Microreview

The role of Rab27a in the regulation of neutrophil function

Sergio D. Catz*
Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA.

Summary

Neutrophils are central regulators of the innate immune response and help shape the adaptive immune response. Malfunction and unregulated neutrophil activation leads to disease and inflammation. During the host response to infection, neutrophils display several mechanisms of defense mediated by their arsenal of granular proteins. Regulation of granular trafficking, docking and fusion is at the core of the neutrophil defense response to pathogens. The small GTPase Rab27a has emerged as a central regulator of the neutrophil response through its tight control of vesicular trafficking and degranulation. This review focuses on the latest research that has led to the characterization of Rab27a as an essential regulator of neutrophil function.

Introduction

Neutrophils are important mediators of the innate immune response and modulators of the adaptive immune response (Mantovani et al., 2011). They play central roles in the host response to infections and in the development of acute and chronic inflammation (Amulic et al., 2011). In addition, recent research has highlighted important roles for neutrophils in the development of metabolic disease (Talukdar et al., 2012), cancer (Shojaei et al., 2008) and autoimmunity (Nemeth and Mocsai, 2012).

The main function of neutrophils is to combat bacterial and fungal infections. In the presence of infection, they display an array of defense mechanisms that include adhesion to the activated endothelium (Andonegui et al., 2003), diapedesis and migration to the infection foci (Johnston et al., 1999), engulfment of pathogens (Lee et al., 2003), secretion of microbicidal products into the phagosome or the extracellular space (Catz, 2013) and the formation of extracellular traps (NETs) (Brinkmann et al., 2004), which help to contain the infection and kill pathogens. In resting neutrophils, their microbicidal molecules are segregated and stored in secretory organelles (Borregaard and Cowland, 1997) that are maintained distant from the plasma membrane by a molecular barrier that includes cortical actin (Johnson et al., 2012), thus protecting the host from uncontrolled activation. In response to adequate stimuli, neutrophils are able to mobilize their granules and secrete their cargoes either to the extracellular milieu or into the phagosome, thus generating a hostile microenvironment to favour the killing of pathogenic microbes.

The subcellular compartments of mature human neutrophils have been classified in four types of mobilizable organelles: azurophilic granules, specific granules, gelatinase granules, and secretory vesicles (Borregaard et al., 1993; Borregaard and Cowland, 1997). In addition, multivesicular bodies (MVB, late endosomes) constitute independent neutrophil subcellular compartments that are able to undergo exocytosis as well as to fuse with the phagosome (Dahlgren et al., 1995). Neutrophil secretory organelles contain a battery of molecules that contribute to the precise implementation of all neutrophil functions. They are characterized by different protein content and variable tendency to undergo exocytosis (Borregaard and Cowland, 1997). A detailed list of neutrophil granular proteins can be found in previous works (for example, see Borregaard and Cowland, 1997; Lominadze et al., 2005; Rorvig et al., 2013).

Due to the central roles played by granular proteins in the modulation of neutrophil functions and the innate immune response, the regulation of vesicular trafficking is of fundamental importance in neutrophils (Catz, 2013). Thus, neutrophil secretory organelles are in constant movement in a process that is both energy consuming and tightly regulated. Through the mechanism of vesicular trafficking, neutrophils maintain vesicles in a dynamic state, thus increasing the likelihood of specific interactions...
with acceptor membranes, an essential process for the development of subsequent fusion events. Vesicular trafficking in eukaryotic cells including neutrophils is regulated by Rab GTPases and their specific effectors, which define the identity of subcellular membranes and organelles (extensively reviewed in Pfeffer, 2001; Seabra et al., 2002). Here, we focus on the roles of the small GTPase Rab27a and its effectors Munc13-4 and JFC1 (Slp1) in the regulation of vesicular trafficking and specific associated functions in neutrophils (Fig. 1). The topic of the molecular mechanisms regulated by Munc13-4 and JFC1 has been discussed extensively in previous works (Brzezinska et al., 2008; Johnson et al., 2010b; 2012; Catz, 2013) and will be visited here briefly for some of the neutrophil functions.

