

Tyrosine kinase signaling pathways in neutrophils

Krisztina Futosi^{1,2} | Attila Mócsai^{1,2}

¹Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

²MTA-SE "Lendület" Inflammation Physiology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

Correspondence

Attila Mócsai, Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary
Email: mocsai.attila@med.semmelweis-univ.hu

Summary

Neutrophils play a critical role in antimicrobial host defense, but their improper activation also contributes to inflammation-induced tissue damage. Therefore, understanding neutrophil biology is important for the understanding, diagnosis, and therapy of both infectious and inflammatory diseases. Neutrophils express a large number of cell-surface receptors that sense extracellular cues and trigger various functional responses through complex intracellular signaling pathways. During the last several years, we and others have shown that tyrosine kinases play a critical role in those processes. In particular, Src-family and Syk tyrosine kinases couple Fc-receptors and adhesion receptors (integrins and selectins) to various neutrophil effector functions. This pathway shows surprising similarity to lymphocyte antigen receptor signaling and involves various other enzymes (e.g. PLC γ 2), exchange factors (e.g. Vav-family members) and adapter proteins (such as ITAM-containing adapters, SLP-76, and CARD9). Those mediators trigger various antimicrobial functions and play a critical role in coordinating the inflammatory response through the release of inflammatory mediators, such as chemokines and LTB₄. Interestingly, however, tyrosine kinases have a limited direct role in the migration of neutrophils to the site of inflammation. Here, we review the role of tyrosine kinase signaling pathways in neutrophils and how those pathways contribute to neutrophil activation in health and disease.

KEYWORDS

neutrophils, Fc-receptors, integrins, protein kinases/phosphatases, inflammation, signal transduction

1 | INTRODUCTION

Neutrophils (also known as neutrophilic granulocytes, polymorphonuclear cells, or PMN) are the most abundant circulating leukocytes that constitute a critical component of the antimicrobial immune response, mainly as part of the innate arm of the immune system.^{1–3} Neutrophils are short-lived, terminally differentiated myeloid cells that are transcriptionally mostly inactive and function primarily as suicide effector cells of the immune response. However, recent studies indicate that neutrophils also participate in the organization of the immune and inflammatory reaction, including coordination of adaptive immunity.^{2,4}

Neutrophils express a diverse set of cell-surface receptors that are involved in sensing microbial invasion, as well as the inflammatory environment.⁵ Neutrophil cell-surface receptors include various G-protein-coupled receptors (e.g. chemokine, formyl peptide, C5a and LTB₄ receptors), Fc-receptors (mainly low-affinity Fc γ -receptors, and, in the case of human neutrophils, Fc α -receptors), integrins (primarily the β_2 integrins LFA-1 and Mac-1), selectins, cytokine receptors, and innate immune receptors (e.g. Toll-like receptors, C-type lectins, and intracellular pattern recognition receptors).⁵ Those receptors ensure that neutrophils sense, and respond to, signs of microbial invasion and other inflammatory changes in the extracellular environment.

Extracellular cues trigger an array of functional responses of neutrophils (Fig. 1). Those responses ensure that neutrophils accumulate at the site of infection/inflammation, mount a proper and

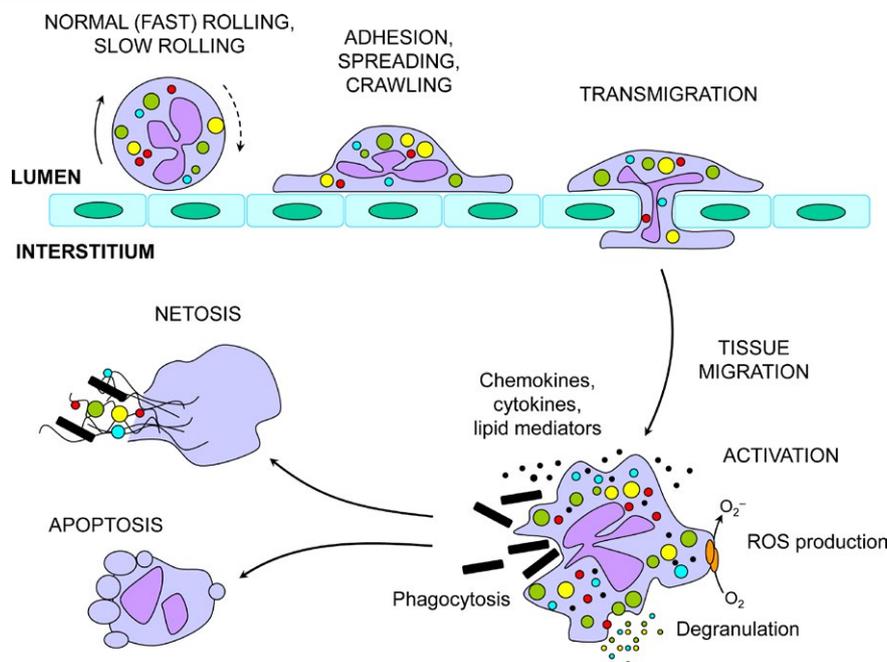


FIGURE 1 Overview of neutrophil activation. At the site of inflammation, neutrophils become activated and then adhere to and migrate through the inflamed endothelium. Within the tissues, neutrophils use their diverse effector functions to eliminate the invading pathogens and to coordinate the inflammatory reaction. At the end of their life cycle, neutrophils die by apoptosis or a specific form of cell death, NETosis. ROS, reactive oxygen species

pathogen-specific antimicrobial response, and participate in the coordination of the inflammatory reaction. Neutrophil recruitment proceeds through a complex and multi-step process that is initiated by enhanced selectin-mediated interactions followed by integrin-mediated firm adhesion and transendothelial migration.^{6,7} Antimicrobial functions of neutrophils recruited to the site of infection include phagocytosis of the pathogens (mostly after opsonization with specific immunoglobulins or complement fragments), entrapment within neutrophil extracellular traps (NETs), and biochemical processes including oxidative damage and proteolytic cleavage, through the activation of the NADPH oxidase and the release of diverse granule proteins.^{2,3} Furthermore, a number of recent studies indicate that neutrophils are not just mere effector cells of the immune response, but strongly contribute to the organization of the immune and inflammatory reactions,^{1–3} including the amplification of their own recruitment and activation,^{8–10} cooperation with other immune cells,¹¹ and even directing the adaptive immune response.^{1,4} The latter functions are mechanistically mediated by the release of a number of proinflammatory mediators (chemokines, cytokines, and lipid mediators) from activated neutrophils.^{9,12}

Neutrophil cell-surface receptors are coupled to antimicrobial responses through very complex intracellular signal transduction pathways.^{5,13} While many of those pathways are reminiscent of similar pathways in other cells, there are a number of unique characteristics specific for neutrophils, and a number of pathways were identified, or first characterized in primary cells, within the neutrophil compartment.^{14–17}

Among the signaling pathways activated and functioning within neutrophils, tyrosine phosphorylation pathways have received particularly much attention. This is especially true for non-receptor tyrosine

kinase pathways triggered by the cooperative activation of Src-family tyrosine kinases and the Syk tyrosine kinase. Analysis of that pathway in neutrophils allowed the characterization of the role of Src-family kinases in integrin signaling in primary cells,^{14,18} one of the first descriptions of the now widely accepted functional overlap between different members of the same protein family,^{8,14} the identification of a novel integrin signaling pathway reminiscent of immunoreceptor signal transduction,^{16,17,19} dissection of the signaling pathways leading from β_2 integrins to cell migration and adhesion-dependent cellular activation,^{8,16,17,20} characterization of the receptors and signaling pathway triggered by neutrophil Fc γ -receptors,^{8,20,21} mapping intracellular signaling during neutrophil mediated *in vivo* inflammatory reactions,^{8,9,20,22–24} and the first direct demonstration of an *in vivo* functional importance of neutrophil gene expression and neutrophil-derived chemokine/cytokine release.⁹

We have contributed to many of the above studies, primarily by testing neutrophil functions and neutrophil-mediated *in vivo* disease processes in genetically modified experimental mice. Here, we provide an overview of the role of tyrosine kinase signaling pathways in neutrophils with some additional personal insight into the development of the field during the last several years.

2 | TYROSINE KINASE PATHWAYS IN NEUTROPHIL ADHESION AND ADHESION-DEPENDENT ACTIVATION

Recruitment of neutrophils to the site of infection or tissue damage and their activation in the inflamed tissues are critical steps

of host defense and the overall inflammatory process. At the site of inflammation, various inflammatory stimuli direct the transendothelial migration of neutrophils from the intravascular space to the surrounding tissues. Integrins and integrin-mediated adhesive processes are critically involved in that transmigration, as well as in the activation of neutrophils bound to extracellular matrix proteins within the inflamed interstitium.

2.1 | Integrins and integrin activation in neutrophils

Integrins are ubiquitously expressed adhesion receptors critically involved in cell–cell and cell–extracellular–matrix interactions. Integrins are transmembrane heterodimers consisting of α - and β -subunits (Fig. 2). In case of neutrophils, the most critical integrins belong to the β_2 integrin family and consist of a common β_2 -subunit (CD18) and a unique α -subunit (one of three CD11 molecules). Those integrins include LFA-1 ($\alpha_L\beta_2$; CD11a/CD18), Mac-1 ($\alpha_M\beta_2$; CD11b/CD18), and likely less important on neutrophils, gp150/95 ($\alpha_X\beta_2$; CD11c/CD18).¹³ While LFA-1 and gp150/95 are constitutively expressed, the cell-surface expression of Mac-1 is upregulated during neutrophil activation. Both LFA-1 and Mac-1 bind to ICAM-1 on the endothelial surface and mediate the later phases of neutrophil adhesion and transmigration.^{13,25} Integrins have three distinct conformations: (i) the inactive bent form; (ii) the extended form with a closed headpiece that has intermediate affinity; and (iii) the extended form with open headpiece that has high affinity for its endothelial ligand ICAM-1 (Fig. 2). The critical role of those molecules in antimicrobial host defense is indicated by the severe bacterial and fungal infections of human patients suffering from type I leukocyte adhesion deficiency (LAD I) due to genetic defects of the β_2 -subunit CD18.²⁶

Integrins are bidirectional signaling molecules, participating in both signaling initiated from the cytoplasm of the cell (inside-out signaling) and from the extracellular environment (outside-in signaling)²⁷ (Fig. 2). During inside-out signaling, intracellular signals trigger affinity changes and other events leading to increased ligand binding of the integrins. Typical examples of inside-out signaling include the changes triggered by proinflammatory mediators such as selectin ligands, chemokines, classical chemoattractants, or proinflammatory cytokines leading to increased surface expression, as well as increased affinity and avidity of β_2 integrins on the neutrophil cell surface. Indeed, the transition from normal ('fast') rolling of neutrophils over the resting endothelium to a deceleration phase ('slow rolling') is mediated by inside-out signaling triggered by engagement of neutrophil PSGL-1 (and, possibly, CD44 and ESL-1) by endothelial E-selectins, triggering inside-out signaling leading to transition from a low to intermediate affinity conformation of β_2 integrins.¹³ In a next step, chemokines expressed on the inflamed endothelium result in an additional inside-out signal leading to full extension of β_2 integrins with high affinity for ICAM-1. The important role for leukocyte inside-out signaling is indicated by the fact that human patients lacking the inside-out signaling molecule kindlin-3 suffer from type III leukocyte

adhesion deficiency, characterized by severe bacterial infections and other pathologies.²⁶

Binding of extracellular ligands (e.g. ICAM-1 or extracellular matrix molecules) to β_2 integrins results in integrin clustering and outside-in signaling that leads to cell spreading and firm adhesion, followed by several additional steps eventually leading to transmigration through the endothelium (Fig. 2). In addition to supporting transendothelial migration, β_2 integrin-dependent outside-in signaling also results in other effector cell responses such as respiratory burst or exocytosis of intracellular granules, e.g. during locomotion of the cells in the extravascular space.²⁷

Here, we discuss tyrosine kinase signaling pathways involved in outside-in signaling triggered by ligand binding of β_2 integrins (Fig. 2 and Table 1). Mechanisms of inside-out signaling will be discussed later during this review.

2.2 | Tyrosine kinases in neutrophil integrin signaling

Src-family kinases were the first tyrosine kinases to be identified²⁸ and their discovery revolutionized several fields including cancer pathogenesis and cell survival.^{29–31} Based on heterologous expression studies in long-propagated cell lines, it has long been thought that Src-family kinases play an important role in integrin signal transduction in fibroblasts and likely also in other cell types.^{32,33} However, direct *in vivo* evidence for such a role had long been missing. This was not changed by the first publication of Src knockout mice either since those mice had an unexpectedly narrow range of phenotypes, namely osteopetrosis due to dysfunctional osteoclasts.³⁴ Few years after the publication of the Src knockouts, targeted deletion of several other members of the Src kinase family has also been reported.³⁵ In a seminal early article, Lowell et al.³⁶ reported the deletion of Hck and Fgr, two major tyrosine kinases present in neutrophils and other myeloid cells. The most striking observation was that although *Hck*^{-/-} and *Fgr*^{-/-} single knockouts did not show signs of increased susceptibility to *Listeria* infection, *Hck*^{-/-} *Fgr*^{-/-} double knockouts failed to clear the *Listeria* pathogens and succumbed to a fatal *Listeria* infection. Those studies provided the first direct genetic evidence for an *in vivo* role of myeloid-specific Src-family kinases, and they were one of the first reports indicating functional overlap between members of the same protein family *in vivo*.

