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Neutrophil Apoptosis: A Target for Enhancing the Resolution of Inflammation

János G. Filep* and Driss El Kebir

Research Center, Maisonneuve-Rosemont Hospital, Department of Pathology and Cell Biology, University of Montréal, Montréal, Québec, Canada

ABSTRACT

Neutrophils are essential for host defense and their programmed cell death and removal are critical for the optimal expression as well as for efficient resolution of inflammation. Delayed neutrophil apoptosis or impaired clearance of apoptotic neutrophils by macrophages contributes to the progression of chronic inflammation. Under most conditions, neutrophils are exposed to multiple factors and their fate would ultimately depend on the balance between pro-survival and pro-apoptotic signals. Life or death decisions are tightly controlled by a complex network of intracellular signaling pathways. Accumulating data indicate that receptors, such as the formyl peptide receptor 2/lipoxin receptor or β_2-integrins can generate contrasting cues in neutrophils in a ligand-specific manner and suggest a hierarchy among these signals. In this article, we review recent advances on how pro-apoptosis and pro-survival signals interact to determine the fate of neutrophils and the inflammatory response, and highlight novel pharmacological strategies that could be used to enhance the resolution of inflammation by redirecting neutrophils to apoptosis. J. Cell. Biochem. 108: 1039–1046, 2009.

KEY WORDS: NEUTROPHILS; APOPTOSIS; β_2 INTEGRINS; FPR2/ALX RECEPTOR; ASPIRIN-TRIGGERED LIPOXINS; RESOLUTION OF INFLAMMATION

Neutrophils play a central role in innate immunity and are rapidly recruited to sites of infection and injury. However, their many defense mechanisms that destroy invading microorganisms are potentially deleterious to tissues [Nathan, 2006]. Excessive or dysregulated neutrophil responses together with inadequate repair contribute to persisting tissue damage that underlies many chronic inflammatory diseases. A central paradigm of inflammation has been that efficient resolution of inflammation depends on rapid clearance of neutrophils and other inflammatory cells [Savill et al., 2002; Gilroy et al., 2004]. During spontaneous resolution of inflammation, neutrophils undergo apoptosis that renders them unresponsive to inflammatory stimuli and allows recognition and removal by scavenger macrophages [Savill et al., 1989, 2002]. The fate of neutrophils is profoundly influenced by signals from the inflammatory microenvironment by both pro- and anti-apoptotic signals. While enhanced neutrophil survival is essential to fight invading pathogens, inappropriate delay of neutrophil death within tissues may contribute to unwanted damage to the host [Gilroy et al., 2004]. Indeed, delayed neutrophil apoptosis has been detected in patients with inflammatory diseases, including acute respiratory distress syndrome [Matute-Bello et al., 1997], sepsis [Keel et al., 1997], and acute coronary artery disease [Garlichs et al., 2004]. Thus, effective removal of neutrophils concomitant with removal of inflammatory stimuli and activation of pro-resolution mechanisms are critical for minimizing tissue damage and facilitating restoration of normal function [Gilroy et al., 2004; Serhan et al., 2007, 2008]. In this review, we discuss recent findings how pro- and anti-apoptosis signals may interact to regulate the life span of neutrophils and the inflammatory response, and highlight novel pharmacological strategies that could be used to facilitate the resolution of inflammation by redirecting neutrophils to apoptosis.

THE NEUTROPHIL LIFE CYCLE

Neutrophils are the most abundant leukocytes in human blood. Mature neutrophils are terminally differentiated that have the shortest life span (8–20 h) among leukocytes in the circulation and in tissues (1–4 days). Blood neutrophils die via apoptosis even in the absence of any extracellular stimuli. Constitutive neutrophil apoptosis is an essential mechanism for regulating neutrophil homeostasis. It is believed that, once they have discharged their function, extravasated neutrophils within inflamed tissues preferentially undergo apoptosis. This process would prevent release of

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*Correspondence to: Dr. János G. Filep, Research Center, Maisonneuve-Rosemont Hospital, University of Montréal, 5415 Boulevard de l'Assomption, Montréal, QC, Canada H1T 2M4. E-mail: janos.g.filep@umontreal.ca
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cytotoxic products, such as reactive oxygen species (ROS) or proteases that contribute to tissue damage [Nathan, 2006]. Neutrophil apoptosis leads to downregulation of neutrophil functions and expression of "eat-me" signals such as phosphatidylserine residues, so that neutrophils can be recognized and cleared by scavenger macrophages [Savill et al., 1989]. When phagocytosis is impaired, apoptotic neutrophils would undergo secondary necrosis. Within the inflammatory locus, neutrophils can undergo direct necrosis and release of their cytotoxic products would aggravate tissue injury and amplify the inflammatory response. It should be noted that neutrophil granule constituents also play a role in the resolution of inflammation. For instance, cathepsin D through activation of caspase-8 was found to initiate neutrophil apoptosis during the resolution of inflammation [Conus et al., 2008]. Extravasated neutrophils may be removed by additional mechanisms, such as direct expulsion in sputum, feces or urine, or recirculation back to lymph nodes [Schwab et al., 2007]. Human neutrophils may also undergo autophagic-like cell death in response to autoantibodies. However, a role for autophagy in constitutive neutrophil death has been questioned [Luo and Loison, 2008]. ROS-mediated neutrophil death distinct from apoptosis and necrosis is an essential step for the generation of neutrophil extracellular traps that bind and kill invading microorganisms [Fuchs et al., 2007].