Rab27a deficiency and human immunodeficiency

Patients with Rab27a deficiency [Griscelli syndrome type 2 (GS2)] suffer hypopigmentation, hepatosplenomegaly, neutropenia, thrombocytopenia, and immunodeficiency with often fatal viral and bacterial infections (Harfi et al., 1992; Klein et al., 1994). They also develop haemophagocytic syndrome characterized by uncontrolled T-lymphocyte and macrophage activation. GS2 results in death, unless the child receives a successful bone marrow transplant. Defects in Rab27a-deficient haematopoietic cells are characterized by impaired CTLs (Menasche et al., 2000), NK cells (Trambas and Griffiths, 2003), and neutrophil function (Munafò et al., 2007), which are unable to secrete cargo proteins into infected cells or the extracellular milieu. Further analyses using human and murine neutrophils and animal models of Rab27a deficiency helped clarify the mechanisms of these defects. This article reviews the findings from these analyses.

Regulation of neutrophil exocytosis

There is a direct correlation between the function of neutrophil secretory proteins, the role they play in the innate immune response, and the responsiveness of the granules that contain these proteins to secretion stimuli. Thus, while secretory vesicles are mobilized in response to weak stimulation, tertiary, specific and azurophilic granules are mobilized in response to increasingly stronger stimuli (Borregaard and Cowland, 1997), with the highly toxic cargo of azurophilic granules being released at the infection foci where the concentration of the invading microorganism is usually higher. The hierarchy that characterizes the exocytosis of these granules correlates with the different roles of their secretory proteins in the processes of adhesion, migration, chemotaxis, phagocytosis, and production of ROS.

Fig. 1. Rab27a plays a central role in the regulation of multiple neutrophil functions. Several neutrophil functions that are regulated by the small GTPase Rab27a are indicated with arrows and legends, and the main citations are specified. Please refer to the text for other neutrophil functions that either are not regulated by Rab27a or are only regulated under selective conditions including phagocytosis and NET formation.
Rab27a regulates exocytosis of azurophilic granules in neutrophils

Azurophilic (primary) granules contain the most toxic secretory proteins of neutrophils including the pro-oxidative haemo-protein myeloperoxidase (Lehrer et al., 1969), the antimicrobial bactericidal/permeability-increasing protein defensins (Ganz, 2003), and the proteases cathepsin G and elastase (Malemud and Janoff, 1975). Azurophilic granule proteins are largely responsible for the neutrophil innate immune response to infections as deficiencies in these secretory factors have been associated with increased susceptibility to infections in humans and animal models (Lehrer et al., 1969).

A role for the small GTPase Rab27a in azurophilic granule exocytosis was first demonstrated by Munafò and colleagues (Munafò et al., 2007). In that work, the authors identified a subpopulation of exocytosable azurophilic granules, whose function is regulated by Rab27a. Only ∼20% of total azurophilic granules are able to engage in exocytosis, and endogenous Rab27a was detected in a similarly low percentage of azurophilic granules in these studies. Furthermore, downregulation of Rab27a was shown to impair azurophilic granule exocytosis, thus it is proposed that only the Rab27a-containing pool of azurophilic granules is exocytosable and that this subpopulation of granules coexists with a non-releasable pool of azurophilic granules lacking an associated secretory machinery (Munafò et al., 2007). The role of Rab27a in azurophilic granule secretion was further demonstrated in vivo using other mouse models of Rab27a-deficiency: Concrete (Munafò et al., 2007) and ashen (Johnson et al., 2010a). In these works, reduced levels of azurophilic granule markers were detected in plasma from Rab27a-KO mice upon endotoxin insult (Munafò et al., 2007; Johnson et al., 2010a). Furthermore, deficient azurophilic granule exocytosis was demonstrated using inhibitory anti-Rab27a antibodies and permeabilized human neutrophils in a model of stimuli-induced regulated secretion (Brzezinska et al., 2008). Further confirming an important role for Rab27a in the regulation of azurophilic granule exocytosis, it was recently shown that the expression of this small GTPase dramatically increases in promyelocytic cells upon myeloid cell differentiation (Munafò et al., 2007) and that downregulation of Rab27a in human promyelocytic cells impairs exocytosis of azurophilic granule cargoes (Munafò et al., 2007). In addition to Rab27a, neutrophils express the isoform Rab27b, which shares 72% homology with Rab27a at the amino acid level (Johnson et al., 2010a). Rab27a deficiency leads to Rab27b upregulation suggesting that the expression of Rab27 GTPases are linked at the transcriptional or translational levels (Johnson et al., 2010a). Rab27b deficiency neutrophils have a mild impairment of azurophilic granule exocytosis and similar to Rab27a-KO neutrophils, show decreased numbers of azurophilic granules in close proximity to the plasma membrane (Johnson et al., 2010a). However, Rab27b upregulation does not correct the defects in azurophilic granule secretion observed in Rab27a-deficiency, indicating that these GTPases regulate independent steps in neutrophil exocytosis (Johnson et al., 2010a).

