

Mechanisms of Degranulation in Neutrophils

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Abstract

Neutrophils are critical inflammatory cells that cause tissue damage in a range of diseases and disorders. Being bone marrow–derived white blood cells, they migrate from the bloodstream to sites of tissue inflammation in response to chemotactic signals and induce inflammation by undergoing receptor-mediated respiratory burst and degranulation. Degranulation from neutrophils has been implicated as a major causative factor in pulmonary disorders, including severe asphyxic episodes of asthma. However, the mechanisms that control neutrophil degranulation are not well understood. Recent observations indicate that granule release from neutrophils depends on activation of intracellular signalling pathways, including β -arrestins, the Rho guanosine triphosphatase Rac2, soluble NSF attachment protein (SNAP) receptors, the *src* family of tyrosine kinases, and the tyrosine phosphatase MEG2. Some of these observations suggest that degranulation from neutrophils is selective and depends on nonredundant signalling pathways. This review focuses on new findings from the literature on the mechanisms that control the release of granule-derived mediators from neutrophils.

Neutrophils are highly mobile and short-lived white blood cells that are densely packed with secretory granules. They derive from the bone marrow, where they mature in response to appropriate cytokines. Following this, they emigrate from the bone marrow into the blood and circulate to tissues. In healthy individuals, peripheral blood neutrophils make up the majority of white blood cells (40–80%). The lungs form the largest marginated pool of neutrophils in the body. In the airways, neutrophils fulfill an important sentinel role in maintaining sterility. As a major effector cell in innate immunity, neutrophils act as a double-edged sword. If neutrophils are absent (eg, in congenital neutropenia or the more common cyclic

neutropenia), infections result from overgrowth of bacteria and fungi at sites of injury or exposed regions of mucosal tissues. At the other extreme, accumulation and overactivation of neutrophils can be fatal in disorders such as in septic shock or acute respiratory distress. The tissue-damaging effects of neutrophils are completely dependent on the activation of mediator release.

Mediator release is defined as the secretion or production of proinflammatory substances that are derived from intracellular stored granules or synthesized *de novo* on stimulation by receptors. Neutrophils release granule-derived mediators by degranulation, or exocytosis, of membrane-bound secretory granules. The neutrophil also possesses the capacity to release a diverse array of antimicrobial proteins and enzymes intracellularly into membrane-bound organelles, called phagosomes, which contain engulfed small microorganisms. At the same time, neutrophils release reactive oxygen species and cytokines outside the cells to kill extracellular bacteria and recruit additional leukocytes to the region of infection or inflammation.

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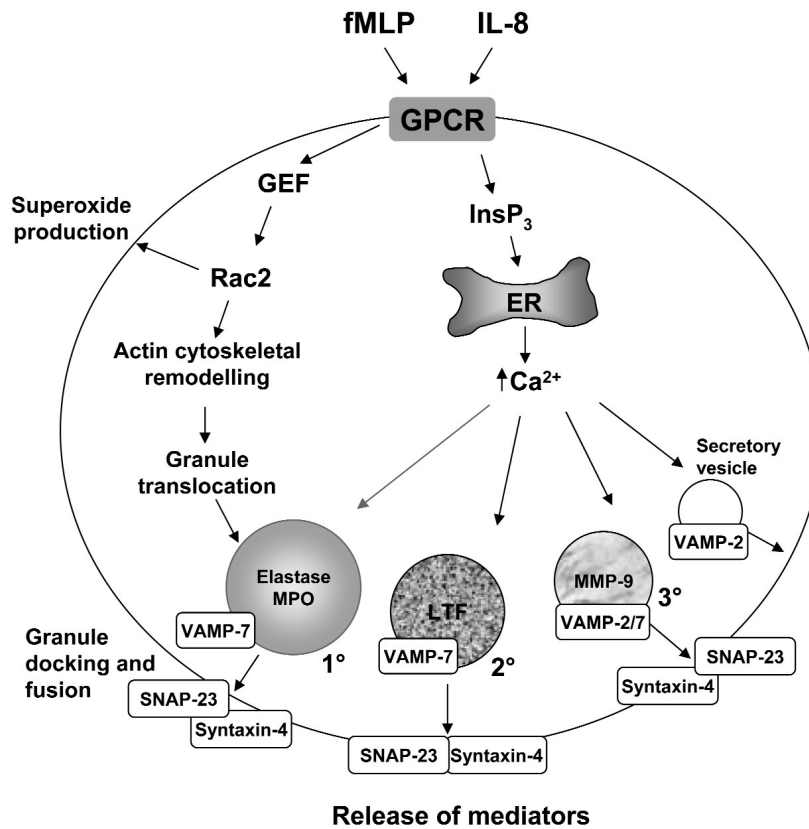