The balance between neutrophil survival and programmed cell death is exquisitely regulated by signals from other blood cells and the inflammatory microenvironment. Red blood cells and platelets suppress neutrophils apoptosis through yet undefined mechanisms [Luo and Loison, 2008]. Pro-inflammatory cytokines, bacterial constituents such as LPS, peptidoglycans and CpG DNA, and the acute-phase proteins modified C-reactive protein and serum amyloid A (SAA) can rescue neutrophils from apoptosis, whereas the presence of pro-apoptotic stimuli such TNF-α and Fas ligand shorten the life span of human neutrophils. Of note, the death rate of neutrophils from Fas (*lpr*) or Fas ligand (*gld*)-deficient mice does not differ from that of wild-type mice [Fecho and Cohen, 1998].

KEY SIGNALING PATHWAYS IN NEUTROPHIL APOPTOSIS

Neutrophil apoptosis is tightly regulated by a complex network of signaling pathways that control expression and degradation of the anti-apoptotic proteins Mcl-1 and A1, the pro-apoptotic proteins Bax and Bad, and activation of caspases. Apoptosis may result from activation of an extrinsic, intrinsic or endoplasmic reticulum stress pathway to cell death. The extrinsic pathway is mediated by ligation of the cell surface death receptors (e.g., TNF and Fas) with formation of the death-inducing signaling complex (DISC), involving adaptor proteins such as Fas-associated death domain (FADD) and cleavage of caspase-8 [Green, 2000]. The intrinsic pathway is mediated through ROS generation, leading to loss of mitochondrial transmembrane potential ($\Delta\Psi_m$), release of cytochrome *c* and apoptosis-inducing factor, assembly of the apoptosome and subsequent activation of caspase-3 [Green, 2000]. Members of the IAP (inhibitor of apoptosis protein) family inhibit both caspase-9 and caspase-3, thereby promoting cell survival [Luo and Loison, 2008]. However, IAPs are expressed at very low levels in human neutrophils and their role requires further clarification. While ROS are recognized as one of the causal mediators of neutrophil death, the mechanism of ROS generation in non-activated senescent neutrophils is not clear. Mature neutrophils contain a low number of mitochondria that may have a role restricted to apoptosis [Maianski et al., 2004]. Lowered threshold requirements for cytochrome *c* and/or elevated Apaf1 expression may compensate for the low levels of cytochrome *c* in neutrophils [Murphy et al., 2003]. Loss of $\Delta\Psi_m$ precedes development of apoptotic morphology. Other proteases, such as calpain, a non-caspase cysteine protease, might also contribute to programmed cell death by generating an active 18 kDa form of the pro-apoptotic factor Bcl-2-associated X protein (Bax) [Altznauer et al., 2004] and by deactivating the pro-survival molecule X-linked IAP (XIAP).

The most extensively studied mechanisms involved in mitochondrial dysfunction are the pathways mediated by the Bcl-2 family of proteins. Human neutrophils express the Bcl-2 homologs A1, Mcl-1, and Bcl-xL. A1 and Mcl-1 appear to be important in maintaining cytokine-regulated survival [Hamasaki et al., 1998; Dzhagalov et al., 2007]. Mcl-1 levels correlates closely with neutrophil survival kinetics [Moulding et al., 1998]. Neutrophils also express the pro-apoptotic Bax, Bak (Bcl-2 homologous antagonist/killer), Bid (BH3-interacting domain death agonist) and Bad (Bcl-2-associated death promoter), which show stable expression over time and are essential components of the apoptotic machinery. Pro-apoptotic Bcl-2 homologs form heterodimers with Mcl-1 or A1, thereby sequestering Mcl-1 and A1 and preventing expression of their anti-apoptotic actions. Phosphorylation of Bad and Bax results in dissociation of the heterodimer, allowing expression of the anti-apoptotic actions of Mcl-1 or A1. Bid and Bax translocates to the mitochondrion and form cytochrome *c* permeant channels [Baines et al., 2007].