In a few seminal early articles, Nathan et al.^{37,38} showed that neutrophil adhesion to integrin-ligand surfaces in the presence of additional proinflammatory stimuli triggers robust activation of the cells characterized by cytoskeletal changes, cell spreading, release of superoxide anions and exocytosis of their granule content. In the mid 1990s, it became evident that such adherent activation leads to tyrosine phosphorylation of a number of molecules.^{39,40} Among others, Src-family kinases including Hck, Fgr, and Lyn were shown to be present in neutrophils^{41–43} and at least some of them became phosphorylated upon integrin-mediated adherent activation of human neutrophils.⁴⁰ Those studies prompted the analysis of the effect of

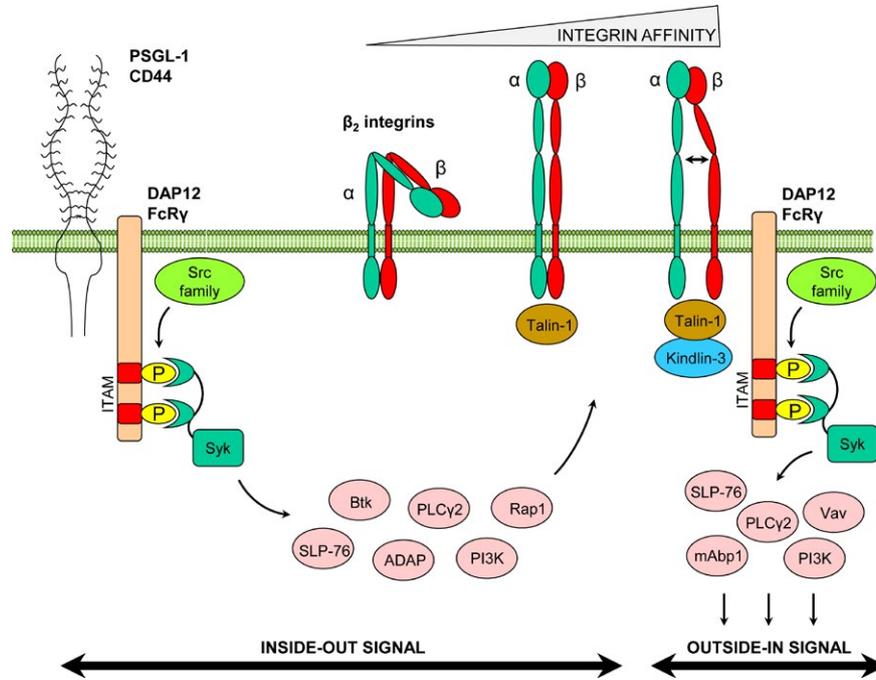


FIGURE 2 Integrin-dependent inside-out and outside-in signaling in neutrophils. Inside-out signaling through selectin ligands and other cell-surface receptors triggers increased affinity and avidity of integrins (inside-out signaling). However, integrin ligation initiates outside-in signaling leading to various effector functions of the cells. Both platelet-selectin glycoprotein ligand 1 (PSGL-1) mediated inside-out signaling and β_2 integrin outside-in signaling utilize an immunoreceptor tyrosine-based activation motif (ITAM) mediated tyrosine kinase signaling pathway

Src-family kinase deficiency on integrin-mediated mouse neutrophil function. Lowell and Berton¹⁴ showed that the combined deficiency of Hck and Fgr strongly reduce adhesion and spreading of neutrophils on integrin-ligand-coated surfaces and adhesion-dependent ROS production. Similar to the *in vivo* Listeria infection models,³⁶ the *Hck*^{-/-} or *Fgr*^{-/-} single mutations did not affect adherent activation of neutrophils.¹⁴ We have extended those first studies to degranulation by showing that *Hck*^{-/-} *Fgr*^{-/-} or Src-inhibitor-treated neutrophils fail to release their secondary granules in response to adherent activation.¹⁸ Taken together, those studies have revealed a critical role for the Src-family kinases Hck and Fgr in integrin-mediated neutrophil activation. Those were also the first studies providing direct genetic evidence for the role of Src-family kinases in integrin signal transduction in primary cells. In later studies, an even more severe defect was found in *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} triple knockout neutrophils, indicating an additional role for the Lyn tyrosine kinase⁴⁴ (and M. Kovács and A. Mócsai, unpublished observations). More recently, we found that the Src/Abl-family kinase inhibitor dasatinib completely blocks adhesion-dependent functional responses of human neutrophils.⁴⁵

Besides Src-family kinases, the Syk tyrosine kinase is also present in neutrophils.⁴⁶ Syk is an SH2 domain-containing non-receptor tyrosine kinase that is a central element of immunoreceptor signaling including signal transduction through B-cell receptors and Fc-receptors.⁴⁷ Syk has been shown to become phosphorylated upon $\alpha_{IIb}\beta_3$ integrin-mediated platelet activation,⁴⁸ during β_1 and β_2 integrin-mediated activation in a human monocytic cell line,⁴⁹ integrin-mediated activation of CHO cells heterologously expressing $\alpha_{IIb}\beta_3$ and Syk,^{50,51} as well as upon integrin-mediated stimulation

of human neutrophils.⁵² Those studies suggested that Syk is a central component of a novel integrin signal transduction pathway.^{50,51} However, direct evidence for a functional role for Syk in integrin signaling in primary cells was still lacking.

Given the proposed role of Syk in integrin signal transduction, we became very interested in testing integrin-mediated functional responses in *Syk*^{-/-} neutrophils. Unfortunately, *Syk*^{-/-} mice die soon after birth, supposedly because of a lymphatic vascular developmental defect due to the lack of Syk in platelets.^{47,53} To overcome that difficulty, we set up an approach to generate chimeric mice with *Syk*-deficient hematopoietic systems by transplanting *Syk*^{-/-} fetal liver cells into lethally irradiated wildtype recipients.¹⁶ *Syk*^{-/-} neutrophils isolated from such bone marrow chimeras failed to respond to various proinflammatory stimuli when plated on β_2 integrin-ligand surfaces such as fibrinogen or recombinant ICAM-1.¹⁶ Defective functional responses included spreading, firm adhesion, respiratory burst, and granule release. Those defects were similar to the ones observed in CD18-deficient neutrophils.¹⁶ Further studies revealed that Syk was activated by upstream mechanisms through CD18 and Src-family kinases. Those results together provided direct genetic evidence for a critical role for Syk in β_2 integrin signal transduction in neutrophils. Interestingly, however, β_2 integrin-mediated chemotactic migration was not affected by the Syk deletion (see below).

We and others have also tested downstream components of the β_2 integrin signaling pathway mediated by Src-family kinases and Syk. Since PLC γ isoforms act downstream of Syk in other cell types, we and others have tested the activation and role of PLC γ isoforms in integrin signaling of neutrophils. The PLC γ 2 isoform was activated

TABLE 1 Integrin-mediated functional responses of neutrophils. Functional responses included adhesion-induced in vitro effector responses (spreading, respiratory burst, and/or degranulation), in vitro Transwell migration, or thioglycollate-induced peritoneal migration (in mixed bone marrow chimeras, whenever available). =, not changed; ↓, partially reduced; ↓↓, strongly reduced; ↓↓↓, completely blocked; n. a., data not available

Genotype	Effector responses (in vitro)	Transwell migration (in vitro)	Peritoneal migration (in vivo)	Reference(s)
<i>Itgb2</i> ^{-/-} (CD18-deficient)	↓↓↓	↓↓	↓↓	16, 23
<i>Itgam</i> ^{-/-} (CD11b-deficient)	↓↓↓	=	=	95, 96, 166 (and M. Kovács and A. Mócsai, unpublished)
<i>Tyrbp</i> ^{-/-} <i>Fcer1g</i> ^{-/-} (DAP12/FcRγ double def.)	↓↓↓	=	=	17
<i>Hck</i> ^{-/-} <i>Fgr</i> ^{-/-} <i>Lyn</i> ^{-/-}	↓↓↓	=	=	8, 16
<i>Syk</i> ^{-/-}	↓↓↓	=	=	16
<i>Plcg2</i> ^{-/-} (PLCγ2-deficient)	↓↓↓	=	=	20, 84
<i>Lcp2</i> ^{-/-} (SLP-76-deficient)	↓↓↓	n.a.	=	58
<i>Vav1</i> ^{-/-} <i>Vav2</i> ^{-/-} <i>Vav3</i> ^{-/-}	↓↓↓	=	=	54, 84
<i>Arhgap35</i> ^{-/-} (p190RhoGAP-deficient)	=	=	=	23
<i>Card9</i> ^{-/-}	=	=	=	9 (and T. Németh and A. Mócsai unpublished)

through Src-family kinases and Syk upon neutrophil adhesion and PLCγ2-deficient neutrophils failed to perform various integrin-mediated functional responses, such as spreading, respiratory burst or granule release.^{20,54} The Vav guanine nucleotide exchange factors for Rho-family small GTPases are also involved in signal transduction downstream of Src-family kinases and Syk in various other cell types.⁵⁵ Both the intact Vav-binding site of Syk and Vav itself are involved in membrane polarization and stabilization of the leading edge in neutrophil-like cell lines and primary mouse neutrophils.^{54,56} Two groups have shown that *Vav1*^{-/-} *Vav3*^{-/-} double knockout or *Vav1*^{-/-} *Vav2*^{-/-} *Vav3*^{-/-} triple knockout neutrophils (but not the single knockouts) fail to mount integrin-mediated spreading, firm adhesion, and respiratory burst.^{54,57} Further downstream signaling is dependent on the SLP-76 adapter since SLP-76-deficient neutrophils had impaired integrin-mediated cellular responses, such as ROS production, adhesion, and spreading.⁵⁸ Taken together, PLCγ2, Vav-family molecules, and SLP-76 are downstream mediators of Src-family kinases and Syk during integrin-mediated neutrophil activation.

In addition to the components of the above signaling pathway, members of the Tec-family tyrosine kinases have also been proposed to participate in integrin-mediated functional responses of neutrophils. Indeed, a recent study has revealed that lack of the Tec-family tyrosine kinase Btk blocks neutrophil responses on a polyvalent integrin-ligand surface and other integrin-mediated functional responses of the cells.⁵⁹ However, interpretation of those findings is hindered by the complex phenotypes of the neutrophil compartment in *Btk*^{-/-} mice.^{60,61} Of further possible components of this signaling pathway, the p190RhoGAP (p190-A) protein, a major Src-family kinase substrate in other cellular systems, was not required for adhesion-mediated functional responses of neutrophils.²³ Additional components of the Syk-mediated integrin signaling pathway may involve the mammalian actin-binding protein mAbp1 and

its interaction partner hematopoietic progenitor kinase 1 (HPK1), though differences in assay conditions complicate the interpretation of those findings.^{62–64}

It should be mentioned that in agreement with Nathan's original report,³⁷ integrin-mediated neutrophil activation is usually triggered by the application of a soluble proinflammatory stimulus (such as TNF-α, chemokines, or TLR ligands), while the cells are adherent to an immobilized integrin-ligand-coated surface (e.g. fibrinogen, FCS, or ICAM-1). However, Berton et al.⁶⁵ reported robust integrin-dependent neutrophil activation by plating neutrophils on immobilized monoclonal antibodies against various integrin chains without any additional stimulus. Those experiments were interpreted as evidence that integrin cross-linking alone (i.e. without a second proinflammatory signal) is capable of triggering maximal activation of neutrophils. We have shown that Syk is indispensable for neutrophil responses triggered by such direct activation by plate-bound monoclonal antibodies against β₁, β₂, and β₃ integrins.¹⁶ In addition, anti-CD18-mediated functional responses of neutrophils were also shown to require Src-family kinases, PLCγ2, Vav-family, and SLP-76.^{44,54,58} Those reports were thought to provide direct evidence for the role of Src-family-Syk-PLCγ2-Vav-SLP-76 pathway in integrin signal transduction in neutrophils. However, our later studies have revealed that anti-integrin antibody-mediated neutrophil activation also requires a second signal through the ligation of low-affinity Fcγ-receptors,⁶⁶ suggesting that integrin cross-linking alone may not be sufficient to trigger full neutrophil activation. Indeed, in case of anti-integrin antibody stimulation, the Fc portion of the antibodies were revealed to provide a second signal through Fcγ-receptors, just like TNF and other mediators in the classical adherent activation system. Since Fc-receptors are expected to signal through the above signaling molecules (see below), it is difficult to tell whether those molecules are required for signaling by integrins, Fc-receptors, or both during neutrophil activation by immobilized anti-integrin antibodies.¹⁶

We and others have also tested the role of the above signaling pathway components in other cell types of hematopoietic origin. Syk was also found to be important for β_2 integrin signaling in monocytes⁶⁷ and for $\alpha_{IIb}\beta_3$ integrin-mediated platelet activation and spreading.⁶⁸ In addition, genetic deficiency of PLC γ 2 or SLP-76 or the combined deletion of Hck, Fgr, Lyn, and Src attenuated platelet spreading and signaling responses on the $\alpha_{IIb}\beta_3$ integrin-ligand fibrinogen.⁶⁸⁻⁷⁰ We proposed and others provided more direct evidence that Syk also plays a critical role in β_3 integrin signal transduction in osteoclasts,⁷¹⁻⁷³ while Src and PLC γ 2 were also shown to be involved in $\alpha_V\beta_3$ integrin signaling in those cells.⁷³⁻⁷⁵

2.3 | Immunoreceptor-like signaling by β_2 and β_3 integrins

One of the main messages of the above studies was that Syk is critically involved in integrin signaling in neutrophils and several other cell types. However, the mechanism of integrin-mediated Syk activation in primary cells was still unclear. Syk activation through binding of its tandem SH2 domains to phosphorylated ITAM motifs is a central component of immunoreceptor (e.g. B-cell receptor and Fc-receptor) signal transduction pathways.⁴⁷ However, integrins do not contain ITAM motifs and integrin-mediated Syk activation appeared to occur in an ITAM-independent manner, at least in heterologous expression systems. Indeed, in CHO cells coexpressing Syk and the platelet integrin $\alpha_{IIb}\beta_3$, integrin-mediated Syk activation did not require functional Syk SH2 domains and was not suppressed by sequestering phosphorylated ITAMs by overexpression of the tandem SH2 domains of Syk.⁵⁰ Those results led to the conclusion that Syk activation through integrins proceeds through a mechanism that is conceptually different from the ITAM-based activation of Syk during immunoreceptor signaling.