In neutrophils, Rab27a function is co-ordinated by two specific effectors: synaptotagmin-like protein 1 (Slp1/JFC1) and Munc13-4 (Brzezinska et al., 2008). As expected, deficiency in either of these two trafficking adaptor molecules impairs azurophilic granule exocytosis (Brzezinska et al., 2008). However, their functions are not redundant as they regulate independent steps in the processes of granule trafficking, docking and fusion.

JFC1 is a Rab27a effector originally isolated from B lymphoblast-derived cDNA library (McAdara-Berkowitz et al., 2001; Strom et al., 2002). Endogenous JFC1 localizes at myeloperoxidase-positive granules in neutrophils (Munafò et al., 2007). In addition, JFC1 colocalizes with Rab27a on vesicles in close proximity to the plasma membrane in granulocytes (Brzezinska et al., 2008). True colocalization was further demonstrated by the similar temporal distribution of JFC1 and Rab27a molecules (Brzezinska et al., 2008) and direct interaction by co-immunoprecipitation analysis (Munafò et al., 2007). A role for Slp1/JFC1 in the regulation of azurophilic granule exocytosis was demonstrated in human neutrophils (Brzezinska et al., 2008), neutrophil-like HL-60 cells (Munafò et al., 2007; Brzezinska et al., 2008) and in JFC1-null murine primary neutrophils (Johnson et al., 2012). In this way, using inhibitory antibodies in permeabilized human neutrophils, and shRNA-mediated JFC1-downregulation in human granulocytic cells, Brzezinska and colleagues showed that interference with JFC1 function inhibits azurophilic granule exocytosis (Brzezinska et al., 2008) while other work showed impaired azurophilic granule exocytosis in JFC1-KO murine primary granulocytes (Sytl−/−) (Johnson et al., 2012). An understanding of the mechanism mediated by JFC1 is now developing. In JFC1-KO neutrophils, azurophilic granules are trapped in cortical actin and unable to reach the plasma membrane to undergo exocytosis (Johnson et al., 2012). In the same work, it was revealed that JFC1 is part of the granule machine necessary to dismantle polymerized actin to facilitate granule movement and exocytosis. The mechanism is mediated by the inhibition of the small GTPase RhoA by the GTPase-activating protein (GAP) GMIP (Johnson et al., 2012).

Munc13-4 was originally identified in goblet cells of the bronchial epithelium and alveolar type II cells (Koch et al., 2000) and later characterized as a Rab27a effector.
granule exocytosis (Brzezinska et al., 2008). A role for Rab27a in the exocytosis of specific granules is also supported by the observation that upregulation of the specific granule marker CD66b at the plasma membrane is impaired upon interference with Rab27a function (Herrero-Turrion et al., 2008). Studies performed with permeabilized human neutrophils and neutrophil-differentiated myeloid cells confirmed that the Rab27a effector Munc13-4 regulates gelatinase granule exocytosis (Brzezinska et al., 2008; Pivot-Pajot et al., 2008). In similar experiments, inhibition of JFC1 failed to prevent MMP-9 secretion (Brzezinska et al., 2008), suggesting that Rab27a regulates azurophilic and gelatinase granule exocytosis in a different fashion, further supporting the importance of the Rab27a effectors in the selective regulation of neutrophil granules.