Figure 1 Rho guanosine triphosphatase and SNAP receptor (SNARE) signalling pathways involved in Ca²⁺-dependent neutrophil degranulation. Receptor binding by a chemoattractant leads to G protein-coupled signal transduction (G protein-coupled receptor [GPCR]) through multiple overlapping intracellular pathways to regulate the selective release of neutrophil granules. Some of these pathways may be non-redundant, for example, through G protein-activated guanine nucleotide exchange factors (GEFs) to activate Rac2, which selectively mobilizes primary granules. ER = endoplasmic reticulum; fMLP = F-Met-Lev-Phe; IL = interleukin; InsP₃ = inositol 1, 4, 5-triphosphate; LTF = lactoperin; MMP = matrix metalloproteinase; MPO = myeloperoxidase; VAMP = vesicle-associated membrane protein.

Excessive neutrophil degranulation is a common feature of many inflammatory disorders, such as severe asphyxial episodes of asthma, acute lung injury, rheumatoid arthritis, and septic shock.¹ A recent study by Brinkmann and colleagues described a novel mechanism by which neutrophils eliminate bacteria.² On activation by a range of mediators, including interleukin-8 (IL-8), lipopolysaccharide, and interferon- α with complement 5a,³ neutrophils were shown to generate a web of extracellular fibres known as neutrophil extracellular traps (NETs), composed of deoxyribonucleic acid (DNA), histones, and antimicrobial granule proteins, which are highly effective at trapping and killing invasive bacteria. The authors proposed that NETs amplified the effectiveness of antimicrobial components by concentrating them in a fibrous network and reducing their exposure to host tissues. Although this report fell short on describing the molecular mechanisms responsible

for NET formation and its association with granular protein, it opened a new horizon in the field of neutrophil biology as it relates to mediator release and bactericidal activity.

Therefore, to attenuate a neutrophilic inflammatory response, an effective therapeutic strategy would be one that is directed at down-regulation of neutrophil degranulation. Recent findings have identified a number of important signalling pathways in neutrophils that may be useful as targets for pharmacologic intervention of degranulation.

Granule Types in Neutrophils

Neutrophils contain at least four different types of granules: (1) primary granules, also known as azurophilic granules; (2) secondary granules, also known as specific granules; (3) tertiary granules; and (4) secretory vesicles (Figure 1). The

primary granules are the main storage site of the most toxic mediators, including elastase, myeloperoxidase, cathepsins, and defensins. The secondary and tertiary granules contain lactoferrin and matrix metalloproteinase 9 (also known as gelatinase B), respectively, among other substances.⁴ The secretory vesicles in human neutrophils contain human serum albumin, suggesting that they contain extracellular fluid that was derived from endocytosis of the plasma membrane. The secondary and tertiary granules have overlapping contents but can be discriminated by their intrinsic buoyant densities when centrifuged on gradient media.⁵ Granules are prevented from being released until receptors in the plasma membrane or phagosomal membrane signal to the cytoplasm to activate their movement to the cell membrane for secretion of their contents by degranulation. This is an important control mechanism as the neutrophil is highly enriched in tissue-destructive proteases.

Degranulation Mechanisms in Neutrophils

When receptor stimulation by a secretagogue occurs, granules translocate to the phagosomal or plasma membrane, where they dock and fuse with the membrane to release their contents. The release of granule-derived mediators from granulocytes occurs by tightly controlled receptor-coupled mechanisms, leading to exocytosis. Exocytosis is postulated to take place in four discrete steps.⁶ The first step of exocytosis is granule recruitment from the cytoplasm to target membrane, which is dependent on actin cytoskeleton remodelling and microtubule assembly.⁷ This is followed by vesicle tethering and docking, leading to contact of the outer surface of the lipid bilayer membrane surrounding the granule with the inner surface of the target membrane. Granule priming then follows to make granules fusion-competent to ensure that they fuse rapidly, and a reversible fusion pore structure develops between the granule and the target membrane. Granule fusion occurs by the expansion of the fusion pore, leading to complete fusion of the granule with the target membrane to release granular contents. In the case of exocytosis, this increases the total surface area of the cell and

exposes the interior membrane surface of the granule to the exterior.