We have attempted to test the above question in primary cells including neutrophils. Our initial approach was to identify ITAM-containing molecules in neutrophils and test Syk activation upon their genetic deletion. Neutrophils express at least two ITAM-containing transmembrane adapter molecules, DAP12 (an adapter of various innate immune receptors in myeloid cells and NK cells), and Fc γ R γ , the common γ -chain of various activating Fc-receptors.⁷⁶ Therefore, we decided to test integrin-mediated functional responses and Syk activation in single- and double-knockout neutrophils lacking DAP12 and/or Fc γ R γ .¹⁷ Although those experiments were initially aimed to be mere controls, they have unexpectedly revealed practically complete defects of integrin-mediated spreading, adhesion, respiratory burst, and degranulation of double-knockout neutrophils lacking both DAP12 and Fc γ R γ , while single knockouts showed an intermediate phenotype.¹⁷ Further biochemical studies have confirmed that adherent activation of neutrophils triggers all critical steps of ITAM-based signaling, including ITAM phosphorylation by Src-family kinases and association of phosphorylated ITAMs with the tandem SH2 domains of Syk.¹⁷ Furthermore, structure-function studies using a retroviral reconstitution approach confirmed that intact Syk SH2 domains and intact ITAM tyrosines are required for integrin-mediated functional

responses of neutrophils.¹⁷ In a parallel study, restoration of integrin-mediated functional responses of Syk^{-/-} neutrophils by retroviral re-expression of Syk also revealed a critical role for both SH2 domains of Syk,⁷⁷ again indicating that Syk activation by neutrophil integrins occurs through an immunoreceptor-like manner. Taken together, those studies unexpectedly revealed that integrin signal transduction in neutrophils occurs through an ITAM-based mechanism through the recruitment of Syk to the phosphorylated ITAMs of the DAP12 and Fc γ R γ adapter molecules. Although it is at present unclear how integrins couple to ITAM-containing adapter molecules, the most likely scenario is that DAP12- and/or Fc γ R γ -associated receptors function as signaling co-receptors upon integrin ligation.¹⁹

We and others have also tested whether a similar, ITAM-based pathway is involved in integrin signaling in other cell types. ITAM-containing adapters, as well as the DAP12 ITAM tyrosines and the Syk SH2 domains, were required for CD18-mediated ERK activation in macrophages.¹⁷ Platelet spreading required both tandem SH2 domains of Syk⁷⁷ and Fc γ RIIA has been shown to mediate ITAM-based outside-in signaling by $\alpha_{IIb}\beta_3$ in human platelets.⁷⁸ We and others have shown that DAP12, Fc γ R γ , and Syk are required for osteoclast development, and likely, $\alpha_V\beta_3$ integrin functions in osteoclastic lineage cells through an ITAM-based mechanisms involving the DAP12 ITAM tyrosines and the Syk SH2 domains.^{71-73,79} Similar ITAM-based integrin activation has later also been shown in dendritic cells⁸⁰ and microglia.⁸¹

Taken together, the above studies unexpectedly revealed a critical role for ITAM-based signaling steps during integrin signal transduction in neutrophils (Fig. 2) and in diverse other cellular lineages of hematopoietic origin.

3 | TYROSINE KINASES IN NEUTROPHIL MIGRATION

Similar to other leukocytes, neutrophils migrate to the site of infection or inflammation in a multistep cascade directed by a series of adhesive interactions between neutrophils and the inflammatory endothelium.^{7,82} We and others have tested the role of tyrosine kinase signaling pathway components in *in vitro* and *in vivo* migration of neutrophils (Table 1).

3.1 | The role of integrins in neutrophil migration

One of the most critical and best known function of β_2 integrins is their role in the migration of leukocytes (including neutrophils) to the site of infection/inflammation. Indeed, the most prominent consequence of β_2 integrin (CD18) deficiency in humans (called type I leukocyte adhesion deficiency) is the development of severe bacterial and fungal infections without pus formation, indicating defective recruitment of neutrophils to the site of infection.²⁶

Previously mentioned studies have indicated that β_2 integrin-mediated non-migratory functional responses of neutrophils (such as adhesion, spreading, respiratory burst, and degranulation) are

mediated by a tyrosine kinase signaling pathway involving Src-family kinases, ITAM-containing adapters, Syk, PLC γ 2, Vav exchange factors, and SLP-76.^{14,16–18,20,54,57,58} The phenotypes seen upon deletion of those molecules was similar to the ones observed in CD18 knockout neutrophils¹⁶ (Table 1). In addition, our own experiments have confirmed a critical role for CD18 in mouse neutrophil migration both in vitro and in vivo.^{8,16,23,56} Syk and Vav are also accumulated at the leading edge of polarized neutrophils and/or HL-60 cells^{16,56} and disruption of their interaction or function interferes with proper lamellipodium formation.⁵⁶ Those results led us to hypothesize that β_2 integrin-mediated migratory responses of neutrophils would also involve components of the above tyrosine kinase signaling pathway. As shown below, this hypothesis did not prove to be correct.

3.2 | Neutrophil migration in in vitro assay systems

As expected from the known role of β_2 integrins in neutrophil migration, the in vitro chemotaxis of CD18-deficient neutrophils through a fibrinogen-coated Transwell membrane toward chemoattractants such as fMLP or LTB₄ was dramatically reduced.^{8,16} Surprisingly, we have found that *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-}, *Syk*^{-/-}, *Plcg2*^{-/-}, and DAP12/FcR γ double-knockout neutrophils migrated normally under similar conditions.^{8,16,17,20,83} In addition, *Plcg2*^{-/-} and *Vav1*^{-/-} *Vav2*^{-/-} *Vav3*^{-/-} neutrophils migrated normally toward C5a in an in vitro Transwell assay⁸⁴ (Table 1).

CD18-deficient neutrophils also failed to migrate toward fMLP or the CXCL1 chemokine KC in an in vitro Zigmund chamber assay.⁵⁶ Although *Syk*^{-/-} neutrophils showed moderately reduced migration under such conditions, that defect was not nearly as severe as the complete defect seen in CD18 deficiency.⁵⁶ Neutrophils with an inducible Syk deletion also migrated normally in a two-dimensional EZ-Taxiscan assay.⁸⁵ In addition, *Vav1*^{-/-} *Vav3*^{-/-} double-knockout neutrophils migrated normally toward fMLP in a Zigmund chamber assay.⁵⁷

We have also tested the role of tyrosine kinases in human neutrophils using a pharmacological approach. Although the Src/Abl-family kinase inhibitor dasatinib blocked various adhesion-induced functional and signaling responses of human neutrophils, it did not affect migration of the cells toward fMLP or CXCL8 (IL-8) through fibrinogen-coated Transwell membranes or Transwells filled with a Matrigel matrix.⁴⁵ Although dasatinib clearly reduced adhesion of fMLP-treated human neutrophils in a flow migration chamber, it did not affect the migration of the adherent cells under such conditions either.⁴⁵ However, dasatinib strongly reduced human neutrophil migration toward fMLP or IL-8 in a Zigmund chamber assay,⁴⁵ indicating possible mechanistic differences between the different migration assays.

Taken together, the overall picture from the above studies was that components of the Src-family-ITAM adapter-Syk-PLC γ 2-Vav-SLP-76 signaling pathway do not play a critical role in various CD18-dependent in vitro migratory responses of neutrophils (Table 1). Those results suggest that β_2 integrins trigger tyrosine kinase-dependent signaling to promote various adhesion-dependent effector functions, and migratory responses that are mostly independent of tyrosine kinase

signaling. It should nevertheless be mentioned that the varying results with the Zigmund chamber assay and the CD18-independent nature of neutrophil migration toward CXCL2 (MIP-2) in an in vitro Transwell assay⁸ indicate further complexities of this question.

Of other signaling pathway components, although Btk has been shown to be required for neutrophil migration in a Zigmund chamber assay,⁵⁹ prior studies did not reveal any major role for Btk in a three-dimensional (Transwell system-related) chemotaxis assay in human neutrophils.⁸⁶

3.3 | In vivo migration of neutrophils

We have also performed a number of experiments to test the signaling requirements for neutrophil migration under in vivo inflammatory conditions. Because of a much more complex environment, the interpretation of such data are also much more challenging. To avoid those obstacles, we have developed and utilized complex experimental approaches to exclude the effect of different environments on neutrophil migration in the different mutant mouse strains.

One of the easiest and most widely used approaches to test neutrophil migration in vivo is the use of a thioglycollate-induced peritonitis model. One of the issues complicating the interpretation of such studies was the controversial data from the literature regarding the role of CD18 in neutrophil migration in this system. While an initial study reported normal accumulation of CD18-deficient neutrophils in the inflamed peritoneum,⁸⁷ another group reported dramatically reduced emigration efficiency of CD18-deficient neutrophils under similar conditions.⁸⁸ The apparent contradiction likely originated from the fact that CD18-deficient mice show a nearly 10-fold increase in circulating neutrophil numbers, leading to strongly different conclusions depending on whether one normalizes the data on circulating neutrophil numbers. Further complicating this issue, possible subclinical infections in immunodeficient (e.g. CD18 knockout) mice could also affect the behavior of neutrophils due to an indirect, environmental effect. Those issues prompted us to develop an in vivo assay system that would allow us to better compare the intrinsic, cell-autonomous migratory capacities of the different mouse strains. To this end, we generated mixed bone marrow chimeras using wildtype and mutant bone marrow cells (carrying different CD45 alleles to allow identification of the two genotypes by flow cytometry) and tested the efficacy of the neutrophils from the two different genotypes to accumulate at the site of inflammation in a competitive migration assay.¹⁶ Since neutrophils from the different genotypes were tested within the same single animal (i.e. under identical conditions) and all data were normalized to the circulating numbers of neutrophils from each genotype, this assay system allowed a very accurate assessment of the intrinsic migratory capacity of neutrophils in vivo.

Using the above assay system, we have shown that CD18-deficient neutrophils have a strong cell-autonomous defect of migration to the inflamed peritoneum,¹⁶ confirming that CD18 is a critical player of neutrophil migration to the site of inflammation (Table 1). Using the same competitive migration assay system, however, neither

Hck^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} triple knockout nor *Syk*^{-/-} neutrophils showed any cell-autonomous defect in peritoneal accumulation.¹⁶ In addition, no defect could be observed in the migration of PLCγ2-deficient neutrophils to the inflamed peritoneum in a similar competitive migration assay either.²⁰ Additional experiments also failed to identify any defects in neutrophil migration in a thioglycollate-induced peritonitis in intact *Hck*^{-/-} *Fgr*^{-/-}, *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-}, *Plcg2*^{-/-}, *Vav1*^{-/-} *Vav2*^{-/-} *Vav3*^{-/-}, or DAP12/FcRγ double-knockout mice or in dasatinib-treated wild-type animals^{17,84,89} (and K. Futosi and A. Mócsai unpublished observations) (Table 1). No effect of inducible *Syk* deletion on neutrophil accumulation in the peritoneum could be observed either.⁹⁰ Taken together, although β₂ integrins are critically involved in neutrophil migration to the inflamed peritoneum in a cell-autonomous manner, components of the Src-family/Syk-mediated tyrosine kinase pathway do not play a substantial role in the same process.

We have also tested aspects of neutrophil migration in other in vivo assays. In agreement with their defective in vitro spreading and adhesion,¹⁶ *Syk*^{-/-} neutrophils showed defective adhesion and spreading in fMLP-superfused cremaster muscle venules.⁹¹ However, their emigration to the perivascular space under similar conditions was only partially reduced,⁵⁶ again indicating a limited contribution of *Syk* to transendothelial neutrophil migration in vivo. In addition, PLCγ2 was also required for neutrophil adhesion and spreading on venous endothelium, but not for the extravasation of the cells in fMLP-superfused cremaster muscles.²⁰ Inducible *Syk* deletion did not impair neutrophil accumulation in the bronchoalveolar lavage under various conditions either.⁹⁰ *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} and *Syk*^{-/-} neutrophils also accumulated normally at the site of tissue damage in the local Shwartzman reaction.⁹² Therefore, tyrosine kinase signaling components are dispensable for neutrophil migration under various in vivo conditions.

We and others have also tested accumulation of neutrophils at the site of inflammation (either tested directly or extrapolated from overall leukocytic infiltration) in immune complex-induced disease models such as the K/B×N serum-transfer arthritis or the reverse passive Arthus reaction. Both β₂ integrins and FcRγ, the signaling chain of activating Fc-receptors, are required for neutrophil accumulation in such immune complex-induced disease models.⁸ However, while β₂ integrins play a cell-autonomous role in neutrophil migration,⁸ Fc-receptors are likely involved in sensing immune complex deposition and generating the inflammatory environment but not in the intrinsic capacity of neutrophils to migrate to the site of inflammation.⁸ Neutrophilic infiltration was strongly reduced in the absence of *Syk*^{22,56,93} or PLCγ2,^{20,84} or in the *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-8} or in the *Vav1*^{-/-} *Vav2*^{-/-} *Vav3*^{-/-84} triple-knockout mice in those models. However, it is likely that those defects were due to the lack of overall inflammation, rather than a direct cell-autonomous role for tyrosine kinase signaling pathway components in neutrophil migration. Indeed, at least in the case of *Syk*^{-/-93} and *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-8} mutants, it has been directly shown that mutant neutrophils are able to migrate to the site of inflammation in mixed bone marrow chimeras subjected to K/B×N serum-transfer arthritis. Those issues will be discussed in further detail in a later section of this review.

Taken together, the above in vitro and in vivo experiments surprisingly indicated that the Src-family-ITAM adapter-Syk-PLCγ2-Vav signaling pathway does not play an important cell-autonomous role in the migration of neutrophils toward inflammatory agents, even under conditions where β₂ integrins had a clear cell-autonomous function (Table 1). It should be noted that some experiments in Zigmond chamber assays^{45,56} did not fit perfectly into this picture. However, since two-dimensional migration assays such as the Zigmond chamber assay tend to overemphasize the role of adhesion⁹⁴ and we found much better correlation of our in vivo findings with other in vitro migration assays,^{8,16,20,45,66} we believe that the Zigmond chamber assay is less accurate than the other in vitro approaches in mimicking in vivo migratory functions of neutrophils.

The lack of a major role of tyrosine kinase signaling in neutrophil migration was a rather unexpected and still poorly understood finding, especially in the light of the critical role of the same pathway in other β₂ integrin-mediated functions and the obvious role of β₂ integrins in neutrophil migration in diverse assay systems. However, it should be noted that even CD18-deficient neutrophils are able to migrate under certain conditions in vitro⁸ and leukocytes are able to migrate in an integrin-independent manner in three-dimensional environments both in vitro and in vivo.⁹⁴ Therefore, it would be tempting to speculate that neutrophils lacking critical tyrosine kinase signaling pathway components are able to migrate simply because the given assay tests integrin-independent migration. However, this is not the case, since CD18-deficient neutrophils are strongly defective under identical conditions, often even tested parallel to the signaling molecule-deficient cells, and in clearly cell-autonomous assay systems.^{8,16} Instead, it seems that β₂ integrins on one hand support adhesion and adhesion-dependent functional responses through a tyrosine kinase signaling pathway, and on the other hand, they participate in neutrophil migration without the need of tyrosine phosphorylation pathways.