### Rab27a regulation of adhesion molecule presentation and exocytosis of secretory vesicles in neutrophils

Neutrophils contain a population of exocytosable organelles called secretory vesicles characterized by the presence of adhesion molecules, receptors and endocytic cargo proteins (Uriarte et al., 2008). They are enriched in α4β1 integrins (CD11b/CD18) as well as in membrane receptors including complement receptor 1, the receptor for formylated peptides and the TLR complex molecule CD14 (Sengelov et al., 1994; Uriarte et al., 2008). Secretory vesicles are rapidly mobilized even in response to weak stimulation. This mechanism increases the number of adhesion molecules at the plasma membrane. Mobilization of secretory organelles that contain the integrin subunit CD11b, a molecule that plays an important role in neutrophil adhesion to the activated endothelium during the innate immune response, was not affected in Rab27a- or Munc13-4-KO neutrophils (Johnson et al., 2010a). Further analysis using Rab27b-KO and Rab27a/b-double KO neutrophils demonstrated that the Rab27 family of small GTPases is not necessary for CD11b upregulation (Johnson et al., 2010a). In fact, other groups suggested that CD11b upregulation at the plasma membrane increases in Rab27a-deficiency (Singh et al., 2012). Also, no differences in the plasma membrane expression of CD11b were observed in vivo, between wild-type, Munc13-4-KO or Rab27a-KO neutrophils either in basal conditions or in response to LPS treatment (Johnson et al., 2011). The expression of CD11a, an adhesion molecule that is constitutively present in the neutrophil plasma membrane, was also normally expressed in Rab27-null neutrophils from LPS-treated or untreated mice. Altogether, these data indicate that Rab27a and Munc13-4 do not regulate the exocytosis of the rapidly releasable secretory vesicles.

### A role for Rab27a in the mobilization of gelatinase and specific granules

Specific and gelatinase granules contain the membrane component of the NADPH oxidase, cytochrome b$_{558}$, composed by the subunits gp91$^{phox}$ and p22$^{phox}$. They also include modulators of the innate and inflammatory responses, such as gelatinase B (MMP-9) (Chakrabarti and Patel, 2005), and lactoferrin (Kjeldsen et al., 1994). Secretory vesicles also play an important role in the innate immune response by providing the β2-integrin family member macrophage antigen 1 (CD11b/CD18) (Sengelov et al., 1994; Uriarte et al., 2008), which are translocated to the plasma membrane upon neutrophil activation by pathogen-derived molecules.

Rab27a co-fractionates with a subpopulation of low density, exocytosable azurophilic granules as well as with gelatinase and specific granules (Munafo et al., 2007). Molecular interference with Rab27a function by means of specific inhibitory antibodies impairs MMP-9 secretion suggesting that Rab27a regulates gelatinase and specific granule exocytosis in addition to modulating azurophilic macrophage antigen 1 (CD11b/CD18)
which contain CD11b and that the constitutive plasma membrane expression of CD11a is also independent of Rab27a.

Neutrophil adhesion to activated surfaces through plasma glycoproteins including fibrinogen is an important mechanism of the innate immune response. Under static conditions, neutrophils bind to fibrinogen in a process mediated by $\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins but not $\beta_2$ integrins (Reinhardt et al., 1997). Rab27a-deficient neutrophils and Munc13-4-KO neutrophils are able to bind to fibrinogen and, in response to physiological stimulation, they increase their binding activity to the same extent as wild-type cells (Johnson et al., 2011). Altogether, these data suggested that the expression of $\beta_1$ integrins in neutrophils is Rab27a-independent.

The hyaluronan receptor CD44, which is constitutively expressed in the plasma membrane of neutrophils, plays an essential role in systemic inflammation by mediating neutrophil infiltration and retention at liver sinusoids (McDonald et al., 2008). Johnson and collaborators showed that the plasma membrane expression of the hyaluronan receptor CD44 is downregulated in Rab27a-KO neutrophils (Johnson et al., 2011). The plasma membrane expression of CD44 was not significantly upregulated by in vivo LPS treatment of either wild-type or Rab27-KO neutrophils (Johnson et al., 2011). As a consequence of low CD44 expression levels and impaired CD44 presentation, binding to hyaluronan, an essential process for neutrophil recruitment to the liver during endotoxemia (McDonald et al., 2008), is impaired in Rab27a-KO neutrophils (Johnson et al., 2011). These data highlight an important role for Rab27a in the constitutive expression of CD44 at the plasma membrane, suggesting that, in addition to exocytosis, Rab27a may control mechanisms that are independent of regulated secretion in neutrophils.

