IRTG - Basic Principles Seminar / Advanced Methods Course (24./25.11.14)

Neutrophil trafficking under flow conditions

Polymorphonuclear neutrophils (PMN) play a central role in acute inflammation, host defense and repair. In order to fulfill their functions, PMN have to extravasate from the blood to sites of lesion following a well-known multistep recruitment cascade (1). Upon initial capturing, PMN roll along the vessel wall of postcapillary venules under hemodynamic shear forces. Both capturing and rolling are mediated by selectins whereas adhesion and postadhesion events are mainly mediated by integrins. Rolling allows sensing of pro-inflammatory signals presented on the luminal surface of the inflamed endothelium including chemokines like e.g. CXCL8. Upon ligand binding, G-protein coupled chemokine receptors (GPCRs) induce intracellular signaling events in PMN resulting in the activation of leukocyte adhesion molecules of the β2 integrin family (CD11/CD18) by a process called inside-out signaling. In the first step, this signal induces extension of β2 integrins with intermediate affinity allowing slow rolling of PMN. In the second step, further activation induces the open conformation of β2 integrins with high ligand binding affinity that is required for firm adhesion of PMN to the endothelial cells by binding to e.g. ICAM-1 (2,3). Upon ligand binding, β2 integrins transduce signals into the cell by outside-in signaling. This process is required for post-adhesion PMN functions namely adhesion strengthening, spreading and intraluminal crawling. All these steps depend on cytoskeletal reorganization and are critical for PMN to resist mechanical forces exerted by the blood flow thereby allowing PMN to reach optimal extravasation sites (3,4).

The aim of this basic principle seminar is to give the IRTG students a brief state-of-the-art overview on the molecular mechanisms of PMN recruitment during inflammation. Moreover, this seminar will prepare the students for the accompanying advanced methods course in which we will focus on the analysis of rolling, induction of firm adhesion, adhesion strengthening as well as intraluminal crawling of PMN under physiological shear stress conditions in vitro using flow chamber assays. Data analysis will be performed using Image J software plugin Manual Tracking and the Chemotaxis and Migration tool.

References:

(1) Intraluminal crawling versus interstitial neutrophil migration during inflammation. Pick R, Brechtefeld D, Walzog B. Mol Immunol. 2013 Aug;55(1):70-5.

(2) The cytokine midkine supports neutrophil trafficking during acute inflammation by promoting adhesion via β2 integrins (CD11/CD18). Weckbach LT, Gola A, Winkelmann M, Jakob SM, Groesser L, Borgolte J, Pogoda F, Pick R, Pruenster M, Müller-Höcker J, Deindl E, Sperandio M, Walzog B. Blood. 2014 Mar 20;123(12):1887-96.

(3) Hematopoietic progenitor kinase 1 (HPK1) is required for LFA-1-mediated neutrophil recruitment during the acute inflammatory response. Jakob SM, Pick R, Brechtefeld D, Nussbaum C, Kiefer F, Sperandio M, Walzog B. Blood. 2013 May 16;121(20):4184-94.

(4) The mammalian actin-binding protein 1 is critical for spreading and intraluminal crawling of neutrophils under flow conditions. Hepper I, Schymeinsky J, Weckbach LT, Jakob SM, Frommhold D, Sixt M, Laschinger M, Sperandio M, Walzog B. J Immunol. 2012 May 1;188(9):4590-601.