Translocation and exocytosis of granules in neutrophils require, as a minimum, increases in intracellular Ca^{2+} , as well as hydrolysis of adenosine triphosphate (ATP) and guanosine triphosphate (GTP). The target molecules for these effectors are numerous and include Ca^{2+} -binding proteins such as annexins and calmodulin and GTP-binding proteins such as G proteins and small monomeric proteins. ATP is used by ATP-hydrolyzing enzymes (adenosine triphosphatases) and kinases, which act by phosphorylating downstream effector molecules. Combined with activation of these effector molecules is reorganization of the actin cytoskeleton, which forms a mesh around the periphery of the cell as a shield against granule docking and fusion. The actin cytoskeletal mesh must be disassembled to allow access of granules to the inner surface of the plasma membrane. It is likely that the process of granule translocation and exocytosis involves activation and recruitment of many different signalling molecules, only some of which are beginning to be identified.

Ca^{2+} Signalling in Exocytosis

Increases in intracellular Ca^{2+} alone are sufficient to induce the release of many of the granule types in neutrophils, particularly if the concentration of Ca^{2+} is elevated to sufficiently high levels by the use of Ca^{2+} ionophores such as A23187 or ionomycin. A hierarchy of granule release exists in response to elevating concentrations of Ca^{2+} .⁸ The order of release is secretory vesicles > tertiary granules > secondary granules > primary granules.^{8,9} The release of each type of granule appears to be regulated by different intracellular signalling pathways. Many neutrophil receptors activate increased Ca^{2+} levels, including the seven transmembrane-spanning G protein-coupled receptors, such as the formyl peptide receptor (that binds to the bacterial tripeptide f-Met-Leu-Phe) and chemokine receptors (such as CXCR1). Although Ca^{2+} is a crucial second messenger in the activation of exocytosis, the specific target molecules for Ca^{2+} in neutrophil degranulation have not yet been identified (see Figure 1).

Phospholipid Signalling in Degranulation

Numerous studies have indicated a role for phospholipids, particularly polyphosphoinositides, in the regulation of neutrophil degranulation. Polyphosphoinositide production, such as phosphatidylinositol bisphosphate (PIP₂), induced by activation of the hematopoietic cell-specific isoform phosphatidylinositol 3-kinase (PI3K)- γ , has been shown to be required for granule exocytosis in permeabilized neutrophil-like cells, HL-60 cells.¹⁰ The intracellular sites of PIP₂ formation in neutrophils are not known, but it is likely to occur both at the plasma membrane and on granule membranes. Regions of PIP₂ enrichment in the membrane form essential binding sites for many intracellular signalling molecules, particularly those that contain pleckstrin homology domains. Phosphatidylinositol transfer protein has been shown to be essential for the transport of phosphatidylinositol to cellular membranes as a substrate for PI3K activity to generate PIP₂ and is also capable of restoring exocytotic responses in HL-60 cells.¹⁰ In addition, a role for phospholipase D has been indicated in neutrophil degranulation, particularly for primary and secondary granule release, as its product, phosphatidic acid, induces the release of these granules.¹¹ Thus, membrane lipids form an essential component of degranulation in neutrophils.

Role for src Family Kinases in Neutrophil Degranulation

Protein phosphorylation is a critical event in neutrophil activation leading from receptor stimulation to exocytosis. Phosphorylation is carried out by kinases, which are themselves frequently activated by phosphorylation by upstream molecules. This specifically involves the attachment of a phosphate molecule, donated by intracellular ATP, to a key site in the effector molecule, leading to conformational changes that cause activation. Receptor stimulation through the formyl peptide receptor by f-Met-Leu-Phe leads to phosphorylation of a wide range of kinases, which then activate their respective effector pathways. Kinases can

be discriminated based on their affinity for different amino acid residues in effector molecules. Thus, serine/threonine kinases and tyrosine kinases have been characterized as distinct types of kinases involved in receptor signalling. Tyrosine kinases are further differentiated for their intrinsic association with the intracellular domain of receptors (receptor tyrosine kinases) or as cytosolic enzymes (nonreceptor tyrosine kinases).

