

Commentary

Neutrophil 'connectivity': key to neutrophil-mediated tissue injury?

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Abstract

Neutrophils use cell surface molecules to communicate with their external environment. These molecules are markers reflecting neutrophil development, activation status and cell function. They are also critically important in controlling neutrophil behavior. Targeting these cell surface molecules is an attractive approach in the treatment of neutrophil-mediated conditions.

Keywords connectivity, inflammation, neutrophils, surface molecules, tissue injury

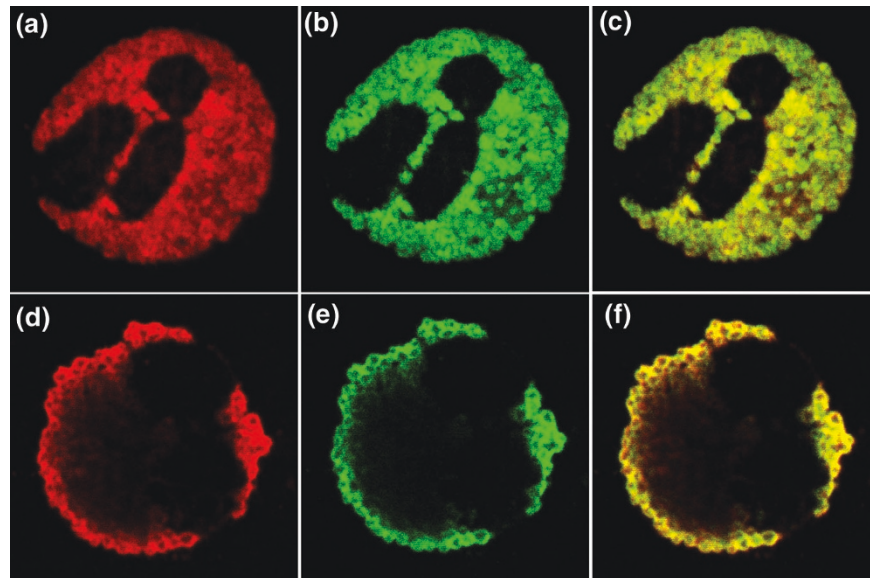
Neutrophils are professional phagocytic cells that provide the host with a first line of defense against acute bacterial and fungal diseases. They sense the focus of infection, they adhere to the endothelium of capillaries and venules adjacent to the inflammatory locus, they migrate through the vessel wall and the interstitial tissues to the infectious site and they phagocytose, kill and digest the invading microorganisms using a large number of proinflammatory mediators and proteolytic enzymes. During the inflammatory process, neutrophils produce factors to ensure their survival in the hostile inflammatory milieu, they recruit additional phagocytes, they inactivate their own toxic products and they induce their own death pathway to prevent damage to normal host tissue. Neutrophils may damage normal tissue, and a number of clinical conditions such as acute respiratory distress syndrome, septicemia with multiorgan failure, ischemia reperfusion injury and rheumatoid arthritis have all been linked to inappropriate neutrophil-mediated tissue damage.

In the present issue of *Critical Care*, Seely and colleagues delineate the important association of neutrophil cell membrane molecules with specific neutrophil function [1]. They argue that neutrophil membrane molecules mediate the processes integral to neutrophil delivery, function and clearance. These surface molecules connect neutrophils to their external environment (connectivity), and alterations in these molecules reflect changes in cell function and behavior

during every stage of the neutrophil's lifespan. Cell surface molecules alter with neutrophil proliferation, differentiation, maturation and the release of cells from the marrow into the circulation. Surface adhesion molecules and chemoattractant receptors are pivotal in determining tissue localization and recruitment of neutrophils, and when in the tissues, cell surface receptors are critical in cell activation and recognition of foreign pathogens. Surface molecules promote phagocytosis of pathogens and foreign material, initiate exocytosis of granules and participate in their final removal from the inflammatory site. Unraveling the relationship between neutrophil surface molecules and their behavior could impact on our understanding of the pathogenesis of neutrophil-mediated diseases and could spearhead potential therapeutic approaches [2].