**Rab27a regulates the NADPH oxidase in neutrophils**

Reactive oxygen species (ROS) are at the core of neutrophil-mediated innate immunity. In neutrophils, ROS production relies on the NADPH oxidase, a multi-subunit enzymatic complex responsible for the monoelectronic reduction of oxygen to produce superoxide anion ($O_2^-$) (Babior, 1995). The importance of the oxidase in the neutrophil-mediated defense against microorganisms is highlighted in the genetic disease CGD (chronic granulomatous disease) (Babior, 1991). In this case, patients with CGD, whose NADPH oxidase is inactive, suffer recurrent bacterial and fungal infections. The NADPH oxidase consists of the cytosolic factors p47$^{phox}$, p67$^{phox}$ and p40$^{phox}$, the membrane-associated cytochrome b$_{558}$ (composed by p22$^{phox}$ and gp91$^{phox}$) and the accessory proteins Rac2 and Rap1a. Upon stimulation by pathogen-associated molecular patterns, the oxidase is activated in a process that involves the mobilization of the membrane-associated-cytochrome b$_{558}$ from tertiary and specific granules to the plasma membrane or the phagosome, depending on the nature of the soluble or particulate stimuli respectively (Babior, 1994; Li et al., 2009).

A role for Rab27a in the activation of plasma membrane-associated NADPH oxidase was first demonstrated by Johnson et al. (2010a) (Fig. 2). In that work, using Rab27a-KO neutrophils from ashen mice, the authors showed that Rab27a-deficiency impairs the kinetics of NADPH oxidase activation in response to the chemotactic peptide fMLP as measured by oxidation of the cell impermeant chemiluminescence reagent isoluminol (Johnson et al., 2010a). In the same study Rab27b was also found to be important for NADPH oxidase activation at the plasma membrane in response to soluble, physiological stimuli. In a different work, Rab27a-KO neutrophils were found to produce isoluminol-dependent ROS in response to opsonized bacteria (Anderson et al., 2010). Several mechanisms may explain these differences. First, the differences could be attributed to the dissimilar mechanisms regulating extracellular oxidative production by neutrophils in response to soluble versus particulate stimuli. For example, phagocytosis-induced exocytosis, a phenomenon that induces the polarized mobilization of azurophilic granules and non-polarized mobilization of specific granules to the plasmalemma upon particle ingestion (Tapper et al., 2002), could mediate plasma membrane upregulation of cyt b$_{558}$ and activation of the NADPH oxidase in a Rab27a-independent manner. In addition, co-activation of neutrophils by various pathogen-associated molecular patterns expressed in bacteria may trigger independent mechanisms of exocytosis thus bypassing Rab27a-dependent cyt b$_{558}$ mobilization. Furthermore, the interpretation of results from experiments involving the use of isoluminol to detect extracellular ROS when triggered by opsonized-bacteria should be taken with care. Thus, since phagocytosis stimulates localized pinocytosis in neutrophils (Botelho et al., 2002), it is likely that focal pinocytosis during particle engulfment induces isoluminol co-internalization and subsequent detection of intracellular ROS in addition to extracellular oxidants. Since phagocytosis of serum-opsonized bacteria by neutrophils is a Rab27a-independent mechanism (see Phagocytosis section below), it would be expected that Rab27a-KO neutrophils produce intracellular, isoluminol-detectable, ROS under phagocytosis conditions. Finally, diffusion of phagosomal H$_2$O$_2$ (Burton et al., 2014), may also contribute to the oxidation of isoluminol in cells challenged by opsonized-bacteria.

The intracellular oxidative response triggered by phagocytosis of opsonized-*Listeria monocytogenes* was
not impaired in the absence of Rab27a expression (Johnson et al., 2010a). Neither was the kinetics of the activation of the NADPH oxidase within the phagosome of Rab27a-KO neutrophils affected, as measured with dichlorodihydro-fluorescein immune-complex conjugate, which detects intraphagosomal ROS (Johnson et al., 2010a). These results indicated that, Rab27a is not involved in neutrophil cytochrome b558 trafficking to the phagosome in neutrophils. These data correlate with studies showing that Rab27a neutrophils are able to phagocyte serum-opsonized bacteria as efficiently as wild-type cells (Monfregola et al., 2012). Furthermore, Rab27a-KO neutrophils undergo phagosomal maturation with similar efficiency as wild-type cells, as determined by analysis of the distribution of endogenous granule markers at the phagosome (Monfregola et al., 2012). This also correlates with results from Anderson and colleagues showing that intracellular ROS production was not affected in Rab27a-deficient neutrophils upon phagocytosis of serum-opsonized Staphylococcus aureus (Anderson et al., 2010). However, in the same work, intracellular ROS production was found to be Rab27a-dependent when trigger by IgG-opsonized particles, which correlates with the observations that phagosomal maturation is Rab27a-independent unless purified IgG is used as opsonin instead of serum (see Catz, 2013, and Phagocytosis section below).