How β₂ integrins fulfill these dual functions is at present unclear. It is in theory possible that adhesion requires active outside-in signaling by β₂ integrins (e.g. to promote cell spreading), whereas cell migration only requires minimal adhesion by β₂ integrins but no active signaling. It is also possible that β₂ integrins trigger two different pathways, one mediated by tyrosine kinases and leading to adhesion, spreading, and related responses, and the other one mediated by yet unidentified mechanisms and leading to cell migration. A third possibility stems from the heterogeneity of β₂ integrins. Indeed, most functions requiring the Src-family-ITAM adapters-Syk-PLCγ2-Vav-SLP-76 pathway are mediated by Mac-1, whereas neutrophil migration is primarily mediated by LFA-1^{95,96} (and M. Kovács and A. Mócsai unpublished observations). Therefore, it is possible that the requirement for tyrosine kinase signaling pathway is a unique feature of Mac-1 signaling, whereas LFA-1 is able to signal in a tyrosine kinase-independent manner. In this context, it is interesting to note that the Zigmond chamber migration assay (which seems to be mediated, at least in part, by Src-family kinases and Syk) appears to be dependent on the Mac-1 integrin.⁵⁹

4 | TYROSINE KINASES IN FC-RECEPTOR SIGNALING OF NEUTROPHILS

Leukocytes have characteristic Fc-receptors on their cell surface which are essential players in the development of various innate and adaptive immune responses against invading pathogens or during autoantibody-induced inflammatory diseases. We have also tested signal transduction by Fc-receptors on neutrophils (Fig. 3 and Table 2).

4.1 | Neutrophil Fc-receptors

Fc-receptors expressed on the surface of neutrophils are involved in a number of cellular functions, including immune complex-mediated cellular activation, immune complex clearance, phagocytosis of opsonized particles, NET formation, and tumor cell killing, as well as in various disease processes including the pathogenesis of arthritis, dermatitis, glomerulonephritis, or anaphylaxis.^{97–102}

Neutrophils express various types of Fc-receptors which can recognize the Fc portion of IgG (in case of Fcγ-receptors) and IgA (in case of Fcα-receptors). Although the dominant Fc-receptor isoforms are low-affinity Fcγ-receptors on both human and mouse neutrophils, the

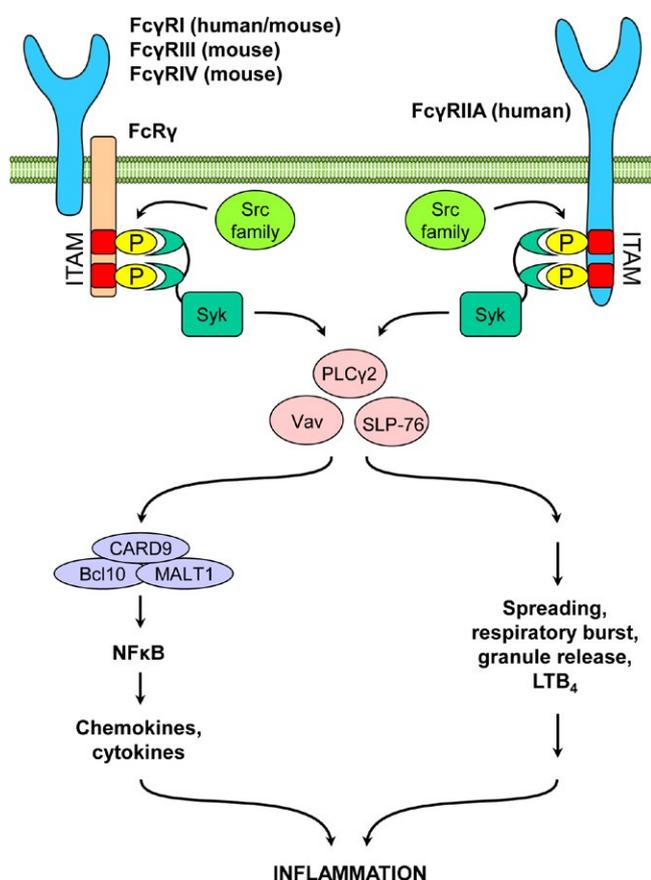


FIGURE 3 Fcγ-receptor signaling in neutrophils. Receptor-proximal signaling by Fcγ-receptors is mediated by an ITAM-dependent tyrosine kinase signaling pathway. Further downstream signaling diverges into CARD9- and NFκB-mediated chemokine and cytokine release and CARD9/NFκB-independent direct responses

actual set of receptors expressed by neutrophils from the two species are quite different¹⁰³ (Fig. 3).

Human neutrophils primarily express the low-affinity Fcγ-receptors FcγRIIA (CD32A), FcγRIIB (CD32B), and FcγRIIB (CD16B).¹⁰³ Of those receptors, FcγRIIA is a single-chain transmembrane protein containing an immunoreceptor tyrosine-based activation (ITAM) motif in the cytoplasmic part of its principal ligand-binding transmembrane (‘α’) chain, whereas FcγRIIB has an inhibitory ITIM motif in a similar position. FcγRIIB is a GPI-anchored receptor with likely no direct signaling function, although it is able to transmit activating signals, supposedly through other cell-surface molecules. Indeed, recognition of IgG immune complexes is believed to proceed through initial capture and tethering by FcγRIIB, followed by the ligation of FcγRIIA which triggers the effector responses of the cells.^{21,104} Human neutrophils also express the Fcα-receptor FcαRI (CD89).^{103,105} FcαRI is also associated with the Fc-receptor γ-chain (FcRγ), the ITAM-bearing transmembrane adapter protein utilized by a number of activating Fc-receptors. Taken together, of the above human receptors, FcγRIIA, FcγRIIB, and FcαRI transmit activating signals, whereas FcγRIIB is an inhibitory receptor.

In contrast to human neutrophils, murine neutrophils express two activating low-affinity Fcγ-receptors, FcγRIII and FcγRIV, both of which associate with the ITAM-containing FcRγ transmembrane adapter.¹⁰³ They also express the ITIM-containing inhibitory FcγRIIB receptor but not Fcα-receptors.

The ITAM-bearing common γ-chain of Fc-receptors (FcRγ) plays a dual role in Fc-receptor biology. On one hand, its ITAM tyrosines become phosphorylated upon receptor cross-linking and are thought to recruit the Syk tyrosine kinase through its tandem SH2 domains, leading to Syk activation and downstream signaling.⁴⁷ This mechanism is very similar to the ITAM-based signaling by lymphocyte antigen receptors, such as the B-cell receptors (BCR) and the T-cell receptors (TCR). On the other hand, since FcRγ primarily associates with Fc-receptors through intramembrane salt bridges, FcRγ is also required for the stabilization of the associated receptors in the plasma membrane. Accordingly, neutrophils (and other cell types) lacking FcRγ fail to express FcRγ-associated receptors (such as the murine FcγRIII and FcγRIV) on their cell surface. In addition, FcRγ is also associated with, and required for the expression and/or signaling of, a number of additional molecules that do not recognize immunoglobulin Fc portions.⁷⁶

Since mice only express FcRγ-associated activating Fc-receptors (i.e. they lack the ITAM-containing single-chain FcγRIIA and the GPI-anchored FcγRIIB proteins), FcRγ-deficient mice are widely used to test the role of activating Fc-receptors and their ITAM-based signaling in experimental mice. In case of neutrophils, FcRγ-deficient animals mimic the simultaneous abrogation of the signaling function of both FcγRIII and FcγRIV. However, care should be taken when interpreting findings obtained with the use of FcRγ-deficient mice. Since FcRγ is not only required for the signaling of activating Fc-receptors but also for their cell-surface expression, phenotypes seen in those animals could either be due to a defective ITAM-based signaling capacity of FcRγ-associated receptors or by the mere absence of those receptors on the cell-surface irrespective of whether they signal through the ITAM motif or not. In addition, it cannot be

TABLE 2 Immune complex-induced functional responses of neutrophils. Mouse neutrophils were activated by plate-bound IgG immune complexes. =, not changed; ↓, partially reduced; ↓↓, strongly reduced; ↓↓↓, completely blocked

Genotype	Respiratory burst	MIP-2 release	LTB ₄ release	Reference(s)
<i>Fcer1g</i> ^{-/-} (FcRγ-deficient)	↓↓↓	↓↓↓	↓↓↓	8, 21
<i>Hck</i> ^{-/-} <i>Fgr</i> ^{-/-} <i>Lyn</i> ^{-/-}	↓↓↓	↓↓↓	↓↓↓	8, 9
<i>Syk</i> ^{-/-}	↓↓↓	↓↓↓	↓↓↓	9
<i>Plcg2</i> ^{-/-} (PLCγ2-deficient)	↓↓↓	↓↓↓	↓↓↓	9, 20
<i>Card9</i> ^{-/-}	=	↓↓	=	9

excluded either that any phenotypes seen in FcRγ-deficient mice are actually due to the absence and/or defective signaling of cell-surface receptors other than Fc-receptors. Nevertheless, the use of FcRγ-deficient mice provides a very good tool to test the functional role of Fc-receptors (in case of neutrophils, mainly Fcγ-receptors) in experimental mice.

In addition to the above receptors, neutrophils also express Fcε-receptors and the structurally unrelated neonatal Fc-receptor FcRn, and upon stimulation, they can also express the high-affinity Fcγ-receptor FcγRI.¹⁰³ The function of those molecules on neutrophils is poorly understood, although FcRn has been proposed to modulate the phagocytic activity of neutrophils.¹⁰⁶

We have decided to test the signal transduction pathway utilized by Fc-receptors on neutrophils. As a first step, we have set up an in vitro assay to stimulate neutrophils by immune complexes. Plating neutrophils on immobilized IgG immune complex-coated surfaces triggered very robust activation of both human and mouse cells, resulting in cell spreading, adhesion, respiratory burst, and degranulation responses.²¹

We then attempted to identify the Fc-receptors involved in this response, particularly because the functional role of mouse neutrophil Fc-receptors and, particularly, the then recently identified FcγRIV was mainly unknown. Activation of mouse neutrophils by immobilized IgG immune complexes was completely abrogated by deletion of the ITAM-containing FcRγ adapter protein,²¹ suggesting a role for activating Fc-receptors. Further studies using genetic deletion and antibody-mediated blocking revealed no role for FcγRI but indicated a critical but overlapping role for FcγRIII and FcγRIV. Indeed, while deleting FcγRIII or blocking FcγRIV alone did not affect neutrophil responses, blocking FcγRIV on FcγRIII-deficient neutrophils completely abrogated neutrophil responses.²¹

We have also performed additional studies to identify the Fc-receptors involved in immune complex-induced activation of human neutrophils. Interestingly, blocking FcγRIIA or FcγRIIB alone was sufficient to abrogate the responsiveness of those cells.²¹

Taken together, low-affinity Fcγ-receptors play a critical role in immune complex-induced neutrophil activation. Interestingly, while a single receptor (either FcγRIII or FcγRIV) was sufficient to trigger

activation of mouse neutrophils, human neutrophil activation required a cooperative activity of two receptors (FcγRIIA and FcγRIIB) under such conditions.

It should also be mentioned that Fcγ-receptors strongly interact with β₂ integrins (primarily Mac-1), and therefore, immune complex-induced functional responses are partially reduced (but not abrogated) in CD18-deficient cells.^{107–109}

4.2 | Signaling by neutrophil Fcγ-receptors

All activating transmembrane Fcγ-receptors contain ITAM motifs in their intracellular parts of the receptor complex, either within the ligand-binding α-chain (FcγRIIA) or in the receptor-associated FcRγ adapter (all other receptors) (Fig. 3). The role of FcRγ in immune complex-induced neutrophil activation²¹ suggested signaling by ITAM-based activation of Syk. However, given that FcRγ-deficient cells do not even express activating Fcγ-receptors, it was not entirely clear whether ITAM-based signaling was truly required for immune complex-induced neutrophil activation. Using an in vivo structure-function analysis, we have found that the FcRγ ITAM motifs were critical for neutrophil activation by immobilized immune complexes (T. Németh and A. M., unpublished observation), confirming the role of the FcRγ ITAM in Fc-receptor signaling. It has previously been shown that *Syk*^{-/-} neutrophils fail to respond to immune complex-induced activation,¹¹⁰ and we have also observed abrogation of various immune complex-induced functional responses of *Syk*^{-/-} mouse neutrophils⁹ (and Z. Jakus and A. Mócsai, unpublished observations). We have also shown that PLCγ2, acting downstream of Syk in the pathway, is also critically involved in immune complex-induced neutrophil activation.²⁰

The above experiments indicated a critical role for FcRγ ITAM-mediated Syk activation and downstream pathways in immune complex-induced neutrophil activation (Fig. 3). However, the mechanism of FcRγ ITAM phosphorylation was still unclear. In case of TCR signal transduction, Src-family kinases are responsible for phosphorylation of the TCR-associated ITAM motifs, leading to recruitment and activation of ZAP-70.¹¹¹ Those studies suggested that Src-family kinases may also be involved in ITAM phosphorylation in other cell types. However, the role of Src-family kinases in ITAM phosphorylation during BCR and Fc-receptor signaling was rather controversial. For example, the combined genetic deficiency of Lyn, Fyn, and Blk did not reduce BCR-induced ITAM phosphorylation¹¹² and Lyn deficiency even led to enhanced BCR signaling and B-cell-mediated autoimmunity.^{113–116} Src-family kinases also played both positive and negative functions in Fc-receptor signaling in myeloid cells such as macrophages or mast cells.^{8,117–120} In case of neutrophils, combined deficiency of Hck and Fgr did not affect soluble immune complex-induced functional responses.¹⁴ It has even been proposed that, in contrast to ZAP-70, Syk is able to directly phosphorylate ITAM tyrosines¹²¹; therefore Src-family kinases may not be as critical for Syk-mediated signaling in B cells and myeloid cells as in ZAP-70-mediated signaling in T cells.

The above issues prompted us to test the role of Src-family kinases in immune complex-induced functional and signaling responses in neutrophils. *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} triple knockout neutrophils were completely defective in their respiratory burst response when plated on immobilized IgG immune complexes.⁸ Analysis of single and double knockouts have revealed strong functional overlap between *Hck*, *Fgr*, and *Lyn* since deletion of all three kinases was required for complete abrogation of this response. The role of Src-family kinases was not restricted to activation of the NADPH oxidase since *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} neutrophils also failed to release proinflammatory mediators, such as the IL-1 β cytokine, the MIP-1 α and MIP-2 chemokines, and the LTB₄ lipid mediator.⁸ Biochemical studies have also indicated complete defect of immune complex-induced Fc γ R ITAM phosphorylation and tyrosine phosphorylation of Syk in *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} neutrophils.⁸ Those results were also in line with the dramatic inhibition of immune complex-induced human neutrophil responses (including respiratory burst, spreading, degranulation, and signaling responses such as Syk phosphorylation) by the Src/Abl-family tyrosine kinase inhibitor dasatinib.⁴⁵

Taken together, the above experiments indicated that Src-family kinases play an indispensable role in neutrophil activation by immobilized IgG immune complexes, primarily by mediating ITAM phosphorylation within the Fc-receptor complex and concomitant activation of the Syk tyrosine kinase (Fig. 3 and Table 2).