There is considerable evidence of the involvement of multiple kinase pathways in moderating the fate of neutrophils. Activation of the MAPK/ERK and phosphoinositide-3-kinase (PI3K) pathways by pro-inflammatory mediators generates survival signals that inhibit the mitochondrial pathway of apoptosis, though these pathways are not essential for constitutive apoptosis [Luo and Loison, 2008]. PI3K generates PtdIns(3,4,5)P_3, which activates Akt and influences NF-κB and cAMP-response-element-binding protein (CREB); these produce anti-apoptotic signals. ERK 1/2 and Akt phosphorylate Bad and Bax, leading to dissociation of phosphorylated Bad and Bax from Mcl-1 [El Kebir et al., 2007]. Akt-mediated downregulation Transient activation of Akt without ERK activation may not be sufficient to delay apoptosis.

The role of the p38 MAPK in the regulation of neutrophil apoptosis has been a matter of controversy, for both pro-apoptosis [Khreiss et al., 2002] and pro-survival [Alvarado-Kristensson et al., 2003] have been reported. Putative pro-survival function of p38 MAPK includes phosphorylation, and therefore, inactivation of caspase-3 and caspase-8 [Alvarado-Kristensson et al., 2003]. On the other hand, constitutive neutrophil apoptosis has been reported to be associated with phosphorylation of p38 MAPK [Khreiss et al., 2002; El Kebir et al., 2007] and p38MAPK could generate a death signal through the reduction of Mcl-1, as observed in sodium salicylate-treated neutrophils. Caspase-3-mediated cleavage

and activation of protein kinase C δ has been identified as one essential effector of constitutive neutrophil apoptosis [Pongracz et al., 1999].

NF-κB-mediated transcription of survival proteins represents another signaling pathway that promotes neutrophil survival. This notion is based on the observations that several pharmacological inhibitors of NF-κB and the fungal metabolite gliotoxin induce neutrophil apoptosis in vitro and can overcome the neutrophil survival effect conferred by LPS or TNF-α [Hallett et al., 2008]. However, evaluation of the impact of NF-κB inhibitors on neutrophils is complicated by the observations that release of survival factors from contaminating monocytes contribute to LPS-mediated neutrophil survival and that TNF-α could exert both anti- and pro-apoptotic actions in a concentration-dependent fashion. As NF-κB has also been implicated in the resolution of inflammation [Lawrence et al., 2001], timing of treatment with NF-κB inhibitors might be critical to avoid interfering with the successful resolution of inflammation in vivo.

The complex network of signaling pathways regulating neutrophil survival and death represents a number of potential targets for pharmacological intervention. Since under most conditions, neutrophils will be exposed to multiple mediators, the fate of neutrophils would ultimately depend on the balance between the pro- and anti-apoptotic cues. Recent evidence indicates that a hierarchy may exist among these signals and suggest that such hierarchy can be exploited to drive the resolution of inflammation by promoting neutrophil apoptosis.

LIGAND-SPECIFIC MODULATION OF NEUTROPHIL APOPTOSIS THROUGH THE FPR2/ALX RECEPTOR

The formyl peptide receptor 2/lipoxin receptor (FPR2/ALX, formerly named as formyl peptide receptor-like 1) is a pleiotropic G-protein-coupled receptor that in addition to the anti-inflammatory lipids lipoxin A_4 (LXA_4) and aspirin-triggered 15-epi-LXA_4 also binds structurally unrelated peptide/protein ligands in vitro, including SAA, glucocorticoid-induced annexin 1 and amyloid β ($A\beta_{42}$) [Ye et al., 2009]. Accumulating evidence indicates that activation of FPR2/ALX may result in potent pro-, or anti-inflammatory responses in a ligand-specific fashion (Fig. 1).

SAA is an acute-phase protein the serum concentrations of which can increase as much as 1,000-fold within 24 h in response to infection or tissue damage. Elevated plasma levels of SAA have been noted in a wide range of pathological conditions and portend a poor prognosis in rheumatoid arthritis [Cunnane et al., 2000] and unstable angina [Johnson et al., 2004]. SAA is also present in atherosclerotic plaques and rheumatoid arthritis synovial tissues, suggesting a potential role for SAA in chronic inflammation.

The precise role of SAA as a modulator of inflammation remains elusive, as it possesses both beneficial and harmful actions. For instance, SAA opsonizes Gram-negative bacteria and enhances phagocytosis, but it is also precursor of amyloid A, the deposit of which causes amyloidosis. SAA, acting through FPR2/ALX, is chemotactic to neutrophils and monocytes, promotes neutrophil adherence to the endothelium and stimulates production of