The *src* family of nonreceptor tyrosine kinases has been implicated in the control of exocytosis of granule products from neutrophils. Three *src* family members, Hck, Fgr, and Lyn, have been shown to be expressed in neutrophils and are activated by f-Met-Leu-Phe receptor stimulation. Interestingly, different granule populations appear to be associated with different *src* kinases. Hck translocates to the primary granule population following cell activation¹² whereas Fgr becomes associated with the secondary granules during exocytosis.¹³ The selective recruitment of *src* kinases indicates that different signalling pathways exist in neutrophils to induce the release of each granule population. Recent studies showed that treatment of human neutrophils with the *src* family inhibitor PP1 led to inhibition of the release of primary granules, secondary granules, and secretory vesicles in response to f-Met-Leu-Phe.¹⁴ Neutrophils isolated from *hck*^{-/-}*fgr*^{-/-}*lyn*^{-/-} triple knockout mice also showed a deficiency in secondary granule release, although it was not possible to determine primary granule release.¹⁴ The deficiency in secondary granule release correlated with reduced p38 mitogen-activated protein (MAP) kinase activity, suggesting that *src* kinases act upstream of p38 MAP kinase. Indeed, treatment of neutrophils with the p38 MAP kinase inhibitor SB203580 led to reduced primary and secondary granule exocytosis in response to f-Met-Leu-Phe. Another kinase inhibitor, PD98059, which blocks extracellular-related kinase (ERK)1/2 activity, did not affect the release of primary and secondary granules or secretory vesicles. These findings indicate that *src* kinases and p38 MAP kinase play a role in regulating the release of granules in response to f-Met-Leu-Phe receptor stimulation in neutrophils and probably act at an early signalling step proximal to the receptor in this process (Figure 2).

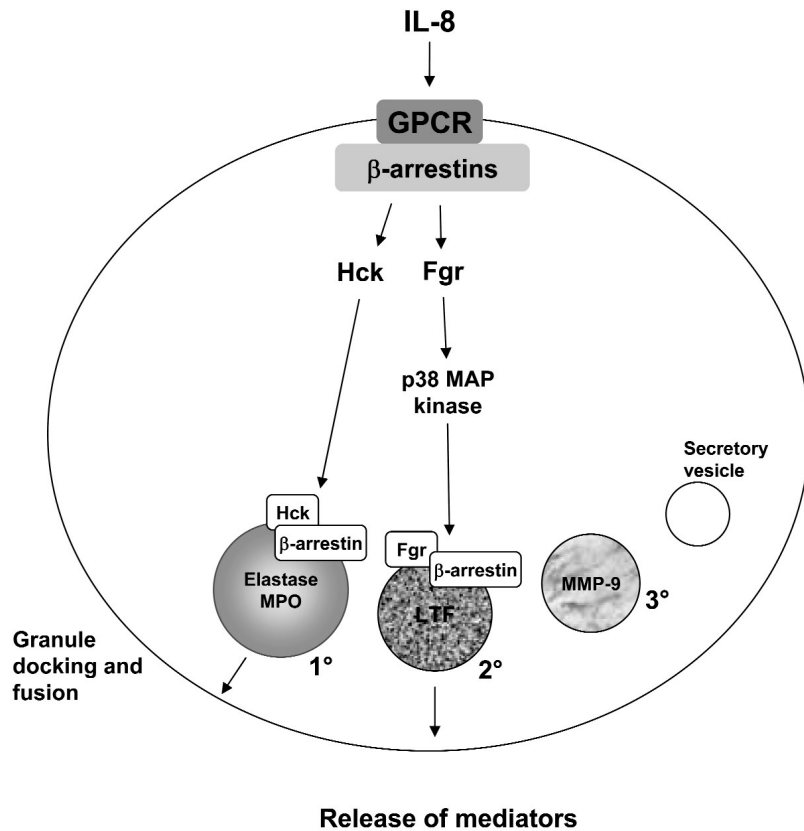


Figure 2 Tyrosine kinases associated with chemokine-induced neutrophil degranulation. Receptor binding leads to direct binding of the G protein-coupled receptor (GPCR) by β -arrestins, which also translocate to primary and secondary granules along with src family kinases Hck and Fgr. IL = interleukin; LTF = ; MAP = mitogen-activated protein; MMP = matrix metalloprotease; MPO = myeloperoxidase.

