Review

Purinergic regulation of neutrophil chemotaxis

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Abstract. Chemotaxis allows polymorphonuclear neutrophils (PMN) to rapidly reach infected and inflamed sites. However, excessive influx of PMN damages host tissues. Better knowledge of the mechanisms that control PMN chemotaxis may lead to improved treatments of inflammatory diseases. Recent findings suggest that ATP and adenosine are involved in PMN chemotaxis. Therefore, these purinergic signaling processes may be suitable targets for novel therapeutic approaches to ameliorate host tissue damage.

Keywords. Neutrophils, inflammation, chemotaxis, purinergic receptors, tissue damage.

Introduction

Up to 70% of all leukocytes in the peripheral blood of healthy adults are polymorphonuclear neutrophils (PMN). PMN play an important role in the innate immune defense. They are well equipped to detect the presence of invading micro-organisms, to accurately locate such invaders at sites of infection, and to rapidly migrate to these sites where PMN kill the invaders by unleashing a powerful set of defensive tools that include reactive oxygen species (ROS), proteolytic enzymes, and bactericidal mediators stored in intracellular granules of PMN or deposited into the extracellular space in DNA-containing structures that have been termed NETs [1–3]. PMN liberate these cytotoxic agents to incapacitate and destroy micro-organisms, but often host tissues are also compromised in the process. In fact, collateral damage to host tissues by excessively activated PMN is a hallmark of chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and asthma [4–6]. During acute inflammatory episodes caused by shock, ischemia, and reperfusion, PMN-induced tissue damage is responsible for severe clinical complications such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) - leading causes of death in trauma patients suffering post-traumatic shock [7–10]. It is unclear what triggers this indiscriminating and overzealous response of PMN. Therapeutic approaches to treat these inflammatory diseases may target a wide range of processes that are involved in the initial activation of PMN or their recruitment to inflamed tissues; other approaches focus on the neutralization of cytotoxic mediators like elastase that are released from activated PMN. Among these potential therapeutic targets, PMN recruitment to inflamed tissues is the most promising. Indeed, a number of drugs have been developed to prevent the sequestration of PMN into affected host tissues [11–15]. Of particular interest is PMN chemotaxis, as this is a key process involved in the recruitment of PMN to sites of inflammation. However, a more complete understanding of the mechanisms that control PMN chemotaxis is required to develop novel therapeutic strategies that effectively reduce chemotaxis and PMN-induced tissue damage.
Life cycle of PMN

PMN are formed by continuous differentiation of hematopoietic stem cells in the bone marrow through a process referred to as granulopoiesis. Myeloblasts differentiate into promyeloblasts, myelocytes, and then metamyelocytes as well as segmented band neutrophils that can be found in circulation during stress [16–19]. Metamyelocytes are the precursors of polymorphonuclear leukocytes, which are commonly referred to as granulocytes, including eosinophils, basophils, and neutrophils (PMN). The newly generated PMN are stored in the bone marrow, from where they can be rapidly released into the bloodstream when needed. This process is tightly regulated, allowing significant increases in granulopoiesis and in the circulating PMN population to facilitate host defense, when PMN turnover can increase ten-fold and PMN counts in peripheral blood can surge to levels several fold higher than under normal conditions. Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (G-CSF), interleukin (IL)-3, IL-23, and IL-17 are key mediators that regulate the production of PMN [20, 21]. In addition to these regulatory mediators, the mechanisms that control the migration of PMN within the bone marrow as well as their delivery to the bloodstream determine the final rate at which PMN are dispatched in response to inflammation or infection.

Extravasation

Upon their release from the bone marrow, PMN can circulate in the vasculature for several hours before they are finally recruited to inflamed tissues in order to pursue microbial invaders or to eliminate dead or dying cells. Numerous different chemokines and chemoattractant mediators that are produced by bacteria or by infected and inflamed tissues control the recruitment of PMN from the bloodstream into affected tissues. Adhesion molecules such as E-, L-, and P-selectins facilitate ever tighter interactions of circulating PMN with the endothelial cell layer lining the blood vessels closest to inflamed or infected host tissues [2, 11]. Initially, P-selectin is rapidly mobilized to the cell surface of the endothelial cell layer coating blood vessels of the microcirculation. Adhesion molecules on PMN such as P-selectin ligand-1 (PSGL-1) are concentrated on the tips of microvilli that protrude from circulating PMN, allowing the PMN to interact with P-selectin, which results in tethering of PMN to the endothelium. Together with the vasodilatation of capillaries in inflamed tissues, this process of tethering slows circulating PMN, resulting in the “rolling” of PMN along the endothelium of the microcirculation. In this microenvironment, PMN can become stimulated by mediators such as platelet-activating factor (PAF), leukotriene B4 (LTB4), and IL-8 that are generated by the inflamed endothelium [11, 22]. Upon binding of these mediators to receptors on the cell surface of PMN, the cells express β1-integrins (CD11/CD18 and LFA-1) stored in intracellular granules as well as a number of additional integrins such as αβ4-integrins [23–25]. With the help of these integrins, PMN bind more tightly to the endothelium by recognition of endothelial adhesion molecules such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecule-1 (VCAM-1) [23–25]. These PMN-endothelial interactions culminate in the firm adherence and spreading of PMN on the endothelium, which allows PMN to penetrate the endothelial cell layer in order to transmigrate into the extravascular space. Extravasation of PMN into the extravascular space is known to occur in a paracellular manner at endothelial cell junctions where molecules such as platelet endothelial adhesion molecule-1 (PECAM-1), expressed at these regions of endothelial cell layers, appear to play important roles in facilitating transmigration [26]. In addition to this paracellular transmigration process, transcellular migration of PMN across the endothelial cell layer can also occur. In the latter case, PMN seem to penetrate and travel through individual endothelial cells at non-junctional locations without impairing endothelial cell integrity [24, 25]. Once in the extravascular space, PMN migrate upstream in chemotactic gradient fields generated by chemotactic mediators that are released at inflamed sites (Fig. 1).

PMN chemotaxis

In contrast to random cell migration (chemokinesis), chemotaxis denotes the ability of cells to migrate in a directed fashion upstream in a chemotactic gradient field. This process requires coordinated and directional cytoskeletal rearrangements, mediated via F-actin polymerization, that produce pseudopods at the leading edge in the direction of the chemotactic gradient field [27]. In order to produce appropriate pseudopods that result in chemotaxis rather than merely in random membrane ruffling or in cell spreading, F-actin polymerization is restricted to the leading edge, while extension of the leading edge is synchronized with myosin responses that induce the retraction of cell membrane at the receding edge (uropod). This is accomplished through contractile forces generated by myosin motor proteins and the consequent interactions with actin filaments at...