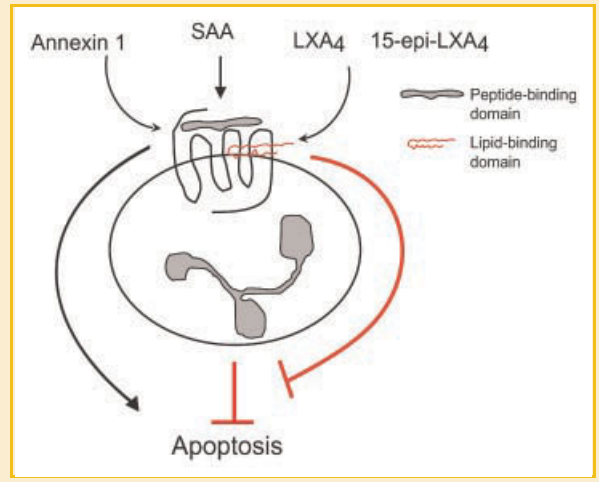

Fig. 1. Regulation of neutrophil apoptosis via the formyl peptide receptor 2/lipoxin A_4 receptor (FPR2/ALX). FPR2/ALX recognizes a variety of ligands, which induce opposing biological responses. Serum amyloid A (SAA) generates survival signals and suppresses the constitutive death program. By contrast, the glucocorticoid-regulated protein annexin 1 accelerates apoptosis. While the anti-inflammatory lipids, LXA_4 and aspirin-triggered 15-epi-LXA_4 by themselves have no effect on neutrophil apoptosis/survival, they effectively override the anti-apoptosis signal from SAA and redirect neutrophils to apoptosis, thereby shortening their life span. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cytokines [Chiang et al., 2006; Ye et al., 2009]. More recent studies have identified additional receptors for SAA, including the class B type I scavenger receptor CLA-1, receptor for advanced glycation end product (RAGE) and Toll-like receptor 2 that are involved in regulation of cholesterol metabolism and production of selected cytokines, including G-CSF [Ye et al., 2009]. SAA at clinically relevant concentrations delays constitutive apoptosis [El Kebir et al., 2007] and suppresses anti-Fas antibody-induced neutrophil apoptosis [Christenson et al., 2008], suggesting that even modest increases in serum SAA over baseline level are sufficient to signal pro-survival cues for neutrophils. SAA rescues neutrophils from constitutive apoptosis in ERK-, and PI3K-mediated prevention of mitochondrial dysfunction and repression of caspase-3 activity [El Kebir et al., 2007]. These actions can be blocked with the FPR antagonist N-t-Boc-Phe-Leu-Phe-Leu-Phe (Boc2), indicating involvement of the FPR2/ALX receptor [Ye et al., 2009]. The nucleotide receptor P2X7 has also been implicated in mediating SAA suppression of neutrophil apoptosis [Christenson et al., 2008]. However, involvement of this receptor is uncertain because human neutrophils express P2X7 at a very low level and oxidized ATP, which was used to block the SAA action, effectively dampens inflammation in P2X7-deficient mice.

LXA_4 and 15-epi-LXA_4 are typically generated by transcellular biosynthesis. In particular, inhibition of cyclooxygenase-2 by aspirin or atorvastatin results in conversion of arachidonate to 15R-HETE that can be converted by neutrophils and other cells to 15-epi-LXA_4 (also denoted ATL or aspirin-triggered LXA_4) [Serhan et al., 2008]. LXA_4 and 15-epi-LXA_4 are increasingly recognized for their potent dual anti-inflammatory and pro-resolution actions. Lipoxins

stimulate activation and recruitment of monocytes/macrophages and inhibit neutrophil activation, chemotaxis, adhesion, transmigration, and tissue accumulation [Serhan et al., 2007, 2008]. The observation that accumulation of PGE_2 induces a switch in lipid mediator synthesis from a predominantly 5-lipoxygenase activity to a 15-lipoxygenase activity generating LXA_4 parallel with the resolution of inflammation [Levy et al., 2001] led to the suggestion that initiation of an inflammatory response also signals to activate subsequent pro-resolution mechanisms [Serhan et al., 2008]. While neither LXA_4 nor 15-epi-LXA_4 per se interferes with the neutrophil apoptotic machinery, they facilitate non-phlogistic phagocytosis of apoptotic neutrophils [Godson et al., 2000] In vitro, 15-epi-LXA_4 overrides the apoptosis-delaying effect of SAA and redirects neutrophil to apoptosis, indicating a therapeutical potential for this lipid [El Kebir et al., 2007]. 15-epi-LXA_4 markedly attenuates SAA-induced ERK 1/2 and Akt activation, ultimately leading to collapse of mitochondrial function and activation of caspase-3 [El Kebir et al., 2007].