β -Arrestin Function in Regulating Exocytosis

The family of scaffolding proteins, β -arrestins, may be required for activating signalling pathways leading to exocytosis of primary and secondary granules in neutrophils.¹⁵ β -Arrestins are a group of cytosolic phosphoproteins that were previously characterized for their role in endocytosis of ligand-bound chemokine receptors, particularly CXCR1, which is the high-affinity receptor for the neutrophil chemotactic factor IL-8. β -Arrestins act by uncoupling activated G protein-coupled receptors from their associated heterotrimeric G proteins and binding directly to the cytoplasmic tail of the CXCR1 receptor.^{15,16} Dominant negative mutants of β -arrestin were shown to inhibit the release of granules following transfection of a rat mast cell line (RBL cells) that serves as a model for neutrophil degranulation.¹⁵ Interestingly, β -arrestins also associate with the primary and secondary granules in IL-8-activated neutrophils, and they do

so by binding to Hck and Fgr, respectively.¹⁵ Thus, β -arrestins act at two sites in the cell during chemokine activation: one site at the receptor in the plasma membrane and a second on granule membranes (see Figure 2).

Requirement for Guanosine Triphosphatases in Exocytosis

Exocytosis requires binding of GTP to intracellular effector molecules as the addition of the nonhydrolyzable analog GTP γ S to permeabilized or patch-clamped neutrophils leads to secretion of granule-derived mediators.¹⁷ This suggests that GTP-binding proteins, including guanosine triphosphatases (GTPases), may be involved in granule translocation and exocytosis. To date, over 100 different types of GTPases have been identified, with heterotrimeric G proteins and *ras*-related monomeric GTPases being two of the most comprehensively studied families of regu-

latory GTPases. Whereas heterotrimeric G proteins typically bind to the plasma membrane to transduce receptor signals to the cytoplasm, the superfamily of *ras*-related GTPases can reside in the cytoplasm, in actin cytoskeleton, or on membranes in the cell to fulfill a regulatory role in cell activation. *Ras*-related GTPases are important switches for turning on or off a signalling event. They are switched on by binding to high-energy GTP, which is cleaved to form guanosine diphosphate to activate the next effector molecule in the signalling pathway. Binding to GTP induces the association of many cytosolic GTPases to membrane or cytoskeletal sites within the cell.

Ras-related GTPases can be divided into several subfamilies based on their homology at the amino acid level. One particular group of *ras*-related GTPases is the Rho subfamily of GTPases, which serves a role in regulating actin cytoskeletal rearrangement and in the release of reactive oxygen species. Remodelling of the actin cytoskeleton is critical for allowing a diverse range of cellular activities to occur, including cell motility (chemotaxis), phagocytosis, and exocytosis. The three prototypical members of the Rho GTPase subfamily are Rho, Rac, and Cdc42.^{18–20} Rac is present as three different isoform proteins: Rac1, Rac2, and Rac3. The functions of Rac1 and Rac2 in superoxide generation and chemotaxis are well established in neutrophils.²¹ Rho GTPases are also substrates for a number of bacterial toxins, including *Clostridium difficile* toxin B and *Clostridium sordellii* lethal toxin, which act by glucosylating Rho GTPases.^{22,23}

Rac1 and Rac2 possess 92% homology in their amino acid sequences and differ mainly in the final 10 amino acids in their carboxyl termini. Both isoform proteins are expressed in neutrophils, although human neutrophils express more Rac2 than Rac1.²⁴ It is because of this high homology that they serve functionally interchangeable roles in actin cytoskeletal remodelling and regulation of the release of reactive oxygen species by activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in neutrophils.^{25–27} Interestingly, because of sequence variation in a short carboxyl terminal sequence, Rac2 is the preferential activator of NADPH oxidase in neu-

trophils.²⁸ Human neutrophils translocate most of their Rac protein to intracellular sites of NADPH oxidase activation following stimulation of respiratory burst,²⁹ suggesting that the neutrophil oxidase preferentially produces reactive oxygen species at intracellular sites.

