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Review

Intraluminal crawling versus interstitial neutrophil migration during inflammation

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ABSTRACT

Site-directed trafficking of polymorphonuclear neutrophils (PMN) to their target regions within the tissue is an important prerequisite for efficient host defense during the acute inflammatory response. This process requires intraluminal crawling of PMN on the activated endothelial cells to their extravasation sites. Upon transendothelial diapedesis, PMN migrate in the interstitial tissue to sites of inflammation. These crucial steps within the recruitment cascade are defined as intraluminal crawling and interstitial migration. In this review, we will focus on the molecular mechanisms that control and fine-tune these migratory processes and discuss the role of adhesion molecules of the β_2 integrin (CD11/CD18) family for these cellular functions.

1. Introduction

Polymorphonuclear neutrophils (PMN) play an important role in innate immunity by protecting the host against invading microorganisms. During the acute inflammatory response, PMN are the first leukocytes to arrive at sites of inflammation in order to eliminate microbial pathogens or to remove cell debris by phagocytosis. In addition to immediate host defense, PMN are also involved in wound healing and repair. Growing evidence supports the concept that PMN also contribute to the control of the adaptive immune response by modulating, e.g. the function of B and T lymphocytes (Nathan, 2006; Pillay et al., 2012; Puga et al., 2012). Aside from acute immune responses, PMN are involved in the progression of chronic inflammatory diseases including autoimmune arthritis and atherosclerosis (Ionita et al., 2010; Nemeth and Mocsai, 2012; Soehnlein, 2012).

After generation and maturation in the bone marrow, PMN circulate in the blood until they are captured by activated endothelial cells of postcapillary venules in the inflamed tissue. Recent data provide evidence that PMN may live longer than 1–2 days, but their half-life in the circulation is thought to be relatively short spanning approximately 6–8 h (Pillay et al., 2010; Summers et al., 2010). Upon senescence, non-recruited PMN are removed from the circulation by, e.g. Kupffer cells in the liver or resident tissue macrophages in the spleen (Saverymuttu et al., 1985; Shi et al., 1996). More recent findings suggest that PMN reenter the bone marrow for clearance under homeostatic conditions (Furze and Rankin, 2008; Martin et al., 2003). In this review, we primarily focus on the mechanisms of PMN trafficking from the blood into the tissue during the acute inflammatory response. During this process, PMN leave the intravascular compartment where they are exposed to flow conditions and enter no-flow regions, e.g. the perivascular and the interstitial compartment. On their migratory route through completely different environments, these cells can rely on sophisticated migratory modes such as mechanotaxis during intraluminal crawling and chemotaxis during interstitial migration.

The signals guiding PMN from the blood into the inflamed tissue can be generated by resident macrophages, damaged tissue cells, bacteria and other sources. In the case of infection, microbe-derived moieties, so called pathogen-associated molecular patterns (PAMPs), are recognized by pattern recognition receptors (PRRs) like toll-like receptors (TLRs) and C-type lectin receptors (CLRs). The PRRs are expressed by, e.g. macrophages, dendritic cells and also by non-immune cells. Upon ligand recognition, these receptors induce the expression and secretion of proinflammatory cytokines such as IL-1β and TNFα resulting in the activation of endothelial cells lining the postcapillary venules (Takeuchi and Akira, 2010). Moreover, PRRs play an important role during sterile inflammation as they also recognize host-derived non-infectious material which is released upon cellular damage, e.g. as a result of trauma or chemically induced injury. Similar to PAMPs, these so called damage-associated molecular patterns (DAMPs) have the ability to

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activate pro-inflammatory pathways and may also lead to chronic inflammation or autoimmune diseases (McDonald et al., 2010), for detailed review see Chen and Nunez (2010) and references within.

The activation of endothelial cells by proinflammatory mediators represents the prerequisite for efficient PMN infiltration of the tissue. The recruitment process of PMN critically depends on the ability of these cells to crawl on the luminal site of the activated endothelial cells to junctional extravasation sites under shear stress forces exerted by blood flow. Upon extravasation, PMN sense the gradient of the chemoattractants and migrate to sites of lesion. Thus, the ability of PMN to migrate in different microenvironments including the vasculature and the interstitial space is indispensable for a successful innate immune response. The critical prerequisite for cell movement is the induction of shape change and polarization, both of which are controlled by internal and external signal transduction cascades leading to highly dynamic remodeling of the cytoskeleton. What are the molecular mechanisms that regulate directed migration of PMN namely intraluminal crawling and interstitial migration?

2. Intraluminal crawling in the context of PMN recruitment

Emerging imaging technologies still provide new insights into the complex dynamics of leukocyte motility within the body (Germain et al., 2012; Megens et al., 2011). In the current model, PMN trafficking to sites of lesion relies on a canonical recruitment cascade of consecutive cellular steps, namely selectin-mediated capturing and rolling of PMN on the inflamed vessel wall, followed by arrest and firm adhesion via adhesion molecules of the β_2 integrin (CD11/CD18) family. In addition to their adhesive functions, β_2 integrins serve as signaling receptors. Ligand binding of the β_2 integrins triggers outside–in signaling processes leading to cytoskeletal reorganization which is important for post-adhesion functions including, e.g. adhesion strengthening, spreading and intraluminal crawling (Abram and Lowell, 2009). For a complete overview of the recruitment cascade please refer to other reviews (Borregaard, 2010; Ley et al., 2007; Schymeinsky et al., 2007a; Simon and Green, 2005). Here, we will summarize the relevance of the dual function of the β_2 integrins namely to mediate adhesive interactions and to transmit signals into the cell for intraluminal crawling of PMN under flow conditions in response to inflammation (Fig. 1).