Unlike SAA, another FPR2/ALX ligand annexin 1, a glucocorticoid-regulated phospholipid-binding protein, was found to accelerate neutrophil apoptosis [Perretti and D'Acquisto, 2009]. This was unexpected in view of the anti-apoptotic action of glucocorticoids. However, extravasated neutrophils and macrophages express and release annexin 1 in a glucocorticoid-independent manner [Perretti and D'Acquisto, 2009]. Studies with chimeric annexin 1 proteins point towards a role for the N-terminal region of annexin 1 in promoting neutrophil apoptosis [Perretti and D'Acquisto, 2009] though contribution of another sequence (corresponding to residues 246–258, also known as antiflammin-2) in annexin 1, which inhibits neutrophil adhesion cannot be ruled out. Caspase-3-mediated externalization of annexin 1 promotes clustering of phosphatidylserine receptors, ensuring efficient engulfment of apoptotic cells [Perretti and D'Acquisto, 2009]. Thus, lipoxins and annexin 1 might have overlapping functions in promoting removal of apoptotic cells. Additionally, the protective actions of 15-epi-LXA_4 and annexin 1 (or the annexin 1 mimetic peptide Ac2-26) to limit neutrophil trafficking and to prevent neutrophil-mediated tissue injury are additive in the mouse models of mesenteric ischemia-reperfusion and myocardial infarction [Perretti et al., 2002]. These findings indicate interconnected actions as well as functional redundancies in endogenous anti-inflammation circuits during resolution of inflammation.

The molecular basis for how FPR2/ALX differently responds to various ligands remains enigmatic. 15-epi-LXA_4 stimulates limited phosphorylation of p38 MAPK, but this is insufficient to affect neutrophil apoptosis [El Kebir et al., 2007]. Direct inhibition of SAA binding to FPR2/ALX by 15-epi-LXA_4 is unlikely, because these ligands bind to distinct pockets on the receptor [Chiang et al., 2006]. Whether binding to such pockets leads to different conformational changes in the receptor remains to be investigated. It is possible that 15-epi-LXA_4 may generate a yet unidentified negative signal that blocks SAA-stimulated ERK and Akt activation. This hypothesis is supported by the observations that LXA_4/15-epi-LXA_4 attenuates peroxynitrite signaling in human neutrophils [József et al., 2002] probably via the regulation of presqualane diphosphate accumulation.

BETA-2 INTEGRINS MODULATE NEUTROPHIL APOPTOSIS AND OUTCOME OF INFLAMMATION

The β_2 integrin Mac-1 (CD11b/CD18) is best known for mediating neutrophil adhesion and trans-migration across the endothelium and for other adhesion-dependent neutrophil function, including phagocytosis of opsonized pathogens, binding to fibrinogen, immune complexes and platelets [Mayadas and Cullere, 2005]. Engagement of Mac-1 with its endothelial counter-receptor ICAM-1 during transmigration or Mac-1-mediated neutrophil adherence to fibrinogen signals survival cues for neutrophils [Watson et al., 1997]. The delay in apoptosis is attributed to PI3K/Akt and MAPK/ERK-mediated inhibition of the mitochondrial pathway of apoptosis. By contrast, ligation of Mac-1 in the presence of TNF or Fas ligand accelerates apoptosis [Mayadas and Cullere, 2005]. Pro-apoptotic stimuli and phagocytosis of opsonized bacteria promote NADPH oxidase-stimulated release of ROS, which leads to activation of SHIP (Src-homology 2 (SH2)-containing inositol 5-phosphatase) that hydrolyzes products of PI3K through the Src kinase Lyn. This signaling pathway would ultimately lead to decreased Akt activation. This purported mechanism might be particularly relevant in vivo under conditions of high neutrophil accumulation in tissues. ROS generated during phagocytosis activate caspase-8, a signature of receptor-mediated cell death. Caspase-8-mediated activation caspase-3 overcomes Mac-1 ligation-generated survival signals, eventually favoring cell death (Fig. 2).

Recent studies have identified myeloperoxidase (MPO), the most abundant granule enzyme in neutrophils, as a ligand for Mac-1 [Johansson et al., 1997; Lau et al., 2005]. Parallel increases in plasma MPO level and neutrophil surface expression of MPO were detected in patients with inflammatory diseases, including sepsis, ischemia-reperfusion and acute coronary syndromes compared with healthy controls [Klebanoff, 2005]. MPO catalyzes the formation of hypochlorous acid, a potent oxidant that has been implicated in killing bacteria and tissue destruction through induction of necrosis and apoptosis [Klebanoff, 2005]. MPO binding to Mac-1 induces neutrophil activation [Lau et al., 2005], signals to rescue neutrophils from constitutive apoptosis in vitro and prolongs neutrophil-mediated acute lung injury in mice [El Kebir et al., 2008]. These latter responses to MPO are independent of its catalytic activity.

MPO binding to Mac-1 evokes phosphorylation of p38 MAPK, ERK 1/2 and PI3-kinase and NF-κB activation [Lau et al., 2005; El Kebir et al., 2008]. Activation of p38 MAPK triggers superoxide formation by NADPH oxidase and induces transcription of genes involved in acute inflammatory responses that are primarily initiated by NF-κB. It is worth noting that MPO triggers release of elastase and MPO from the granules and upregulates surface expression of Mac-1 on neutrophils [Lau et al., 2005], implying an autocrine and paracrine mechanism for perpetuation of the inflammatory response (Fig. 3).