Inflammation and stress accelerate neutrophil production, shorten their maturation time in the marrow and allow immature neutrophils to enter the circulation. This marrow response results in heterogeneity in the expression of surface receptors on circulating neutrophils such as the lipopolysaccharide receptor CD14, Toll-like receptors, CD16 and CD11/CD18 [2-4]. The CD11/CD18, Toll-like receptors and CD14 receptors have been implicated in the pathophysiology of septicemia and septic shock [5]. Interaction of lipopolysaccharide with these transmembrane receptors transduces intracellular activation signal through a signaling complex comprising heat-shock protein 70 and

Figure 1



Confocal laser scanning microscopy images of immunostained peripheral blood neutrophils of asthmatic subjects. Representative images of human neutrophils stained with Alexa-conjugated secondary antibody (red) to detect elastase, and BODIPY-FL-conjugated secondary antibody (green) to detect immunoreactivity against CD63. **(a)** Resting peripheral blood neutrophil labeled with Alexa indicating elastase immunoreactivity. **(b)** Resting peripheral blood neutrophil labeled with BODIPY-FL indicating CD63 immunoreactivity. **(c)** Combined image of CD63 and elastase immunostaining in resting cells. **(d)** IL-8-activated (50 ng/ml) peripheral blood neutrophil labeled with Alexa indicating elastase immunoreactivity. **(e)** IL-8-activated (50 ng/ml) peripheral blood neutrophil labeled with BODIPY-FL indicating CD63 immunoreactivity. **(f)** Combined image of CD63 and elastase immunostaining in IL-8-activated (50 ng/ml) peripheral blood neutrophil. Original magnification, 63×10 for all images.

heat-shock protein 90, chemokine receptor 4 and growth differentiation factor 5 [6].

Furthermore, we have shown that neutrophils newly released from the bone marrow express higher levels of L-selectin, a molecule that contributes to the recruitment of neutrophils to a site of inflammation [7]. L-selectin expression on neutrophils decreases as they age in the circulation with neutrophils in the circulation [8]. This unique L-selectin heterogeneity of expression determines which neutrophils participate in the inflammatory response and which neutrophils are destined to be removed from the circulating pool. Bone marrow stimulation induced by infection or smoking cigarettes causes a skip in cell division and a rapid transit of neutrophils through the mitotic stage. This leads to the production of neutrophils with higher granule numbers and greater destructive capabilities [9,10].

The primary granules in neutrophils are formed at an early stage (promyelocytic) in the mitotic pool in the marrow, and the number of granules is reduced by mitosis as the cells pass through the mitotic stage. These granules contain proteolytic enzymes such as myeloperoxidase, which is critical for neutrophil reactive oxygen radical production. Other granule proteolytic enzymes such as elastase, proteinase 3, cathepsin G and metalloproteinases are also formed early during the mitotic phase of development in the

marrow. We suspect that neutrophils released during marrow stimulation contain higher levels of these potentially destructive proteolytic enzymes that play a pivotal role in inappropriate neutrophil-mediated tissue injury associated with infection and sepsis.

Targeting these potentially damaging cells using surface molecules (such as L-selectin, CD11/CD18) may have therapeutic benefits to minimize tissue damage in conditions such as sepsis or with ischemia-reperfusion injury. Blocking antibodies against the β_2 -integrin prevented the ischemia-induced renal infiltration of granulocytes and reduced infarct size in experimental models, with human studies still controversial and ongoing [11,12]. Immune complex diseases such as glomerulonephritis, immune vasculitis, arthritis and systemic lupus [13] are similarly characterized by neutrophilic inflammation, and targeting surface molecules such as Fc γ receptors or surface myeloperoxidase may potentially provide novel therapeutic strategies in the treatment of autoantibody-triggered inflammation.

Recently discovered molecules such as lipid rafts and tetraspanins have generated renewed interest in the studies of the cell membrane. It is thought that signaling events taking place in immune cells including neutrophils occur in specialized membrane domains called lipid rafts. Lipid rafts function as platforms for the formation of multicomponent

transduction complexes and they may represent clinically relevant potential targets for immune regulation [14]. It has been shown in human neutrophils that localization of Fc γ RII to lipid rafts is important for the activation of Src family protein tyrosine kinases to initiate the tyrosine phosphorylation cascade leading to superoxide generation [14].

The tetraspanin superfamily also seems to be an important component of cell surface molecules. Although their precise function is not known, data from knockout mice suggest that they play a major role in membrane biology. One of the well-documented properties is their ability to facilitate the formation of multimolecular complexes (tetraspanin web) in which a number of molecules such as integrins are included [15]. Studies from our laboratory showed that the tetraspanin CD63 may be involved in neutrophil exocytosis (Fig. 1).

The study of neutrophil surface molecule functions and kinetics has advanced our understanding of the pivotal role of neutrophil 'connectivity' to their behavior and their ability to induce tissue damage through the release of damaging mediators. Future studies should focus on understanding the role of surface molecules in the multistep regulatory mechanisms in neutrophil behavior to limit the harm and tissue injury caused by neutrophil-derived mediators.

Competing interests

None declared.

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