr Opin Immunol*, *14*, 798-802.

[154] Tan Sardjono, C., Mottram, P. L. & Hogarth, P. M. (2003). The role of FcgammaRIIa as an inflammatory mediator in rheumatoid arthritis and systemic lupus erythematosus. *Immunol Cell Biol*, *81*, 374-381.

[155] Belostocki, K., Park, M. S., Redecha, P. B., Masuda, E., Salmon, J. E. & Pricop, L. (2005). FcgammaRIIa is a target for modulation by TNFalpha in human neutrophils. *Clin Immunol*, *117*, 78-86.

[156] Wijngaarden, S., van Roon, J. A., van de Winkel, J. G., Bijlsma, J. W. & Lafeber, F. P. (2005). Down-regulation of activating Fcgamma receptors on monocytes of patients with rheumatoid arthritis upon methotrexate treatment. *Rheumatology (Oxford)*, *44*, 729-734.

[157] Fairhurst, A. M., Wallace, P. K., Jawad, A. S. & Goulding, N. J. (2007). Rheumatoid peripheral blood phagocytes are primed for activation but have impaired Fc-mediated generation of reactive oxygen species. *Arthritis Res Ther*., *9*, R29.

[158] Aitman, T. J., Dong, R., Vyse, T. J., Norsworthy, P. J., Johnson, M. D., Smith, J., Mangion, J., Roberton-Lowe, C., Marshall, A. J., Petretto, E., Hodges, M. D., Bhangal, G., Patel, S. G., Sheehan-Rooney, K., Duda, M., Cook, P. R., Evans, D. J., Domin, J., Flint, J., Boyle, J. J., Pusey, C. D. & Cook, H. T. (2006). Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature*, *439*, 851-855.

[159] Fanciulli, M., Norsworthy, P. J., Petretto, E., Dong, R., Harper, L., Kamesh, L., Heward, J. M., Gough, S. C., de Smith, A., Blakemore, A. I., Froguel, P., Owen, C. J., Pearce, S. H., Teixeira, L., Guillevin, L., Graham, D. S., Pusey, C. D., Cook, H. T., Vyse, T. J. & Aitman, T. J. (2007). FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat Genet*, *39*, 721-723.

[160] Willcocks, L. C., Lyons, P. A., Clatworthy, M. R., Robinson, J. I., Yang, W., Newland, S. A., Plagnol, V., McGovern, N. N., Condliffe, A. M., Chilvers, E. R., Adu, D., Jolly, E. C., Watts, R., Lau, Y. L., Morgan, A. W., Nash, G. & Smith, K. G. (2008). Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med*, *205*, 1573-1582.

[161] Floto, R. A., Clatworthy, M. R., Heilbronn, K. R., Rosner, D. R., MacAry, P. A., Rankin, A., Lehner, P. J., Ouwehand, W. H., Allen, J. M., Watkins, N. A. & Smith, K. G. (2005). Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. *Nat Med*, *11*, 1056-1058.

[162] Flores-Borja, F., Kabouridis, P. S., Jury, E. C., Isenberg, D. A. & Mageed, R. A. (2007). Altered lipid raft-associated proximal signaling and translocation of CD45 tyrosine phosphatase in B lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum*, *56*, 291-302.

[163] Yuan, F. F., Wong, M., Pererva, N., Keating, J., Davis, A. R., Bryant, J. A. & Sullivan, J. S. (2003). FcgammaRIIA polymorphisms in Streptococcus pneumoniae infection. *Immunol Cell Biol*, *81*, 192-195.

[164] Yee, A. M., Phan, H. M., Zuniga, R., Salmon, J. E. & Musher, D. M. (2000). Association between FcgammaRIIa-R131 allotype and bacteremic pneumococcal pneumonia. *Clin Infect Dis.*, *30*, 25-28.

[165] Rodriguez, M. E., van der Pol, W. L., Sanders, L. A. & van de Winkel, J. G. (1999). Crucial role of FcgammaRIIa (CD32) in assessment of functional anti-Streptococcus pneumoniae antibody activity in human sera. *J Infect Dis*, *179*, 423-433.

[166] Bredius, R. G., Derkx, B. H., Fijen, C. A., de Wit, T. P., de Haas, M., Weening, R. S., van de Winkel, J. G. & Out, T. A. (1994). Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. *J Infect Dis.*, *170*, 848-853.

[167] Platonov, A. E., Shipulin, G. A., Vershinina, I. V., Dankert, J., van de Winkel, J. G. & Kuijper, E. J. (1998). Association of human Fc gamma RIIa (CD32) polymorphism with susceptibility to and severity of meningococcal disease. *Clin Infect Dis*, *27*, 746-750.

[168] Nicu, E. A., Van der Velden, U., Everts, V., Van Winkelhoff, A. J., Roos, D. & Loos, B. G. (2007). Hyper-reactive PMNs in FcgammaRIIa 131 H/H genotype periodontitis patients. *J Clin Periodontol*, *34*, 938-945.

[169] Raknes, G., Skeie, G. O., Gilhus, N. E., Aadland, S. & Vedeler, C. (1998). FcgammaRIIA and FcgammaRIIIB polymorphisms in myasthenia gravis. *J Neuroimmunol*, *81*, 173-176.

[170] Grillo-Lopez, A. J., White, C. A., Varns, C., Shen, D., Wei, A., McClure, A. & Dallaire, B. K. (1999). Overview of the clinical development of rituximab: first monoclonal antibody approved for the treatment of lymphoma. *Semin Oncol*, *26*, 66-73.

[171] Looney, R. J. (2005). B cells as a therapeutic target in autoimmune diseases other than rheumatoid arthritis. *Rheumatology (Oxford)*, *44 Suppl 2*, ii13-ii17.

[172] Nakagawa, T., Natsume, A., Satoh, M. & Niwa, R. Nonfucosylated anti-CD20 antibody potentially induces apoptosis in lymphoma cells through enhanced interaction with FcgammaRIIIb on neutrophils. *Leuk Res.*, *34*, 666-671.

[173] Isnardi, I., Lesourne, R., Bruhns, P., Fridman, W. H., Cambier, J. C. & Daeron, M. (2004). Two distinct tyrosine-based motifs enable the inhibitory receptor FcgammaRIIB to cooperatively recruit the inositol phosphatases SHIP1/2 and the adapters Grb2/Grap. *J Biol Chem.*, *279*, 51931-51938.