MPO delays constitutive apoptosis in human neutrophils through simultaneous activation of ERK 1/2 and Akt, leading to accumulation of Mcl-1 and suppression of the mitochondrial pathway of apoptosis [El Kebir et al., 2008]. Acute increases in plasma MPO to clinically relevant levels results in prolongation of the life span of

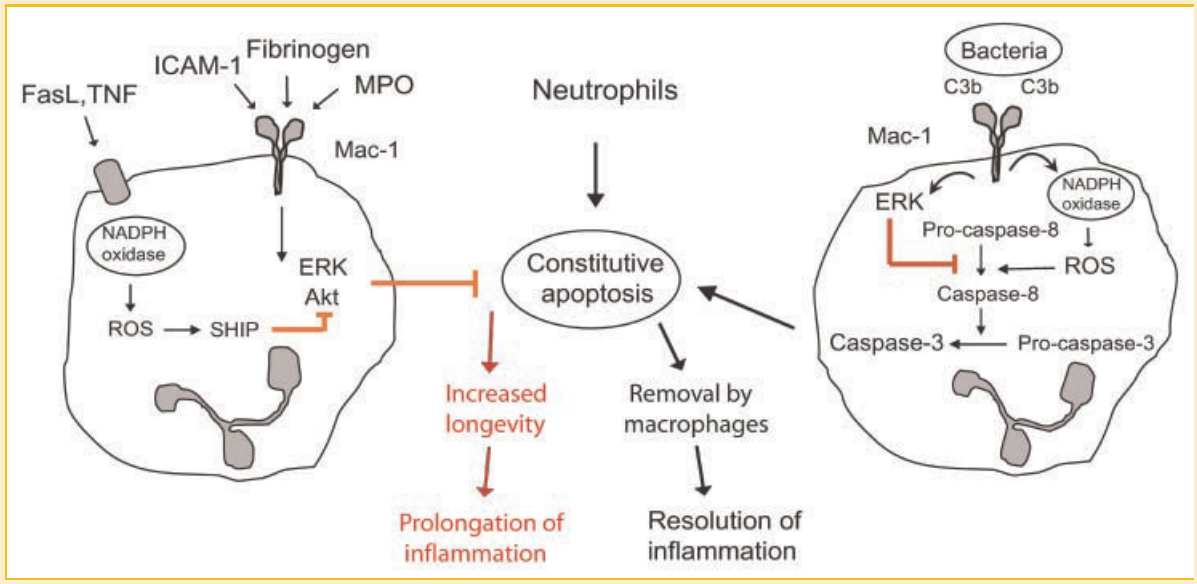

Fig. 2. Ligand-specific outside-in signaling through β_2 integrins generates opposing cues for survival in neutrophils. Clustering or ligation of the β2-integrin Mac-1 (CD11b/CD18) by ICAM-1, fibrinogen or myeloperoxidase (MPO) delays constitutive apoptosis through activation of the survival proteins Akt and ERK. Of note, the MPO action is independent of its catalytic activity. Simultaneous exposure of neutrophils to Fas ligand or TNF-α shifts the balance towards cell death. Fas ligand or TNF-α triggers ROS generation, which through activation of Lyn and SHIP leads to Akt inhibition and induction of apoptosis. Mac-1-dependent phagocytosis of complement C3b-opsonized bacteria leads to ROS generation and subsequent activation of caspase-8 and caspase-3. This overrides the ERK-mediated survival signal and results in apoptosis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

rat neutrophils through suppression of apoptosis as assayed ex vivo [El Kebir et al., 2008]. MPO also suppresses apoptosis in extravasated neutrophils in a mouse model of spontaneously resolving, carrageenan-induced pulmonary inflammation, resulting in persisting lung injury [El Kebir et al., 2008]. Interestingly, MPO-deficient mice exhibit lower pulmonary bacterial colonization, reduced lung injury, and greater survival following injection of live *E. coli* [Brovkovych et al., 2008] and show reduced renal neutrophil accumulation and tissue injury during ischemia/reperfusion [Matthijsen et al., 2007]. Absence of MPO-derived oxidant production during *E. coli* septicemia is consistent with prevention of lung injury. It is not known whether MPO deficiency could affect the longevity of neutrophils and thus contribute to protection against lung injury.