Of other tyrosine kinase pathway components, the Vav-family members Vav1 and Vav3 were involved in regulating immune complex-induced respiratory burst and other functional responses of neutrophils.¹²² In addition, SLP-76 deficiency reduced, though did not abrogate, the respiratory burst and Ca²⁺ influx of neutrophils stimulated by aggregated immune complexes.^{58,123} The PI3K β and PI3K δ isoforms of PI3-kinases were also involved in immune complex-induced neutrophil activation in an overlapping manner.²⁴ However, similar to integrin signaling, the p190RhoGAP (p190-A) protein was not required for immune complex-induced functional responses of neutrophils.²³ In addition, Btk-deficient neutrophils have been shown to react poorly to stimulation with immune complex stimulation,⁵⁹ although the interpretation of those findings was complicated by the complex phenotypes of *Btk*^{-/-} neutrophils.^{60,61}

Taken together, neutrophil activation by IgG immune complexes proceeds through a tyrosine kinase pathway triggered by phosphorylation of ITAM tyrosines within the receptor complex of low-affinity Fc γ -receptors (e.g. the Fc γ R ITAM tyrosine within the Fc γ RIII/Fc γ RIV complex), followed by recruitment and activation of Syk and downstream signaling through PLC γ 2, Vav-family members, SLP-76, and PI3-kinases (Fig. 3 and Table 2).

5 | TYROSINE KINASE SIGNALING IN NEUTROPHIL-DEPENDENT IN VIVO DISEASE MODELS

Having established a critical role for the Src-family-ITAM adapter-Syk-PLC γ 2-Vav-SLP-76 pathway in both integrin and Fc-receptor

signal transduction in neutrophils, we and others aimed to reveal the role of this pathway in neutrophil-mediated in vivo inflammatory processes.

Neutrophils are critically involved in a number of in vivo disease processes in experimental mice which mimic various aspects of human diseases and are dependent on integrin and/or Fc γ -receptor function.

5.1 | Neutrophil-mediated in vivo inflammatory reactions

One of the neutrophil-dependent in vivo inflammation models is the localized Shwartzman reaction, a thrombohemorrhagic vasculitis triggered by consecutive intradermal application of LPS followed by LPS or TNF- α .^{92,124} The model mimicks complement-mediated vasculitis reactions observed in systemic lupus erythematosus patients. This reaction is critically dependent on complement activation and the recognition of C3b fragments by Mac-1 (CD11b/CD18) on the surface of neutrophils.^{92,124}

Other widely used models of neutrophil-dependent in vivo inflammation include the K/B \times N serum-transfer arthritis, autoantibody-mediated blistering skin diseases, and the reverse passive Arthus reaction.¹²⁵⁻¹²⁷ These models are triggered by in vivo deposition of IgG immune complexes, leading to neutrophil infiltration and activation, and concomitant tissue damage. They mimic autoantibody-induced pathologies in various human inflammatory diseases, such as the effector phase of rheumatoid arthritis, the blistering skin diseases such as bullous pemphigoid or epidermolysis bullosa acquisita, or autoantibody-mediated glomerulonephritis. These models are thought to be mediated by Fc γ -receptors but not by Mac-1^{8,128,129} and are, therefore, widely accepted in vivo models of Fc-receptor-mediated functional responses of neutrophils. It should nevertheless be mentioned that the above models do depend on β_2 integrins.^{23,126,129} However, at least in the case of K/B \times N serum-transfer arthritis, that requirement is due to the role of LFA-1 in neutrophil migration, not of Mac-1 in neutrophil activation^{129,130} (though the situation may be different in the case of skin dermatitis, such as experimental bullous pemphigoid¹³¹).

5.2 | Signaling in neutrophil-mediated in vivo inflammation

Analysis of the role of tyrosine kinase pathway components in the Shwartzman reaction revealed that the myeloid Src-family kinases *Hck*, *Fgr*, and *Lyn*, as well as Syk, were critically involved in the local hemorrhage and fibrin deposition.⁹² In addition, myeloid-specific deletion of SLP-76 strongly reduced neutrophil-mediated tissue damage in the same model.^{123,132} Interestingly, however, the accumulation of neutrophils was not affected by the *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} or the *Syk*^{-/-} mutation, or by the myeloid-specific deletion of SLP-76, providing further in vivo evidence that the Src-family-Syk-SLP-76 pathway does not play a major direct role in neutrophil migration.⁹²

TABLE 3 K/B×N serum-transfer arthritis in different mouse strains. Clinical signs of arthritis development were tested following arthritogenic K/B×N serum injection. =, not changed; ↓, partially reduced; ↓↓, strongly reduced; ↓↓↓, completely blocked

Genotype	K/B×N serum-transfer arthritis	Reference(s)
<i>Fcer1g</i> ^{-/-} (FcRγ deficient)	↓↓↓	8, 128, 167
<i>Itgb2</i> ^{-/-} (CD18 deficient)	↓↓	23, 129
<i>Itgal</i> ^{-/-} (CD11a deficient)	↓↓	129
<i>Itgam</i> ^{-/-} (CD11b deficient)	=	129
<i>Hck</i> ^{-/-} <i>Fgr</i> ^{-/-} <i>Lyn</i> ^{-/-}	↓↓↓	8
<i>Syk</i> ^{-/-}	↓↓↓	22
<i>Plcg2</i> ^{-/-} (PLCγ2 deficient)	↓↓↓	20, 84
<i>Lcp2</i> ^{-/-} (SLP-76 deficient)	↓↓	132
<i>Vav1</i> ^{-/-} <i>Vav2</i> ^{-/-} <i>Vav3</i> ^{-/-}	↓↓↓	84
<i>Arhgap35</i> ^{-/-} (p190RhoGAP deficient)	=	23
<i>Card9</i> ^{-/-}	↓	9

We and others have also tested the role of various tyrosine kinase signaling pathway components in autoantibody-induced, immune complex-mediated disease models. As expected from the supposed role of Fcγ-receptors, K/B×N serum-transfer arthritis could not be induced in the absence of the ITAM-bearing FcRγ adapter^{8,128} (Table 3). Using a transgenic structure-function study, we have also found that the FcRγ ITAM tyrosines were required for K/B×N serum-transfer arthritis development (T. Németh and A. M., unpublished observations). Bone marrow chimeras lacking Syk in the hematopoietic compartment²² and mice with a conditional deletion of Syk in the myeloid compartment or in neutrophils⁹³ were protected from K/B×N serum-transfer arthritis. Similarly, inducible Syk deletion strongly reduced the development of collagen antibody-induced arthritis, another autoantibody-induced arthritis model in mice.⁸⁵ In addition, *Syk*^{-/-} bone marrow chimeras were also completely protected from anti-collagen VII antibody-induced skin blistering and inflammation (C. Sitaru, T. Németh and A. M., unpublished observations).

Of further downstream signaling molecules, we and others have shown that PLCγ2 is critical for K/B×N serum-transfer arthritis development.^{20,84} Similarly, *Vav1*^{-/-} *Vav2*^{-/-} *Vav3*^{-/-} mice were also shown to be completely protected from K/B×N serum-transfer arthritis.⁸⁴ In addition, SLP-76-deficient mice showed also nearly complete defect in arthritis development in the same model.¹³² However, similar to integrin and Fc-receptor signaling, p190RhoGAP was not required for autoantibody-induced arthritis.²³ Interestingly, *Btk*-deficient mice also showed normal arthritis development in the K/B×N serum-transfer model,¹³³ indicating either that Tec-family kinases are not involved in this process or that another Tec-family member is able to compensate for the lack of *Btk* in experimental mice.

As in the case of Fc-receptor signaling in vitro, a critical further question was whether Src-family kinases are involved in neutrophil-mediated, autoantibody-induced inflammatory reactions. Therefore, we tested

those disease models in mice lacking myeloid-specific Src-family kinases. *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-} mice were completely protected from K/B×N serum-transfer arthritis, anti-collagen VII antibody-mediated blistering skin inflammation, as well as from the reverse passive Arthus reaction.⁸ In case of K/B×N serum-transfer arthritis, all single- and double-knockout mutants have also been tested, revealing that all three kinases have to be deleted to obtain complete protection from arthritis, indicating a strong functional overlap between *Hck*, *Fgr*, and *Lyn*.⁸

5.3 | How are tyrosine kinases involved in arthritis development?

Having established a critical role for tyrosine kinase signaling pathway components in autoantibody-induced inflammatory disease models, we and others attempted to perform additional experiments to reveal further mechanistic details about the observed findings. These experiments focused on the role of Src-family kinases and Syk in the K/B×N serum-transfer arthritis experiments (Fig. 4 and Table 3).

One of the first questions was which cellular lineage had to express tyrosine kinases. The fact that bone marrow chimeras with hematopoietic-specific Syk expression was completely protected from arthritis development²² pointed to a hematopoietic lineage. The K/B×N serum-transfer arthritis model depends on a number of hematopoietic lineages, including neutrophils^{134,135} and macrophages.¹³⁶ In addition, a role for platelets,¹³⁷ as well as for mast cells,^{138,139} have been proposed, although the latter one has later been questioned by other groups.^{93,140–142} Therefore, we and others tested the role of Syk in different hematopoietic lineages during K/B×N serum-transfer arthritis. Inflammation was completely abrogated by deletion of Syk either in the entire myeloid compartment or in neutrophils only⁹³ (and T. Németh and A. Mócsai, unpublished observations). However, no defect could be observed in mice lacking Syk in platelets or mast cells, despite effective deletion of Syk from the respective lineages (T. Németh and A. M., unpublished observations). Taken together, those studies suggested that tyrosine kinases expressed within neutrophils (and, possibly, macrophages) are critical for autoantibody-induced arthritis development.

Our next question was how tyrosine kinases within neutrophils contribute to the disease pathogenesis. Histological and flow cytometric studies have indicated that neutrophils lacking components of the Src-family-FcRγ-Syk-PLCγ2-Vav-SLP-76 pathway fail to accumulate at the site of inflammation in the K/B×N serum-transfer model.^{8,20,22,84,93,132} This was similar to what was observed in CD18-deficient animals^{8,129} and suggested that the tyrosine kinase pathway was required for CD18-dependent neutrophil migration. This would have also been in line with the role of those signaling molecules in other CD18-dependent neutrophil functions.^{16,18,20,54,58} However, given the normal in vitro migration of *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-}, *Syk*^{-/-}, *Plcg2*^{-/-}, and other neutrophils discussed above,^{8,16,17,20,45,57,83} and the normal in vivo migration of some of those mutant neutrophils under certain conditions, we and others decided to perform more detailed experiments to clarify this issue, by combining the above-mentioned mixed

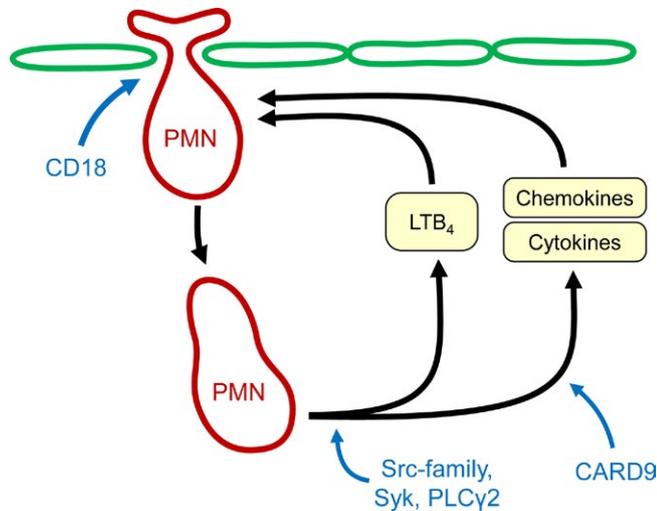


FIGURE 4 Molecular regulation of neutrophil recruitment. Neutrophil migration through the endothelium requires CD18, the common β chain of β_2 integrins. Activated neutrophils at the site of inflammation participate in the orchestrating of inflammatory reactions by amplifying neutrophil recruitment through LTB_4 , chemokines, and cytokines. Components of a tyrosine kinase signaling pathway are differentially involved in various aspects of that feedback amplification loop

bone marrow chimeric approach with the K/B \times N serum-transfer arthritis model.^{8,93}

In a control experiment, mixed bone marrow chimeras with hematopoietic cells from two different wildtype donors (carrying CD45.1 and CD45.2 markers) showed similar ratios of neutrophils derived from the two donors in the blood and the synovial infiltrate.⁸ However, chimeras having both wildtype and CD18-deficient hematopoietic tissues showed a much lower percentage of CD18-deficient neutrophils in the synovial infiltrate than in the peripheral blood,⁸ indicating strong cell-autonomous defects of CD18-deficient neutrophils in their accumulation at the site of inflammation. Importantly, however, in chimeras having both wildtype and $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ hematopoietic tissues, we have observed equal percentages of $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ neutrophils in the blood and the synovial infiltrate,⁸ indicating that the $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mutation did not affect the accumulation of neutrophils at the site of inflammation. Similar results were seen when comparing the accumulation of wildtype neutrophils with those with a neutrophil-specific deletion of Syk.⁹³ These results indicated that, in contrast to CD18, Src-family kinases and Syk do not play a cell-autonomous role in the accumulation of neutrophils at the site of inflammation.

Given the apparent contradiction between defective accumulation of $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ neutrophils at the site of inflammation in intact $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mice but normal accumulation of the same cells in the same arthritis model when wildtype neutrophils were also present,⁸ we speculated that the defect in intact $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mice could be due to defective generation of the inflammatory, chemotactic environment. Indeed, $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mice subjected to K/B \times N serum-transfer arthritis failed to develop signs of an inflammatory environment such as accumulation of IL-1 β , various chemokines

(including the IL-8 homolog MIP-2), the LTB_4 lipid chemoattractant, or ROS species.⁸ Since an inflammatory (chemoattractant) microenvironment is critical for leukocyte recruitment, those results provided mechanistic explanation for the defective neutrophil accumulation in the synovial area of intact $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mice subjected to K/B \times N serum-transfer arthritis (Fig. 4).

We next attempted to identify the source of some of those chemoattractants. To this end, we tested the release of various cytokines, chemokines, and the LTB_4 lipid mediator from myeloid cells plated on immobilized immune complexes as a surrogate for in vivo immune complex deposition. Interestingly, we have observed that immune complex stimulation triggered a robust release of various inflammatory mediators (including MIP-2 and LTB_4) from wildtype neutrophils but no such response could be observed in $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ samples (Table 3). Given the fact that both MIP-2 and LTB_4 are strong chemoattractants for neutrophils, these results indicated a positive feedback loop whereby immune complex-induced activation of neutrophils at the site of inflammation triggers release of chemoattractants that attract further neutrophils, providing a feedback amplification pathway for neutrophil recruitment and activation.¹⁰ Breaking this amplification loop either by interfering with the intrinsic migratory capacity of neutrophils by deleting CD18 or by blocking the release of inflammatory mediators from neutrophils encountering immune complexes both result in abrogation of the entire amplification process, providing mechanistic explanation for the similar in vivo arthritis phenotypes of the CD18-deficient and $Hck^{-/-} Fgr^{-/-} Lyn^{-/-}$ mice^{8,23,129} (Fig. 4).