In spite of their high homology, however, Rac1 and Rac2 are divergent in their functions in certain types of cellular activities.^{30–32} We have determined that Rac2 serves a crucial and selective role in degranulation from neutrophils.³² Gene deletion of Rac2 led to a profound degranulation defect in neutrophils, with a complete loss of primary granule release from murine bone marrow neutrophils. Release of granule enzymes from secondary and tertiary granule was normal in Rac2^{-/-} neutrophils, indicating a selective role for Rac2 in primary granule exocytosis. Rac2^{-/-} neutrophils express normal or even elevated levels of Rac1,^{28,33,34} further suggesting that Rac2 serves a unique and distinct role from Rac1 in regulating translocation and exocytosis of granules. In addition, although Rac2^{-/-} neutrophils showed a loss of primary granule release, p38 MAP kinase phosphorylation was still evident in response to f-Met-Phe-Leu stimulation. This is in contrast to the findings of Mocsai and colleagues, who demonstrated an important role for p38 MAP kinase in primary granule release by the use of chemical inhibitors.¹⁴

Rac2^{-/-} neutrophils also failed to translocate primary granules to the cell membrane during f-Met-Leu-Phe stimulation.³² Thus, the defect in primary granule exocytosis in these cells lies in the translocation machinery required to move the granules to the membrane for docking and fusion. The translocation of granules is likely to require actin cytoskeleton remodelling and/or microtubule movements, and Rac2 has been shown to induce the formation of F-actin, which is required for chemotaxis.³³ Indeed, Rac2^{-/-} neutrophils did not bind as well as their wild-type counterparts to adhesion molecules.³³ Identification of downstream effector molecules of Rac2 that are responsible for regulating actin cytoskeletal remodelling and/or microtubule rearrangements will be important in identifying the pathway(s) associated with Rac2-mediated primary granule release (see Figure 1).

SNARE Molecule Binding in Exocytosis from Neutrophils

The final step of exocytosis involves the mutual recognition of secretory granules and target membranes, which is postulated to involve a set of intracellular receptors that guide the docking and fusion of granules. This led to the formation of the SNAP receptor (SNARE) paradigm, which states that secretory vesicles possess membrane-bound receptor molecules that allow their binding by another set of membrane-bound receptors in target membranes.³⁵

Studies on yeast and neuronal cells have yielded significant insights into highly conserved components of a fusion complex of membrane-bound proteins proposed to be essential for vesicular docking and fusion in all cell types, known as SNAREs.^{35,36} The prototypical members of this complex are vesicle-associated membrane protein (VAMP)-1 (also known as synaptobrevin 1), syntaxin 1, and synaptosome-associated protein of 25 kD (SNAP-25). The exocytotic SNARE complex consists of a vesicular SNARE VAMP, which binds to plasma membrane target SNAREs syntaxin 1 and SNAP-25. The fusion of membranes is proposed to depend on cytosolic *N*-ethylmaleimide-sensitive factor (NSF) and α -, β -, or γ -SNAP (soluble NSF-attachment protein)-mediated disassembly of the SNARE complex.³⁵

During binding, SNARE molecules form a coiled-coil structure with four separate α -helices contributed by three different molecules. The binding region associated with the four α -helices is known as the SNARE motif. The stability of the bonds within the SNARE structure is such that it is resistant to treatment with detergents such as sodium dodecyl sulphate.³⁷

SNARE molecules are exquisitely sensitive to cleavage by clostridial neurotoxins containing zinc endopeptidase activity, in particular, tetanus toxin (TeNT) and botulinum toxin serotypes (BoNT/A, B, C, D, E, F, and G).³⁸ The effects of these toxins on intracellular SNARE molecules are likely to be the molecular basis of spastic and flaccid paralysis induced by tetanus and botulinum toxin poisoning, respectively. TeNT and BoNT holotoxins are only able to enter neuronal cells since their heavy chain components require

a ganglioside-binding site on the cell surface, lacking in nonneuronal cells.³⁸ Other isoforms of SNAREs have been identified in cells outside the neuronal system (syntaxin 4 and SNAP-23)³⁹ whereas VAMP-2 expression is widely distributed between neuronal and nonneuronal tissues.⁴⁰ In addition, VAMP-4,⁴¹ VAMP-5,⁴² and the TeNT-insensitive isoforms VAMP-7 (formerly known as TeNT-insensitive VAMP or TI-VAMP)⁴³⁻⁴⁶ and VAMP-8 have been characterized in nonneuronal tissues.⁴⁷⁻⁴⁹