Downregulation of Mac-1 expression and inhibition of neutrophil adhesion are important components of the anti-inflammatory activities of LXA_4 and 15-epi-LXA_4 [Serhan et al., 2008]. In addition, 15-epi-LXA_4 can also override the Mac-1-mediated "outside-in" survival signal, thus redirecting neutrophils to programmed cell death both in vitro and in vivo. In particular, 15-epi-LXA_4 administered at the peak of inflammation promoted resolution of both exogenous and endogenous MPO-mediated acute lung injury in mice [El Kebir et al., 2009]. Attenuation of MPO-generated survival signals and induction of loss of Mcl-1 expression are likely the critical events in redirecting neutrophils to apoptosis by 15-epi-LXA_4 (Fig. 3). Neutrophil mitochondria emerge as potential targets for attenuating acute lung injury. Thus, inhibition of mitochondrial complex I with metformin [Zmijewski et al., 2008] or induction of collapse of mitochondrial transmembrane potential with 15-epi-LXA_4 [El Kebir et al., 2009] enhances the resolution of lung inflammation. Consistently, inhibition of ERK or Bcl-xL promotes, whereas inhibition of Bax prevents resolution of carrageenan-induced pleurisy [Sawatzki et al., 2006]. 15-epi-LXA_4 promotes recruitment of monocytes/macrophages into the lung and facilitates phagocytosis of apoptotic neutrophils and other cells [El Kebir et al., 2009], consistent with tissue repair [Godson et al., 2000]. By attenuating upregulation of Mac-1 expression and MPO release, 15-epi-LXA_4 could interrupt the MPO-sustained autocrine/paracrine pro-inflammatory amplification loop [El Kebir et al., 2009]. In addition, 15-epi-LXA_4 also inhibits vascular permeability and release of the pro-inflammatory cytokines, further supporting its pro-resolution role. Interestingly, a single injection of 15-epi-LXA_4 was sufficient to accelerate resolution of inflammation, indicating that once 15-epi-LXA_4 overcame pro-survival signals, neutrophil apoptosis would progress even in the absence of 15-epi-LXA_4.

CDK INHIBITION ENHANCES RESOLUTION OF NEUTROPHIL-MEDIATED INFLAMMATION

Having completed the cell cycle, circulating neutrophils rest in G0 phase. Intriguingly, human neutrophils possess cyclin-dependent kinase (CDK) 1 and CDK2 [Rossi et al., 2006] that are generally thought to control exclusively the fate of proliferating cells. CDK1 and CDK2 protein levels remain stable throughout ageing, indicating that these kinases are not targeted for degradation during constitutive apoptosis [Rossi et al., 2006]. The same study