6 | GENE EXPRESSION CHANGES IN NEUTROPHILS – SIGNALING AND IN VIVO RELEVANCE

The above experiments indicated that Fc-receptor-mediated activation of neutrophils triggers the release of chemokines, cytokines, and lipid mediators through a receptor-proximal tyrosine kinase pathway. However, further downstream components of this pathway and the divergence point between proteinaceous (chemokines and cytokines) or lipid chemoattractant (LTB_4) release were unclear. Similar to Fc-receptors, a number of innate immune receptors signal through an ITAM-like signaling pathway involving the Syk tyrosine kinase.⁴⁷ In those pathways, NF κ B-mediated upregulation of chemokine and cytokine expression (but not other responses such as phagocytosis or inflammasome activation) are dependent on the Bcl10/CARD9/MALT1 adapter complex in macrophages and dendritic cells.⁴⁷ Although no function of this complex in neutrophils and very limited information of it in non-infectious inflammation was then known, we hypothesized that CARD9 may link immune complex recognition to chemokine/cytokine production in neutrophils and neutrophil-dependent in vivo inflammation (Figs 3 and 4, and Tables 2 and 3).

While CARD9-deficient neutrophils showed normal short-term functional responses such as respiratory burst or degranulation, they showed robust reduction in NF κ B activation, in the upregulation of

overall gene expression, and in the release of various chemokines and cytokines upon stimulation by immobilized IgG immune complexes⁹ (Table 2). Interestingly, *Card9*^{-/-} neutrophils showed normal immune complex-induced LTB₄ production,⁹ indicating dissection of the signaling pathways leading to chemokine/cytokine and LTB₄ release at the level of CARD9 (Fig. 3). It should be noted that *Hck*^{-/-} *Fgr*^{-/-} *Lyn*^{-/-}, *Syk*^{-/-}, and *Plcg2*^{-/-} neutrophils were completely defective in all those responses,⁹ indicating that Src-family kinases, Syk, and PLCγ2 likely act upstream of CARD9 in this pathway. Neutrophils lacking Bcl10 or MALT1 showed in vitro phenotypes similar to that of the *Card9*^{-/-} cells.⁹

We have also tested the role of CARD9 in autoantibody-induced in vivo inflammation. *Card9*^{-/-} mice were partially but significantly protected from K/B×N serum-transfer arthritis and form anti-collagen VII antibody-induced dermatitis⁹ (Table 3). Again, in vivo accumulation of chemokines and cytokines was defective in *Card9*^{-/-} mice, whereas the LTB₄ levels at the inflammatory site were similar to those in wild-type mice.⁹ Those results again indicated that CARD9 acts as a divergence point between autoantibody-induced chemokine/cytokine and LTB₄ release (Fig. 3).

Taken together, we have identified CARD9 as an important molecule linking immunoreceptors to chemokine and cytokine signaling both in neutrophils and in in vivo inflammatory disease models.

The identification of the role of CARD9 in chemokine and cytokine release but not in other functional responses of neutrophils (such as respiratory burst, degranulation, migration, or LTB₄ release) provided a unique opportunity to test the role of neutrophil-derived chemokines and cytokines in in vivo inflammation. Neutrophils had long been thought to be simple effector cells of the immune response. However, it later became clear that neutrophils are able to undergo significant stimulus-induced gene expression changes and to produce and release various chemokines and cytokines.^{12,143} However, the in vivo function of neutrophil-derived chemokines and cytokines has been difficult to test. On one hand, deletion or depletion of neutrophils,^{125,135} or neutrophil-specific conditional deletion of genes with wide cellular function such as Syk,⁹³ provided approaches that were specific for neutrophils but abrogated all or most neutrophil functions, not just gene expression changes and chemokine/cytokine production. On the other hand, germline deletion of CARD9⁹ blocked gene expression changes and chemokine/cytokine production but was not specific for neutrophils. We, therefore, aimed to combine those two approaches and delete CARD9 in a neutrophil-specific manner. That way, we could test the in vivo effect of abrogating gene expression changes and chemokine/cytokine release from neutrophils without affecting other neutrophil responses.

In order to address the above question, we have generated mice specifically lacking CARD9 in the neutrophil compartment (termed *Card9*^{ΔPMN} mice) by Cre-lox-mediated conditional gene deletion and subjected them to autoantibody-induced in vivo inflammation models along with germline CARD9 knockout (*Card9*^{-/-}) mice.⁹ Interestingly, the *Card9*^{ΔPMN} and the *Card9*^{-/-} mutations both caused substantial, though incomplete defect in K/B×N serum-transfer arthritis, and there was no difference between the arthritis phenotypes of the *Card9*^{ΔPMN}

and the *Card9*^{-/-} mice.⁹ In addition, the *Card9*^{ΔPMN} and *Card9*^{-/-} mice showed similar level of (partial) defect in anti-collagen VII induced skin inflammation.⁹

Taken together, the above results indicate that the partial defect of autoantibody-induced arthritis and dermatitis in *Card9*^{-/-} mice was primarily due to CARD9 deficiency in the neutrophil compartment. More importantly, since *Card9*^{ΔPMN} mice had a specific defect of gene expression changes and chemokine/cytokine productions specifically within the neutrophil compartment, those results provided the most direct evidence so far for an important role for neutrophil gene expression changes and neutrophil-derived chemokines and cytokines in the in vivo inflammation process (Fig. 4).

7 | TYROSINE KINASES IN SIGNALING BY OTHER RECEPTORS

Besides the best-known integrins and Fc-receptors, tyrosine kinases have also been proposed to participate in signaling by other receptors in neutrophils. However, much less detail is available on those issues within neutrophils. Here, we provide a brief summary of those other signaling pathways, also highlighting the limited contributions from our own group. The authors are referred to our recent review on this topic for further detail.⁵

7.1 | Tyrosine kinases in GPCR signaling

Neutrophils express and utilize a wide range of G-protein-coupled receptors, including receptors for formyl peptides, chemokines, LTB₄, complement C5a, and platelet activating factor.⁵ All those receptors signal through G_i-family heterotrimeric G-proteins. Initial pharmacological studies suggested an important role for tyrosine kinases in neutrophil responses triggered by the formyl peptide fMLP^{15,18,144} and later genetic studies have confirmed an important role by the Src-family kinases Hck, Fgr, and Lyn,^{15,18,145} which was also supported by the partial inhibition of fMLP-induced functional responses by the Src/Abl-family inhibitor dasatinib.⁴⁵ However, it is still mostly unclear how Src-family kinases fit into GPCR signal transduction and to what extent the role of Src-family kinases can also be extended to other G_i-coupled receptors of neutrophils, especially in light of the apparently negative regulation of chemokine signaling by the Src-family kinases Hck and Fgr.⁸⁹ In contrast to Src-family kinases, our detailed study did not reveal any role for Syk in GPCR signaling in neutrophils or mast cells⁸³ and a limited set of experiments also failed to reveal such a role for PLCγ2 or p190RhoGAP (p190-A) in neutrophils,^{20,23} although a role for PI3Kβ in LTB₄-mediated amplification of immune complex-induced neutrophil activation has been proposed.²⁴ The Tec-family kinase Btk has also been shown to participate in signal transduction by formyl peptide receptors, in part by coupling GPCR signaling to integrin (Mac-1) inside-out activation.⁵⁹ Further aspects of GPCR signal transduction has been reviewed in Futosi et al.⁵

7.2 | Tyrosine kinases in selectin-mediated signaling pathways

Selectin-selectin ligand interactions ensure reversible adhesive interactions between neutrophils and the endothelium.¹⁴⁶ Selectin-mediated signaling also triggers inside-out activation of the β_2 integrin LFA-1, leading to deceleration of the rolling neutrophils and, eventually, to integrin-mediated firm adhesion. One of the main receptors involved in this process is PSGL-1, a neutrophil cell-surface receptor primarily recognizing endothelial E-selectins on the inflamed endothelium. Interestingly, PSGL-1-mediated inside-out activation of β_2 integrins is mediated by a tyrosine kinase pathway similar to outside-in signaling of β_2 integrins. This pathway is triggered by the activation of Src-family kinases (Hck, Fgr and Lyn), leading to phosphorylation of the ITAM-containing adapter molecules DAP12 and FcR γ , followed by recruitment and activation of the Syk tyrosine kinase.¹⁴⁷⁻¹⁴⁹ In addition, ERM family proteins may also function as linkers between PSGL-1 and Syk through their ITAM-like motif.¹⁵⁰ Further downstream components of the PSGL-1-mediated pathway leading to increased neutrophil activation involved SLP-76, Btk, ADAP, PLC γ 2, and PI3K γ , eventually triggering two parallel pathways leading to recruitment of talin-1 to the β subunit of integrin cytoplasmic tail and concomitant increase in LFA-1 affinity.^{59,151-154}

7.3 | Tyrosine kinases in signaling by C-type lectins

C-type lectins comprise a family of innate immune receptors involved in the recognition of various microbial ligands (such as fungal β -glucans), as well as host-derived danger signals. Neutrophils express a number of C-type lectins, including Dectin-1, Mincle, MDL-1, Mcl, and CLEC-2.⁵ In other cell types (primarily macrophages and dendritic cells), several C-type lectins have been shown to signal through a tyrosine kinase-mediated, ITAM-like pathway, utilizing ITAM motifs in receptor-associated transmembrane adapters (e.g. FcR γ and DAP12 in the case of Mincle and MDL-1, respectively) or dual hemITAM motifs in dimerized single-chain C-type lectins (e.g. Dectin-1, CLEC-2). C-type lectin-mediated antimicrobial functions involve Syk and CARD9 in other myeloid lineages.¹⁵⁵⁻¹⁶⁰ Unfortunately, there is very little information on the role of this pathway in neutrophils. Dectin-1 activates Mac-1 and both are involved in antifungal activity of neutrophils through Vav-family proteins and, supposedly, Src-family kinases, Syk, and PLC γ 2.¹⁶¹ CARD9 is also involved in antifungal responses of human neutrophils, although the mechanism involved is poorly understood.^{162,163} Neutrophil responses to *Aspergillus fumigatus* hyphae also required Syk and PI3K β /PI3K δ .¹⁶⁴ However, other details of the tyrosine kinase pathways involved in C-type lectin signaling in neutrophils are poorly understood.

7.4 | Tyrosine kinases in cytokine receptor signaling

Neutrophils express a number of different cytokine receptors, including members of the conventional cytokine receptors (including

the type I cytokine receptors for IL-4, IL-6, IL-12, IL-15, G-CSF, and GM-CSF, and the type II receptors for IFN- α , IFN- β , IFN- γ , and IL-10), as well as IL-1- and TNF-family receptors.⁵ Of those, tyrosine kinases are likely involved in signaling by conventional cytokine receptors. Most of our current knowledge about cytokine receptor signaling originates from studies on cell types other than neutrophils. It is generally believed that cytokine receptors are signaling through activation of the Jak-family tyrosine kinases (Jak1, Jak2, Jak3, and Tyk2) which then phosphorylate and activate members of the STAT transcription factor family.¹⁶⁵ Although there is certain specificity between the different Jak and STAT proteins with respect to the different cytokine receptors, the details of how overlapping signaling pathways can trigger receptor-specific functional responses is poorly understood. There are also very few genetic (e.g. knockout) studies in neutrophils that could provide deeper understanding of this pathway in neutrophils. For further details, the authors are referred to recent reviews in the field.^{5,165}

8 | CONCLUDING REMARKS

Our understanding of neutrophil biology during the last 10-15 years has dramatically improved, in large part due to the *in vitro* and *in vivo* analysis of knockout mouse strains, allowing the investigation of the effect of genetic manipulation of neutrophils hardly available by other approaches. Signal transduction studies have revealed the role of tyrosine kinase signaling pathways in various neutrophil functions, in particular those triggered by cellular adhesion and immune complex deposition. Those pathways have also proved to be involved in neutrophil-mediated *in vivo* inflammatory disease processes, such as autoantibody-induced arthritis or dermatitis models. Given the critical role of neutrophils in various human diseases, understanding the biology of these cells at the molecular level is expected to contribute to better understanding of various human diseases and may even point to possible future strategies of therapeutic intervention.