Neutrophils have been reported to express many of the SNARE isoforms so far identified. In an early report, neutrophils were shown to express syntaxin 4 and VAMP-2.⁵⁰ VAMP-2 was localized to tertiary granules and CD35⁺ secretory vesicles, and VAMP-2⁺ vesicles translocated to the plasma membrane during Ca²⁺ ionophore stimulation. By reverse transcriptase–polymerase chain reaction, the messenger ribonucleic acid encoding syntaxins 1A, 3, 4, 5, 6, 7, 9, 11, and 16 have been identified in human neutrophils and a neutrophil-differentiated cell line (HL-60).⁵¹ SNAP-23 and syntaxin 6 appear to be important in regulating neutrophil secondary granule exocytosis using antibodies against these molecules in electroporated cells stimulated with Ca²⁺ and GTP γ S.⁵² Finally, the addition of antibodies to VAMP-2 and syntaxin 4 to electroporated neutrophils blocked Ca²⁺ and GTP γ S-induced exocytosis.⁵³ Exocytosis in the latter two articles was measured by flow cytometric analysis of granule markers CD63 (primary granules) and CD66b (secondary granules), which are up-regulated on the cell surface during stimulation. It was shown that anti-VAMP-2 blocked secondary granule CD66b up-regulation in response to Ca²⁺ and GTP γ S whereas there was no inhibition of CD63⁺ primary granule release with antibody against VAMP-2. In summary, although VAMP-2 was shown to be involved in secondary granule exocytosis, there are no reports describing a VAMP isoform associated with primary granule exocytosis. This would appear to be a significant gap in our understanding of the mechanisms of degranulation in these cells as primary granules are specifically enriched in bactericidal and cytotoxic mediators, including elastase and myeloperoxidase.

We recently determined that VAMP-7 is highly expressed in all neutrophil granule populations and that it may be an essential component for SNARE-mediated exocytotic release of primary, secondary, and tertiary granule release.⁵⁴ Inhibition of VAMP-7 by low concentrations of specific anti-VAMP-7 antibody prevented the release of myeloperoxidase, lactoferrin, and matrix metalloprotease 9 in streptolysin-O-permeabilized human neutrophils. These findings indicate that VAMP-7 may play a promiscuous role in controlling regulated exocytosis of numerous granule populations. This is compatible with the recent observations that SNARE molecules are capable of binding multiple cognate and noncognate partners.⁵⁵ Thus, SNARE isoforms are likely to play a crucial role in the regulation of granule fusion in neutrophils (see Figure 1).

Other Potential Regulatory Molecules of Exocytosis in Neutrophils

Recent findings have suggested a role for a protein tyrosine phosphatase MEG2 in the regulation of neutrophil degranulation. Neutrophils express MEG2 in their primary, secondary, and tertiary granules, which translocates to the phagosomal membrane on phagocytosis of serum-opsonized iron beads.⁵⁶ MEG2 was recently shown to be a phosphatase required for dephosphorylation of NSF, the cytosolic ATPase that is required to cycle SNARE proteins between bound and unbound conformations to allow repeated cycles of membrane fusion.⁵⁷ This study demonstrated for the first time that NSF possesses a tyrosine residue that is phosphorylated and that dephosphorylation triggers the binding of another cytosolic protein, α -SNAP, which is also required for SNARE cycling, to promote vesicular fusion. Cells expressing a dephosphorylated form of mutant NSF exhibited substantial enlargement of their granules, suggesting that the dephosphorylated NSF remained bound to α -SNAP to allow repeated homotypic granule fusion and enlargement of the granules in the cells. Transfection of a phosphomimicking mutant of NSF was shown to inhibit the secretion of IL-2 from Jurkat T cells.⁵⁷ In addition, MEG2 was shown to be activated by polyphosphoinosi-

tides, particularly PIP2,⁵⁶ suggesting that MEG2 is directly associated with the membrane fusion event in granule fusion.

Summary

These recent experimental observations reveal that a large group of intracellular signalling molecules exists to regulate translocation of granules to the cell membrane for docking and fusion to release their contents. Many of these molecules are already natural targets for bacterial toxins to inhibit their function, which highlights their important role in regulating bactericidal mediator release. It may be possible to exploit the use of bacterial toxins as a tool to prevent or modulate neutrophil degranulation. Neutrophil degranulation is an important event in inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Products of neutrophil degranulation, including the high-molecular-weight form of matrix metalloprotease 9 specific to neutrophils, have been shown to increase in proportion to asthma severity in the airways of asthmatic patients.⁵⁸ Moreover, neutrophils and their products are strongly associated with early pathogenesis of COPD.⁵⁹ Further analysis of the signalling pathways that are specifically activated to induce the release of different granule populations in neutrophils may create opportunities for the development of drugs that will prevent degranulation from neutrophils in airway diseases and inflammatory disorders.

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