Rab27a does not regulate bacterial phagosomal maturation in neutrophils

Early studies showed that during phagocytosis of opsonized zymosan particles, neither Rab27a nor its effector JFC1/Slp1 are recruited to the phagosomal membrane, in conditions that show normal phagosomal maturation as demonstrated by the recruitment of LAMP proteins (Munafo et al., 2007). In addition, Rab27a-KO neutrophils showed normal phagosomal maturation with timely recruitment of azurophilic granule proteins to zymosan containing phagosomes (Munafo et al., 2007). Concordantly, opsonized zymosan, serum-opsonized live Pseudomonas aeruginosa and S. aureus triggered intraphagosomal, peroxidase-dependent, ROS production in Rab27a-deficient cells as efficiently as in...
wild-type cells (Anderson et al., 2010; Johnson et al., 2010a; Monfregola et al., 2012). This correlates with the observations that the absence of Rab27a does not affect phagosomal maturation during serum-opsonized bacterial phagocytosis (Monfregola et al., 2012), and that the delivery of p22\textsuperscript{fluc} or azurophilic granule proteins to the phagosomal membrane is not impaired in Rab27-deficient neutrophils (Monfregola et al., 2012). Other studies suggested that Rab27a plays an inhibitory rather than a positive regulatory role in phagocytosis by macrophages (Yokoyama et al., 2012). Thus, Munc13-4 regulates azurophilic granule secretion into the phagosomal compartment in neutrophils (Monfregola et al., 2012), highlighting Rab27a-independent functions for Munc13-4 during the innate immune response.

Rab27a and NETs formation

Elastase and MPO, two cargo proteins from azurophilic granules, are frequently found on NETs where they contribute to extracellular trap-dependent bacterial and fungal killing (Brinkmann et al., 2004; Papayannopoulos et al., 2010). However, the mechanisms regulating azurophilic cargoes localization on NETs remains unknown. Studies from my laboratory showed that neutrophils deficient in the secretory protein Rab27a efficiently produce neutrophil extracellular traps (Munafo et al., 2009). In addition, secretory proteins were found decorating NETs in Rab27a-KO neutrophils despite a dramatic defect in the exocytosis of azurophilic granule proteins in this model (Munafo et al., 2009). These data suggest that secretory proteins reach NETs by mechanisms that are independent of Rab27a. Conversely, a recent work using HL-60 cells showed that Rab27a downregulation prevents PMA-induced nuclear shape change and Sytox Green nuclear staining, interpreted as deficient NET formation (Kawakami et al., 2014). This may suggest that Rab27a is important for chromatin decondensation in HL-60 cells (Kawakami et al., 2014), although extracellular DNA fibers were not directly analysed. These data also differ from results obtained with Munc13-4-knockout neutrophils which form NETs decorated with azurophilic granule proteins (Monfregola et al., 2012) and efficiently trap live bacteria (Monfregola et al., 2012). Differences between neutrophils and HL-60 cells in NET formation have previously been reported, with HL-60 cells being identified as poor NET producers (Munafo et al., 2009; Papayannopoulos and Zychlinsky, 2009; Remijsen et al., 2011).

NETs production in response to P. aeruginosa was twofold higher in Munc13-4-KO than wild-type neutrophils (Monfregola et al., 2012). In addition, immunofluorescence analysis of Munc13-4-KO neutrophils showed endogenous azurophilic granule cargo proteins in puncta, in close proximity to trapped bacteria (Monfregola et al., 2012). However, differences in extracellular bacterial killing between wild-type and Munc13-4-deficient cells were observed in neutrophils treated with DNase I after the killing incubation period, suggesting that a small number of bacteria were trapped but not killed by Munc13-4-KO NETs (Monfregola et al., 2012). Altogether, although NETs are produced by both Rab27a- and Munc13-4-KO neutrophils, NET-dependent killing may be impaired in Munc13-4 deficiency.