PMN express three different β_2 integrins namely LFA-1 (CD11a/CD18, $\alpha_L\beta_2$), Mac-1 (CD11b/CD18, $\alpha_M\beta_2$) and gp150/95 (CD11c/CD18) consisting of a common β subunit (CD18) and a non-covalently associated α subunit (CD11a, CD11b, CD11c).

The most important β_2 integrins for PMN trafficking are LFA-1 and Mac-1 (Luo et al., 2007). The physiological relevance of β_2 integrins for PMN recruitment is evident in patients with an inherited defect of the CD18 gene resulting in a lack of β_2 integrin expression. These patients suffer from leukocyte adhesion deficiency type I (LAD I) which is characterized by impaired PMN trafficking and activation that gives rise to recurrent bacterial infections (Anderson et al., 1985; Bowen et al., 1982). Similar to humans, PMN recruitment during inflammation is severely compromised in CD18 deficient mice (Walzog et al., 1999).

During the inflammatory process, the transition from initial capturing and rolling of PMN to firm arrest is mediated by the sequential induction of conformational changes of the β_2 integrins (Evans et al., 2009). Three distinct conformations have been identified of which the bent form with low ligand affinity is predominantly found in non-activated circulating PMN. During rolling, endothelial P- and E-selectin bind to P-selectin glycoprotein ligand-1 (PSGL-1) on the cell surface of PMN leading to a conformational shift towards an extended LFA-1 conformation via inside–out signaling (Chesnutt et al., 2006; Kuwano et al., 2010; Zarbock et al., 2007). This conformation allows LFA-1 binding to its ligand intracellular adhesion molecule-1 (ICAM-1) with intermediate affinity and enables slow rolling of PMN on the endothelium. During the transition from slow rolling to arrest, e.g. chemokine receptors such as CXCR2 on PMN interact with chemokines like CXCL1 presented by the endothelium resulting in further activation of inside–out signaling processes leading to a second conformational shift by partial dissociation of the α and β subunits of the extended LFA-1. This generates an open extended conformation of LFA-1 with high ligand affinity and thereby allows firm PMN arrest (Lefort and Ley, 2012; Moser et al., 2009; Nishida et al., 2006). In addition, changes in lateral surface motility and clustering of β_2 integrins further increase the avidity for the ligand (Carman and Springer, 2003). Recent studies by Sundd et al. led to the identification of slings, membrane tethers wrapped around PMN rolling at high shear stress (10 dyn/cm^2). Slings were found to be rich in LFA-1 that allows binding of the sling to ICAM-2 on the cell body of the same PMN (Sundd et al., 2012).

Endothelial ICAM-1 is critical for sustained adhesive interactions between PMN and the endothelium (Smith et al., 1988, 1989). Although LFA-1 and Mac-1 are both able to bind endothelial ICAM-1, each β_2 integrin plays a distinct role in the recruitment cascade. In a subcutaneous air pouch model, extravasation of CD11a or CD18 deficient PMN was severely decreased, whereas the number of CD11b deficient PMN accumulating in the air pouch was significantly increased (Ding et al., 1999). Similar results were obtained in the model of thiolglycollate-induced peritonitis indicating that LFA-1 plays the predominant role in PMN trafficking to sites of inflammation (Coxon et al., 1996). This is in line with the fact that LFA-1, but not Mac-1 serves as the main receptor of ICAM-1 mediating PMN arrest to activated endothelium (Smith et al., 1989). In the inflamed cremaster model, Phillipson et al. (2006) have demonstrated that PMN crawl perpendicular to or even against the blood flow within inflamed post-capillary venules upon the onset of firm adhesion. The authors concluded that directional crawling of PMN is necessary to find optimal emigration sites under flow conditions. New evidence suggests a role for an intravascular gradient of chemoattractants which are immobilized on endothelial heparin sulfate guiding the crawling PMN toward their transmigration sites in vivo (Massena et al., 2010). During intraluminal crawling, Mac-1, but not LFA-1 plays the predominant role. This could be demonstrated by normal adhesion of Mac-1 deficient PMN to inflamed venules of the cremaster muscle upon TNFα stimulation. However, in the absence of Mac-1, intraluminal crawling was severely compromised and led to delayed emigration probably because junctional extravasation sites could not be reached by PMN (Phillipson et al., 2006). Therefore, transcellular emigration routes were taken alternatively, which are non-optimal. In contrast, most of LFA-1 deficient PMN failed to adhere in inflamed venules but those that did were able to crawl (Phillipson et al., 2006). A recent study demonstrated that the overall ability of PMN to migrate under flow conditions depends on binding of Mac-1 to ICAM-1 (Hepper et al., 2012). However, the capability of PMN to crawl against the direction of flow seems to involve high affinity LFA-1 that allows calcium influx via Orai1 (Dixit et al., 2011). Thus, LFA-1-mediated slow leukocyte rolling and firm adhesion precedes Mac-1-dependent crawling under shear stress while LFA-1 signaling seems to promote the ability of PMN to migrate against the direction of flow.

ICAM-1 binding induces $\beta_2$integrin-mediated outside–in signaling in PMN (Hepper et al., 2012; Walzog et al., 1996). This signaling cascade proceeds via the phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) bearing transmembrane adapters (like DAP12 or the Fc receptor γ chain) by members of the Src tyrosine kinase family, leading to the