ACKNOWLEDGEMENTS

We apologize to colleagues whose work could not be cited due to space limitation and/or our primary focus on mouse knockout studies. This work was supported by the Lendület program of the Hungarian Academy of Sciences (grant no. LP2013-66/2013 to A. M.). A. M. was a recipient of a Wellcome Trust International Senior Research Fellowship (grant no. 087782). K. F. was a recipient of a Hungarian National Excellence Program (TÁMOP 4.2.4. A/1-11-1-2012-0001) research fellowship co-funded by the EU's European Social Fund.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

REFERENCES

- Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol.* 2011;11:519–531.
- Mócsai A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med.* 2013;210:1283–1299.
- Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. *Annu Rev Pathol.* 2014;9:181–218.
- Weber FC, Németh T, Csepregi JZ, et al. Neutrophils are required for both the sensitization and elicitation phase of contact hypersensitivity. *J Exp Med.* 2015;212:15–22.
- Futosi K, Fodor S, Mócsai A. Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol.* 2013;17:638–650.
- Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* 2013;13:159–175.
- Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity.* 2014;41:694–707.
- Kovács M, Németh T, Jakus Z, et al. The Src family kinases Hck, Fgr, and Lyn are critical for the generation of the in vivo inflammatory environment without a direct role in leukocyte recruitment. *J Exp Med.* 2014;211:1993–2011.
- Németh T, Futosi K, Sitaru C, Ruland J, Mócsai A. Neutrophil-specific deletion of the CARD9 gene expression regulator suppresses autoantibody-induced inflammation in vivo. *Nat Commun.* 2016;7:11004.
- Németh T, Mócsai A. Feedback amplification of neutrophil function. *Trends Immunol.* 2016;37:412–424.
- Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. *Blood.* 2014;124:710–719.
- Tecchio C, Micheletti A, Cassatella MA. Neutrophil-derived cytokines: facts beyond expression. *Front Immunol.* 2014;5:508.
- Mócsai A, Walzog B, Lowell CA. Intracellular signalling during neutrophil recruitment. *Cardiovasc Res.* 2015;107:373–385.
- Lowell CA, Fumagalli L, Berton G. Deficiency of Src family kinases p59/61^{hck} and p58^{c-fgr} results in defective adhesion-dependent neutrophil functions. *J Cell Biol.* 1996;133:895–910.
- Mócsai A, Jakus Z, Vántus T, Berton G, Lowell CA, Ligeti E. Kinase pathways in chemoattractant-induced degranulation of neutrophils: The role of p38 mitogen-activated protein kinase activated by Src family kinases. *J Immunol.* 2000;164:4321–4331.
- Mócsai A, Zhou M, Meng F, Tybulewicz VL, Lowell CA. Syk is required for integrin signaling in neutrophils. *Immunity.* 2002;16:547–558.
- Mócsai A, Abram CL, Jakus Z, Hu Y, Lanier LL, Lowell CA. Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. *Nat Immunol.* 2006;7:1326–1333.
- Mócsai A, Ligeti E, Lowell CA, Berton G. Adhesion-dependent degranulation of neutrophils requires the Src family kinases Fgr and Hck. *J Immunol.* 1999;162:1120–1126.
- Jakus Z, Fodor S, Abram CL, Lowell CA, Mócsai A. Immunoreceptor-like signaling by β_2 and β_3 integrins. *Trends Cell Biol.* 2007;17:493–501.
- Jakus Z, Simon E, Frommhold D, Sperandio M, Mócsai A. Critical role of phospholipase Cy2 in integrin and Fc receptor-mediated neutrophil functions and the effector phase of autoimmune arthritis. *J Exp Med.* 2009;206:577–593.
- Jakus Z, Németh T, Verbeek JS, Mócsai A. Critical but overlapping role of Fc γ RIII and Fc γ RIV in activation of murine neutrophils by immobilized immune complexes. *J Immunol.* 2008;180:618–629.
- Jakus Z, Simon E, Balázs B, Mócsai A. Genetic deficiency of Syk protects mice from autoantibody-induced arthritis. *Arthritis Rheum.* 2010;62:1899–1910.
- Németh T, Futosi K, Hably C, et al. Neutrophil functions and autoimmune arthritis in the absence of p190RhoGAP: generation and analysis of a novel null mutation in mice. *J Immunol.* 2010;185:3064–3075.
- Kulkarni S, Sitaru C, Jakus Z, et al. PI3K β plays a critical role in neutrophil activation by immune complexes. *Sci Signal.* 2011;4:ra23.
- Schymeinsky J, Mócsai A, Walzog B. Neutrophil activation via β_2 integrins (CD11/CD18): molecular mechanisms and clinical implications. *Thromb Haemost.* 2007;98:262–273.
- Hanna S, Etzioni A. Leukocyte adhesion deficiencies. *Ann N Y Acad Sci.* 2012;1250:50–55.
- Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. *Annu Rev Immunol.* 2009;27:339–362.
- Hunter T. Discovering the first tyrosine kinase. *Proc Natl Acad Sci USA.* 2015;112:7877–7882.
- Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene.* 2004;23:7906–7909.
- Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol.* 2009;6:587–595.
- Aleshin A, Finn RS. SRC: a century of science brought to the clinic. *Neoplasia.* 2010;12:599–607.
- Miranti CK, Brugge JS. Sensing the environment: a historical perspective on integrin signal transduction. *Nat Cell Biol.* 2002;4:E83–E90.
- Playford MP, Schaller MD. The interplay between Src and integrins in normal and tumor biology. *Oncogene.* 2004;23:7928–7946.
- Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell.* 1991;64:693–702.
- Lowell CA, Soriano P. Knockouts of Src-family kinases: stiff bones, wimpy T cells, and bad memories. *Genes Dev.* 1996;10:1845–1857.
- Lowell CA, Soriano P, Varmus HE. Functional overlap in the src gene family: inactivation of hck and fgr impairs natural immunity. *Genes Dev.* 1994;8:387–398.
- Nathan CF. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest.* 1987;80:1550–1560.
- Nathan C, Sriml S, Farber C, et al. Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. *J Cell Biol.* 1989;109:1341–1349.
- Fuortes M, Jin WW, Nathan C. Adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumor necrosis factor. *J Cell Biol.* 1993;120:777–784.
- Berton G, Fumagalli L, Laudanna C, Sorio C. β_2 integrin-dependent protein tyrosine phosphorylation and activation of the FGR protein tyrosine kinase in human neutrophils. *J Cell Biol.* 1994;126:1111–1121.
- Mohn H, Le Cabec V, Fischer S, Maridonneau-Parini I. The src-family protein-tyrosine kinase p59^{hck} is located on the secretory granules in human neutrophils and translocates towards the phagosome during cell activation. *Biochem J.* 1995;309(Pt 2):657–665.
- Gutkind JS, Robbins KC. Translocation of the FGR protein-tyrosine kinase as a consequence of neutrophil activation. *Proc Natl Acad Sci USA.* 1989;86:8783–8787.
- Yan SR, Fumagalli L, Berton G. Activation of p58^{c-fgr} and p53/56^{lyn} in adherent human neutrophils: evidence for a role of divalent cations in regulating neutrophil adhesion and protein tyrosine kinase activities. *J Inflamm.* 1995;45:297–311.
- Pereira S, Zhou M, Mócsai A, Lowell C. Resting murine neutrophils express functional α_4 integrins that signal through Src family kinases. *J Immunol.* 2001;166:4115–4123.
- Futosi K, Németh T, Pick R, Vántus T, Walzog B, Mócsai A. Dasatinib inhibits proinflammatory functions of mature human neutrophils. *Blood.* 2012;119:4981–4991.

46. Asahi M, Taniguchi T, Hashimoto E, Inazu T, Maeda H, Yamamura H. Activation of protein-tyrosine kinase p72^{Syk} with concanavalin A in polymorphonuclear neutrophils. *J Biol Chem.* 1993;268:23334–23338.
47. Mócsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol.* 2010;10:387–402.
48. Clark EA, Shattil SJ, Ginsberg MH, Bolen J, Brugge JS. Regulation of the protein tyrosine kinase pp72^{Syk} by platelet agonists and the integrin $\alpha_{IIb}\beta_3$. *J Biol Chem.* 1994;269:28859–28864.
49. Lin TH, Rosales C, Mondal K, Bolen JB, Haskill S, Juliano RL. Integrin-mediated tyrosine phosphorylation and cytokine message induction in monocytic cells. A possible signaling role for the Syk tyrosine kinase. *J Biol Chem.* 1995;270:16189–16197.
50. Gao J, Zoller KE, Ginsberg MH, Brugge JS, Shattil SJ. Regulation of the pp72^{Syk} protein tyrosine kinase by platelet integrin $\alpha_{IIb}\beta_3$. *EMBO J.* 1997;16:6414–6425.
51. Miranti CK, Leng L, Maschberger P, Brugge JS, Shattil SJ. Identification of a novel integrin signaling pathway involving the kinase Syk and the guanine nucleotide exchange factor Vav1. *Curr Biol.* 1998;8:1289–1299.
52. Yan SR, Huang M, Berton G. Signaling by adhesion in human neutrophils: activation of the p72^{Syk} tyrosine kinase and formation of protein complexes containing p72^{Syk} and Src family kinases in neutrophils spreading over fibrinogen. *J Immunol.* 1997;158:1902–1910.
53. Abtahian F, Guerriero A, Sebzda E, et al. Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk. *Science.* 2003;299:247–251.
54. Graham DB, Robertson CM, Bautista J, et al. Neutrophil-mediated oxidative burst and host defense are controlled by a Vav-PLC γ 2 signaling axis in mice. *J Clin Invest.* 2007;117:3445–3452.
55. Turner M, Billadeau DD. Vav proteins as signal integrators for multi-subunit immune-recognition receptors. *Nat Rev Immunol.* 2002;2:476–486.
56. Schymeinsky J, Sindrilaru A, Frommhold D, et al. The Vav binding site of the non-receptor tyrosine kinase Syk at Tyr 348 is critical for β_2 integrin (CD11/CD18)-mediated neutrophil migration. *Blood.* 2006;108:3919–3927.
57. Gakidis MA, Cullere X, Olson T, et al. Vav GEFs are required for β_2 integrin-dependent functions of neutrophils. *J Cell Biol.* 2004;166:273–282.
58. Newbrough SA, Mócsai A, Clemens RA, et al. SLP-76 regulates Fc γ receptor and integrin signaling in neutrophils. *Immunity.* 2003;19:761–769.
59. Volmering S, Block H, Boras M, Lowell CA, Zarbock A. The neutrophil Btk signalosome regulates integrin activation during sterile inflammation. *Immunity.* 2016;44:73–87.
60. Honda F, Kano H, Kanegane H, et al. The kinase Btk negatively regulates the production of reactive oxygen species and stimulation-induced apoptosis in human neutrophils. *Nat Immunol.* 2012;13:369–378.
61. Fiedler K, Sindrilaru A, Terszowski G, et al. Neutrophil development and function critically depend on Bruton tyrosine kinase in a mouse model of X-linked agammaglobulinemia. *Blood.* 2011;117:1329–1339.
62. Schymeinsky J, Then C, Sindrilaru A, et al. Syk-mediated translocation of PI3K δ to the leading edge controls lamellipodium formation and migration of leukocytes. *PLoS ONE.* 2007;2:e1132.
63. Schymeinsky J, Gerstl R, Mannigel I, et al. A fundamental role of mAbp1 in neutrophils: impact on β_2 integrin-mediated phagocytosis and adhesion in vivo. *Blood.* 2009;114:4209–4220.
64. Jakob SM, Pick R, Brechtel D, et al. Hematopoietic progenitor kinase 1 (HPK1) is required for LFA-1-mediated neutrophil recruitment during the acute inflammatory response. *Blood.* 2013;121:4184–4194.
65. Berton G, Laudanna C, Sorio C, Rossi F. Generation of signals activating neutrophil functions by leukocyte integrins: LFA-1 and gp150/95, but not CR3, are able to stimulate the respiratory burst of human neutrophils. *J Cell Biol.* 1992;116:1007–1017.
66. Jakus Z, Berton G, Ligeti E, Lowell CA, Mócsai A. Responses of neutrophils to anti-integrin antibodies depends on costimulation through low affinity Fc γ Rs: full activation requires both integrin and nonintegrin signals. *J Immunol.* 2004;173:2068–2077.
67. Vines CM, Potter JW, Xu Y, et al. Inhibition of β_2 integrin receptor and Syk kinase signaling in monocytes by the Src family kinase Fgr. *Immunity.* 2001;15:507–519.
68. Oberfell A, Eto K, Mócsai A, et al. Coordinate interactions of Csk, Src, and Syk kinases with $\alpha_{IIb}\beta_3$ initiate integrin signaling to the cytoskeleton. *J Cell Biol.* 2002;157:265–275.
69. Judd BA, Myung PS, Leng L, et al. Hematopoietic reconstitution of SLP-76 corrects hemostasis and platelet signaling through $\alpha_{IIb}\beta_3$ and collagen receptors. *Proc Natl Acad Sci USA.* 2000;97:12056–12061.
70. Wonerow P, Pearce AC, Vaux DJ, Watson SP. A critical role for phospholipase C γ 2 in $\alpha_{IIb}\beta_3$ -mediated platelet spreading. *J Biol Chem.* 2003;278:37520–37529.
71. Mócsai A, Humphrey MB, Van Ziffle JA, et al. The immunomodulatory adapter proteins DAP12 and Fc receptor γ -chain (Fc γ R γ) regulate development of functional osteoclasts through the Syk tyrosine kinase. *Proc Natl Acad Sci USA.* 2004;101:6158–6163.
72. Faccio R, Zou W, Colaianni G, Teitelbaum SL, Ross FP. High dose M-CSF partially rescues the Dap12^{-/-} osteoclast phenotype. *J Cell Biochem.* 2003;90:871–883.
73. Zou W, Kitaura H, Reeve J, et al. Syk, c-Src, the $\alpha_V\beta_3$ integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J Cell Biol.* 2007;176:877–888.
74. Epple H, Cremasco V, Zhang K, Mao D, Longmore GD, Faccio R. Phospholipase C γ 2 modulates integrin signaling in the osteoclast by affecting the localization and activation of Src kinase. *Mol Cell Biol.* 2008;28:3610–3622.
75. Kertész Z, Gyori D, Körmendi S, et al. Phospholipase C γ 2 is required for basal but not oestrogen deficiency-induced bone resorption. *Eur J Clin Invest.* 2012;42:49–60.
76. Fodor S, Jakus Z, Mócsai A. ITAM-based signaling beyond the adaptive immune response. *Immunol Lett.* 2006;104:29–37.
77. Abtahian F, Bezman N, Clemens R, et al. Evidence for the requirement of ITAM domains but not SLP-76/Gads interaction for integrin signaling in hematopoietic cells. *Mol Cell Biol.* 2006;26:6936–6949.
78. Boylan B, Gao C, Rathore V, Gill JC, Newman DK, Newman PJ. Identification of Fc γ R11a as the ITAM-bearing receptor mediating $\alpha_{IIb}\beta_3$ outside-in integrin signaling in human platelets. *Blood.* 2008;112:2780–2786.
79. Koga T, Inui M, Inoue K, et al. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature.* 2004;428:758–763.
80. Graham DB, Stephenson LM, Lam SK, et al. An ITAM-signaling pathway controls cross-presentation of particulate but not soluble antigens in dendritic cells. *J Exp Med.* 2007;204:2889–2897.
81. Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A. Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. *J Neurosci.* 2008;28:8138–8143.
82. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol.* 2007;7:678–689.
83. Mócsai A, Zhang H, Jakus Z, Kitaura J, Kawakami T, Lowell CA. G-protein-coupled receptor signaling in Syk-deficient neutrophils and mast cells. *Blood.* 2003;101:4155–4163.
84. Cremasco V, Graham DB, Novack DV, Swat W, Faccio R. Vav/Phospholipase C γ 2-mediated control of a neutrophil-dependent murine model of rheumatoid arthritis. *Arthritis Rheum.* 2008;58:2712–2722.