Chemotaxis

Neutrophil chemotaxis is facilitated by protease release, a process that favours neutrophil detachment during migration (Colvin et al., 2010). Consistent with a role for Rab27a in the secretion of azurophilic granule proteases, Rab27a-KO cells showed a defective chemotactic phenotype, a process associated with deficient protease-mediated uropod detachment (Singh et al., 2012). Thus, using EGFP-Rab27a transgenic neutrophils, Singh and collaborators showed that Rab27a localizes at the uropod during chemotaxis and that treatment of wild-type neutrophils with protease inhibitors mimic the motility defects observed in Rab27a-KO cells (Singh et al., 2012). Neutrophil chemotactic defects where associated with a decreased in vivo response to MIP-2 and LTB4 and impaired in vitro chemotactic response to fMLP (Singh et al., 2012), further supporting a role for Rab27a in chemotaxis.
great importance. In a model of lipopolysaccharide-induced systemic inflammation, Rab27a-deficient mice were resistant to lipopolysaccharide (LPS)-induced death (Johnson et al., 2011). Interestingly, Rab27a-KO mice also showed decreased tumour necrosis factor alpha (TNF-α) plasma levels after LPS administration, which was suggested to be associated with a possible role of Rab27a in TNF-α secretion from immune cells (Johnson et al., 2011). Neutrophil infiltration into lung tissue is a central mechanism in the acute inflammatory response to endotoxemic challenge. However, sequestration of Rab27a-KO neutrophils in lungs after systemic LPS treatment was not different from that observed in wild-type mice (Johnson et al., 2011). Conversely, a role for Rab27a in neutrophil migration into lungs was suggested in a model of local MIP-2 administration, which was associated with a role of Rab27a in the secretion of proteases to facilitate neutrophil detachment and chemotaxis (Singh et al., 2012). Altogether these data indicate that systemic inflammatory processes may bypass the suggested role of Rab27a in MIP-2-dependent neutrophilic infiltration of the lungs in response to local challenge.

Rab27a-deficiency was associated with decreased neutrophil infiltration into the liver (Johnson et al., 2011), a process explained by the reduced expression of the hyaluronan receptor CD44 in neutrophils from Rab27a-KO mice (Johnson et al., 2011), which is necessary for liver invasion (Khan et al., 2004; McDonald et al., 2008). Liver sequestration of neutrophils during endotoxemia required Rab27a but not Munc13-4 (Johnson et al., 2011). Decreased liver infiltration correlated with reduced manifestation of neutropenia in endotoxemic Rab27a-KO mice (Johnson et al., 2011). Conversely, no differences in LPS-induced thrombocytopenia were observed between wild-type and Rab27a-KO mice, suggesting that the TLR4-initiated signalling pathway was not affected in Rab27a-deficiency (Johnson et al., 2011). Based on these data, Rab27a plays a central role in selective and specific mechanisms of tissue infiltration by neutrophils under conditions of systemic inflammation.

In agreement with an important role for Rab27a in neutrophil function during systemic inflammation, Yan and collaborators recently revealed an IL-6 and G-CSF-dependent, STAT3-mediated, mechanism of Rab27a downregulation in mice (Yan et al., 2013). In this model, Rab27a-downregulation correlated with decreased levels of azurophilic proteases secretion and reduced neutrophil cytotoxicity against tumours. In a different work, downregulation of Rab27a function (but not Rab27b) reduced exosome secretion in mammary carcinoma cells, which prevented systemic mobilization of a protumoural population of neutrophils (Bobrie et al., 2012), suggesting that Rab27a downregulation prevents neutrophil pro-inflammatory properties both by direct and by indirect mechanisms.

Due to the central role of Rab27a in azurophilic granule exocytosis, Rab27-KO mice showed very low plasma levels of neutrophil secretory proteins upon endotoxic challenge (Johnson et al., 2011). Importantly, MPO-KO mice showed increase survival in a model of *Escherichia coli* induced sepsis (Brockvych et al., 2008) suggesting that low plasma concentrations of neutrophil secretory proteins is beneficial for the outcome of systemic inflammation during endotoxemia and sepsis. Similarly, mice deficient for Rab27a are protected from LPS-induced death (Johnson et al., 2011). Decreased plasma levels of myeloperoxidase and a correlation with increased survival to LPS-induced systemic inflammation was also observed in Munc13-4-deficient mice (Johnson et al., 2011), supporting the idea that interference with these secretory proteins is potentially beneficial in the context of sepsis.

Conclusions

The small GTPase Rab27a has emerged as a central regulator of several neutrophil functions. Its ability to control steps in vesicular trafficking through interactions with specific effectors determines the dynamics of the activation of several mechanisms that are essential for the process of neutrophil-mediated inflammation, highlighting Rab27a as a target for novel therapeutic approaches for the treatment of systemic inflammation.

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