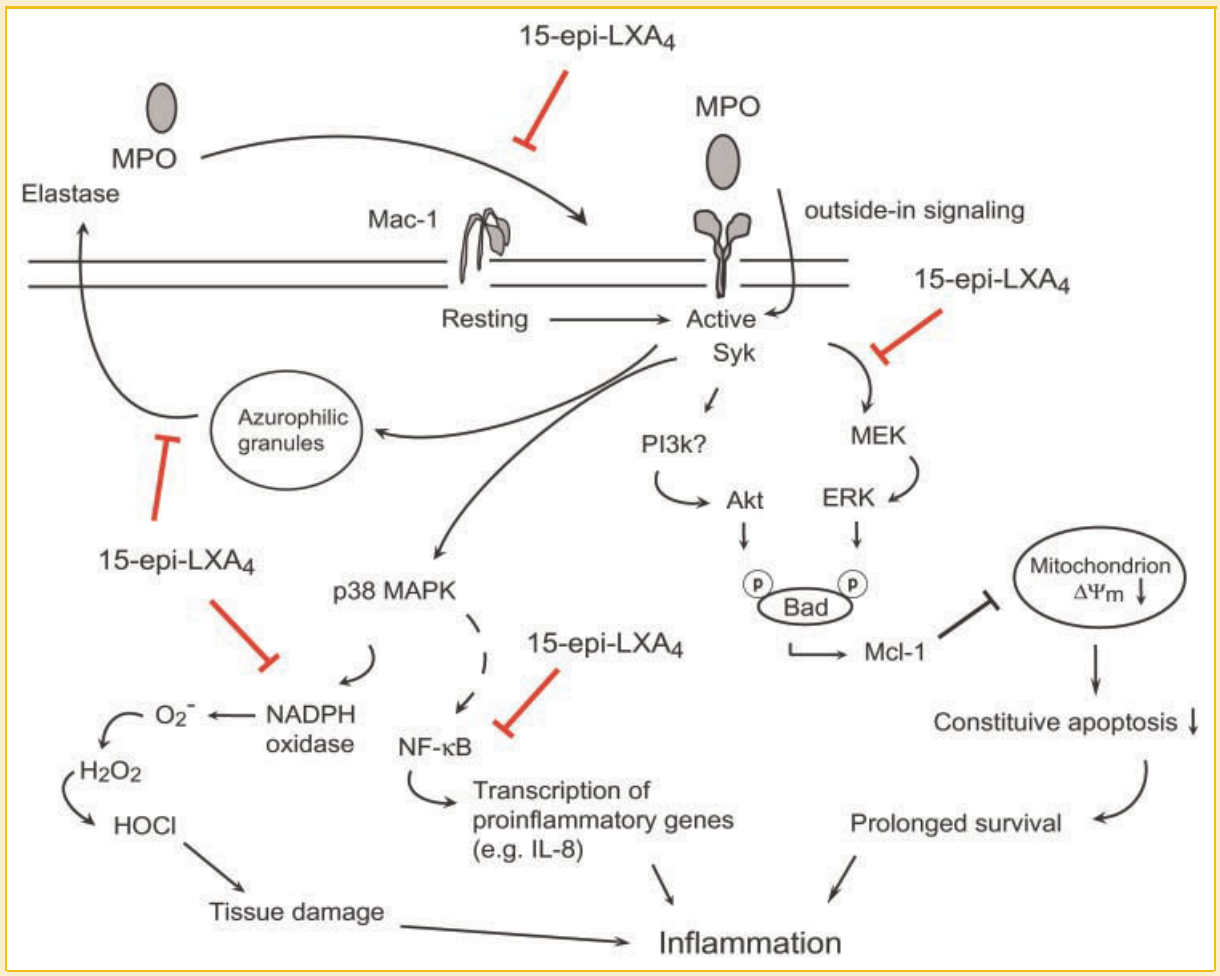

Fig. 3. Aspirin-triggered 15-1pi-LXA_4 inhibits myeloperoxidase (MPO) signaling through the β_2 integrin Mac-1 (CD11b/CD18) in human neutrophils. MPO binding to Mac-1 leads to upregulation of the surface expression of Mac-1, degranulation and activation of NADPH oxidase. H_2O_2 serves as a substrate for MPO to generate HOCl, which is predominantly responsible for tissue damage. MPO induces NF-κB-mediated transcription of pro-inflammatory genes, such as interleukin-8 (IL-8) and TNF-α, and signals through ERK and Akt to suppress constitutive neutrophil apoptosis. These events contribute to aggravation and prolongation of inflammation. 15-epi-LXA_4 could interfere with MPO-mediated pro-inflammatory circuits at multiple points. Thus, 15-epi-LXA_4 downregulates Mac-1 expression, attenuates MPO-evoked degranulation, activation of NADPH oxidase and NF-κB, and by overriding pro-survival cues redirects neutrophils to apoptosis. These actions contribute to promotion of resolution of established inflammation, as it has been observed in murine models. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

showed that the specific CDK inhibitors, R-roscovitine, NG75 and hymenialdisine induce caspase-dependent neutrophil apoptosis even in the presence of diverse pro-survival factors, such as dibutyryl-cAMP, GM-CSF, and LPS. R-roscovitine-induced rapid loss of Mcl-1 is a likely explanation for induction of apoptosis, even though the molecular mechanisms linking CDKs and Mcl-1 remains to be investigated. Administering R-roscovitine at the peak of inflammation was found to accelerate resolution of carrageenan-induced pleurisy, bleomycin-induced lung injury and serum-induced arthritis parallel with induction of neutrophil apoptosis [Rossi et al., 2006]. The same study also showed that CDK inhibition activates programmed cell death in monocytes and macrophages. It is unclear whether such dramatic reductions in macrophage number might hinder effective clearance of apoptotic neutrophils. Thus, the effects of CDK inhibitors on monocytes and macrophages contrast those of 15-epi-LXA_4 [Serhan et al., 2008; El Kebir et al., 2009]. Further elucidation of the mechanisms of action and development of selective inhibitors to different CDKs would help to address this issue.

CONCLUDING REMARKS

During the past years much progress has been made in the characterization of the molecular mechanisms governing neutrophil apoptosis and the removal of apoptotic neutrophils. Accumulating evidence indicates that neutrophil apoptosis is one of critical determinants of the outcome of the inflammatory response and is a potential target for therapeutic interventions. Thus, suppression of neutrophil apoptosis would lead to persisting inflammation, whereas induction of neutrophil apoptosis would enhance resolution of inflammation. Contrasting signaling through the FPR2/ALX and the β_2-integrin Mac-1 triggers important pathways that, among others, are involved in orchestration of neutrophil activation,

survival and death. Importantly, endogenously generated lipid mediators such as LXA_4 and aspirin-triggered 15-epi-LXA_4 as well as pharmacological agents such as CDK inhibitors can also promote neutrophil apoptosis in animal models parallel with enhanced resolution of acute inflammation. This is a fascinating field of research with a plethora of open questions. A better understanding of the molecular mechanisms governing neutrophil apoptosis would provide a rationale basis for developing novel therapeutic strategies for treatment of inflammatory diseases in humans.

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