85. Ozaki N, Suzuki S, Ishida M, et al. Syk-dependent signaling pathways in neutrophils and macrophages are indispensable in the pathogenesis of anti-collagen antibody-induced arthritis. *Int Immunol*. 2012;24:539–550.
86. Broides A, Hadad N, Levy J, Levy R. The effects of Bruton tyrosine kinase inhibition on chemotaxis and superoxide generation in human neutrophils. *J Clin Immunol*. 2014;34:555–560.
87. Mizgerd JP, Kubo H, Kutkoski GJ, et al. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. *J Exp Med*. 1997;186:1357–1364.
88. Walzog B, Scharffetter-Kochanek K, Gaehtgens P. Impairment of neutrophil emigration in CD18-null mice. *Am J Physiol*. 1999;276:G1125–G1130.
89. Zhang H, Meng F, Chu CL, Takai T, Lowell CA. The Src family kinases Hck and Fgr negatively regulate neutrophil and dendritic cell chemokine signaling via PIR-B. *Immunity*. 2005;22:235–246.
90. Wex E, Bouyssou T, Duechs MJ, et al. Induced Syk deletion leads to suppressed allergic responses but has no effect on neutrophil or monocyte migration in vivo. *Eur J Immunol*. 2011;41:3208–3218.
91. Frommhold D, Mannigel I, Schymeinsky J, et al. Spleen tyrosine kinase Syk is critical for sustained leukocyte adhesion during inflammation in vivo. *BMC Immunol*. 2007;8:31.
92. Hirahashi J, Mekala D, Van Ziffle J, et al. Mac-1 signaling via Src-family and Syk kinases results in elastase-dependent thrombohemorrhagic vasculopathy. *Immunity*. 2006;25:271–283.
93. Elliott ER, Van Ziffle JA, Scapini P, Sullivan BM, Locksley RM, Lowell CA. Deletion of Syk in neutrophils prevents immune complex arthritis. *J Immunol*. 2011;187:4319–4330.
94. Lammermann T, Bader BL, Monkley SJ, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature*. 2008;453:51–55.
95. Lu H, Smith CW, Perrard J, et al. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1-deficient mice. *J Clin Invest*. 1997;99:1340–1350.
96. Ding ZM, Babensee JE, Simon SI, et al. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. *J Immunol*. 1999;163:5029–5038.
97. Bruhns P, Jonsson F. Mouse and human FcR effector functions. *Immunol Rev*. 2015;268:25–51.
98. Chen K, Nishi H, Travers R, et al. Endocytosis of soluble immune complexes leads to their clearance by FcγRIIIB but induces neutrophil extracellular traps via FcγRIIA in vivo. *Blood*. 2012;120:4421–4431.
99. Behnen M, Leschczyk C, Möller S, et al. Immobilized immune complexes induce neutrophil extracellular trap release by human neutrophil granulocytes via FcγRIIIB and Mac-1. *J Immunol*. 2014;193:1954–1965.
100. Guyre PM, Campbell AS, Kniffin WD, Fanger MW. Monocytes and polymorphonuclear neutrophils of patients with streptococcal pharyngitis express increased numbers of type I IgG Fc receptors. *J Clin Invest*. 1990;86:1892–1896.
101. Repp R, Valerius T, Sandler A, et al. Neutrophils express the high affinity receptor for IgG (FcγRI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. *Blood*. 1991;78:885–889.
102. Mancardi DA, Albanesi M, Jönsson F, et al. The high-affinity human IgG receptor FcγRI (CD64) promotes IgG-mediated inflammation, anaphylaxis, and antitumor immunotherapy. *Blood*. 2013;121:1563–1573.
103. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood*. 2012;119:5640–5649.
104. Coxon A, Cullere X, Knight S, et al. FcγRIII mediates neutrophil recruitment to immune complexes. A mechanism for neutrophil accumulation in immune-mediated inflammation. *Immunity*. 2001;14:693–704.
105. Aleyd E, Heineke MH, van Egmond M. The era of the immunoglobulin A Fc receptor FcαRI; its function and potential as target in disease. *Immunol Rev*. 2015;268:123–138.
106. Vidarsson G, Stemerding AM, Stapleton NM, et al. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood*. 2006;108:3573–3579.
107. Graham IL, Lefkowitz JB, Anderson DC, Brown EJ. Immune complex-stimulated neutrophil LTB₄ production is dependent on β₂ integrins. *J Cell Biol*. 1993;120:1509–1517.
108. Zhou MJ, Brown EJ. CR3 (Mac-1, α_Mβ₂, CD11b/CD18) and FcγRIII cooperate in generation of a neutrophil respiratory burst: requirement for FcγRIII and tyrosine phosphorylation. *J Cell Biol*. 1994;125:1407–1416.
109. Chen H, Mócsai A, Zhang H, et al. Role for plastein in host defense distinguishes integrin signaling from cell adhesion and spreading. *Immunity*. 2003;19:95–104.
110. Kiefer F, Brumell J, Al-Alawi N, et al. The Syk protein tyrosine kinase is essential for Fcγ receptor signaling in macrophages and neutrophils. *Mol Cell Biol*. 1998;18:4209–4220.
111. Palacios EH, Weiss A. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. *Oncogene*. 2004;23:7990–8000.
112. Saijo K, Schmedt C, Su IH, et al. Essential role of Src-family protein tyrosine kinases in NF-κB activation during B cell development. *Nat Immunol*. 2003;4:274–279.
113. Hibbs ML, Tarlinton DM, Armes J, et al. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell*. 1995;83:301–311.
114. Nishizumi H, Taniuchi I, Yamanashi Y, et al. Impaired proliferation of peripheral B cells and indication of autoimmune disease in lyn-deficient mice. *Immunity*. 1995;3:549–560.
115. Chan VW, Meng F, Soriano P, DeFranco AL, Lowell CA. Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity*. 1997;7:69–81.
116. Lamagna C, Hu Y, DeFranco AL, Lowell CA. B cell-specific loss of Lyn kinase leads to autoimmunity. *J Immunol*. 2014;192:919–928.
117. Abram CL, Lowell CA. The diverse functions of Src family kinases in macrophages. *Front Biosci*. 2008;13:4426–4450.
118. Lowell CA. Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. *Cold Spring Harb Perspect Biol*. 2011;3:a002352.
119. Furumoto Y, Gomez G, Gonzalez-Espinosa C, et al. The role of Src family kinases in mast cell effector function. *Novartis Found Symp*. 2005;271:39–47; discussion 47–53, 95–99.
120. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev*. 2009;228:149–169.
121. Rolli V, Gallwitz M, Wossnig T, et al. Amplification of B cell antigen receptor signaling by a Syk/ITAM positive feedback loop. *Mol Cell*. 2002;10:1057–1069.
122. Utomo A, Cullere X, Glogauer M, Swat W, Mayadas TN. Vav proteins in neutrophils are required for FcγR-mediated signaling to Rac GTPases and nicotinamide adenine dinucleotide phosphate oxidase component p40^{phox}. *J Immunol*. 2006;177:6388–6397.
123. Clemens RA, Lenox LE, Kambayashi T, et al. Loss of SLP-76 expression within myeloid cells confers resistance to neutrophil-mediated tissue damage while maintaining effective bacterial killing. *J Immunol*. 2007;178:4606–4614.
124. Brozna JP. Shwartzman reaction. *Semin Thromb Hemost*. 1990;16:326–332.
125. Wipke BT, Allen PM. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J Immunol*. 2001;167:1601–1608.
126. Chiriac MT, Roesler J, Sindrilaru A, Scharffetter-Kochanek K, Zillikens D, Sitaru C. NADPH oxidase is required for neutrophil-dependent autoantibody-induced tissue damage. *J Pathol*. 2007;212:56–65.

127. Li JL, Lim CH, Tay FW, et al. Neutrophils self-regulate immune complex-mediated cutaneous inflammation through CXCL2. *J Invest Dermatol.* 2016;136:416–424.
128. Ji H, Ohmura K, Mahmood U, et al. Arthritis critically dependent on innate immune system players. *Immunity.* 2002;16:157–168.
129. Watts GM, Beurskens FJ, Martin-Padura I, et al. Manifestations of inflammatory arthritis are critically dependent on LFA-1. *J Immunol.* 2005;174:3668–3675.
130. Monach PA, Nigrovic PA, Chen M, et al. Neutrophils in a mouse model of autoantibody-mediated arthritis: critical producers of Fc receptor γ , the receptor for C5a, and lymphocyte function-associated antigen 1. *Arthritis Rheum.* 2010;62:753–764.
131. Liu Z, Zhao M, Li N, Diaz LA, Mayadas TN. Differential roles for β_2 integrins in experimental autoimmune bullous pemphigoid. *Blood.* 2006;107:1063–1069.
132. Lenox LE, Kambayashi T, Okumura M, et al. Mutation of tyrosine 145 of lymphocyte cytosolic protein 2 protects mice from anaphylaxis and arthritis. *J Allergy Clin Immunol.* 2009;124:1088–1098.
133. Nyhoff LE, Barron B, Johnson EM, et al. Bruton's tyrosine kinase deficiency inhibits autoimmune arthritis but fails to block immune complex-mediated inflammatory arthritis. *Arthritis Rheumatol.* 2016; doi: 10.1002/art.39657. In Press.
134. Wipke BT, Wang Z, Nagengast W, Reichert DE, Allen PM. Staging the initiation of autoantibody-induced arthritis: a critical role for immune complexes. *J Immunol.* 2004;172:7694–7702.
135. Jonsson H, Allen P, Peng SL. Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. *Nat Med.* 2005;11:666–671.
136. Solomon S, Rajasekaran N, Jeisy-Walder E, Snapper SB, Illges H. A crucial role for macrophages in the pathology of K/BxN serum-induced arthritis. *Eur J Immunol.* 2005;35:3064–3073.
137. Boillard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science.* 2010;327:580–583.
138. Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science.* 2002;297:1689–1692.
139. Kneilling M, Hültner L, Pichler BJ, et al. Targeted mast cell silencing protects against joint destruction and angiogenesis in experimental arthritis in mice. *Arthritis Rheum.* 2007;56:1806–1816.
140. Zhou JS, Xing W, Friend DS, Austen KF, Katz HR. Mast cell deficiency in Kit^{W^{sh}} mice does not impair antibody-mediated arthritis. *J Exp Med.* 2007;204:2797–2802.
141. Feyerabend TB, Weiser A, Tietz A, et al. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. *Immunity.* 2011;35:832–844.
142. Mancardi DA, Jönsson F, Iannascoli B, et al. Cutting edge: the murine high-affinity IgG receptor Fc γ R4 is sufficient for autoantibody-induced arthritis. *J Immunol.* 2011;186:1899–1903.
143. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Cassatella MA. The neutrophil as a cellular source of chemokines. *Immunol Rev.* 2000;177:195–203.
144. Mócsai A, Bánfi B, Kapus A, et al. Differential effects of tyrosine kinase inhibitors and an inhibitor of the mitogen-activated protein kinase cascade on degranulation and superoxide production of human neutrophil granulocytes. *Biochem Pharmacol.* 1997;54:781–789.
145. Fumagalli L, Zhang H, Baruzzi A, Lowell CA, Berton G. The Src family kinases Hck and Fgr regulate neutrophil responses to N-formyl-methionyl-leucyl-phenylalanine. *J Immunol.* 2007;178:3874–3885.
146. McEver RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc Res.* 2015;107:331–339.
147. Zarbock A, Lowell CA, Ley K. Spleen tyrosine kinase Syk is necessary for E-selectin-induced $\alpha_L\beta_2$ integrin-mediated rolling on intercellular adhesion molecule-1. *Immunity.* 2007;26:773–783.
148. Zarbock A, Abram CL, Hundt M, Altman A, Lowell CA, Ley K. PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and Fc γ R to induce slow leukocyte rolling. *J Exp Med.* 2008;205:2339–2347.
149. Yago T, Shao B, Miner JJ, et al. E-selectin engages PSGL-1 and CD44 through a common signaling pathway to induce integrin $\alpha_L\beta_2$ -mediated slow leukocyte rolling. *Blood.* 2010;116:485–494.
150. Urzainqui A, Serrador JM, Viedma F, et al. ITAM-based interaction of ERM proteins with Syk mediates signaling by the leukocyte adhesion receptor PSGL-1. *Immunity.* 2002;17:401–412.
151. Block H, Herter JM, Rossaint J, et al. Crucial role of SLP-76 and ADAP for neutrophil recruitment in mouse kidney ischemia-reperfusion injury. *J Exp Med.* 2012;209:407–421.
152. Mueller H, Stadtmann A, Van Aken H, et al. Tyrosine kinase Btk regulates E-selectin-mediated integrin activation and neutrophil recruitment by controlling phospholipase C (PLC) γ 2 and PI3K pathways. *Blood.* 2010;115:3118–3127.
153. Herter JM, Rossaint J, Block H, Welch H, Zarbock A. Integrin activation by P-Rex1 is required for selectin-mediated slow leukocyte rolling and intravascular crawling. *Blood.* 2013;121:2301–2310.
154. Moser M, Legate KR, Zent R, Fassler R. The tail of integrins, talin, and kindlins. *Science.* 2009;324:895–899.
155. Gross O, Gewies A, Finger K, et al. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature.* 2006;442:651–656.
156. LeibundGut-Landmann S, Gross O, Robinson MJ, et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol.* 2007;8:630–638.
157. Werninghaus K, Babiak A, Gross O, et al. Adjuvanticity of a synthetic cord factor analogue for subunit Mycobacterium tuberculosis vaccination requires Fc γ R-Syk-Card9-dependent innate immune activation. *J Exp Med.* 2009;206:89–97.
158. Gross O, Poeck H, Bscheider M, et al. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature.* 2009;459:433–436.
159. Roth S, Ruland J. Caspase recruitment domain-containing protein 9 signaling in innate immunity and inflammation. *Trends Immunol.* 2013;34:243–250.
160. Glocker EO, Hennigs A, Nabavi M, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *N Engl J Med.* 2009;361:1727–1735.
161. Li X, Utomo A, Cullere X, et al. The β -glucan receptor Dectin-1 activates the integrin Mac-1 in neutrophils via Vav protein signaling to promote *Candida albicans* clearance. *Cell Host Microbe.* 2011;10:603–615.
162. Drewniak A, Gazendam RP, Tool AT, et al. Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency. *Blood.* 2013;121:2385–2392.
163. Gazendam RP, van Hamme JL, Tool AT, et al. Two independent killing mechanisms of *Candida albicans* by human neutrophils: evidence from innate immunity defects. *Blood.* 2014;124:590–597.
164. Boyle KB, Gyori D, Sindrilaru A, et al. Class IA phosphoinositide 3-kinase β and δ regulate neutrophil oxidase activation in response to *Aspergillus fumigatus* hyphae. *J Immunol.* 2011;186:2978–2989.
165. O'Shea JJ, Plenge R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity.* 2012;36:542–550.
166. Coxon A, Rieu P, Barkalow FJ, et al. A novel role for the β_2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. *Immunity.* 1996;5:653–666.
167. Corr M, Crain B. The role of Fc γ R signaling in the K/BxN serum transfer model of arthritis. *J Immunol.* 2002;169:6604–6609.