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Neutrophil adhesion and crawling dynamics on liver sinusoidal endothelial cells under shear flow

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ABSTRACT

Neutrophil (polymorphonuclear leukocyte, PMN) recruitment in the liver sinusoid takes place in almost all liver diseases and contributes to pathogen clearance or tissue damage. While PMN rolling unlikely appears in liver sinusoids and Mac-1 or CD44 is assumed to play respective roles during *in vivo* local or systematic inflammatory stimulation, the regulating mechanisms of PMN adhesion and crawling dynamics are still unclear from those *in vivo* studies. Here we developed a two-dimensional *in vitro* sinusoidal model with primary liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) to investigate TNF-α-induced PMN recruitment under shear flow. Our data demonstrated that LFA-1 dominates the static or shear resistant adhesion of PMNs while Mac-1 decelerates PMN crawling on LSEC monolayer. Any one of LFA-1, Mac-1, and CD44 molecules is not able to work effectively for mediating PMN transmigration across LSEC monolayer. The presence of KCs only affects the randomness of PMN crawling. These findings further the understandings of PMN recruitment under shear flow in liver sinusoids.

1. Introduction

Liver is the largest organ with the functions of biosynthesis, metabolism, digestion, detoxification, and immunity. Various infectious or noninfectious factors such as hepatic virus, bacteria, parasite, alcohol intake, ischemia-reperfusion (I/R) and trauma can cause liver inflammation and damage. Almost in all kinds of liver diseases, neutrophils (polymorphonuclear leukocytes, PMNs) serve as an essential cell type for innate immune responses and PMN infiltration into hepatic tissue is widely observed to posses its function as a double-edged sword [1,2]. That is, PMNs can clear those infectious pathogens and damage tissue debris [3–5], whereas inappropriate accumulation of PMNs in liver microcirculation leads to severe tissue injury during drug-induced acute liver inflammation [6–8], I/R damage [9,10], alcohol liver disease [11,12], and other diseases. Thus, characterizing PMN recruitment in liver during hepatitis and liver injury are critical for understanding molecular mechanisms and potential clinical therapy.

PMN recruitment in liver mostly takes place in the capillary-like sinusoids rather than post-capillary venules which are the main locations for leukocyte infiltration in other tissues [13–15]. The liver sinusoid is a specialized capillary network with narrow luminal diameter (7–15 μm), slow blood flow (0.1–1 dyn/cm^2) [16–18], and is lined with fenestrated selectin-deficient liver sinusoid endothelial cells (LSECs) and liver resident macrophage Kupffer cells (KCs) [19]. All these features make the sinusoid a unique passage for flowing PMNs and affect their recruitment. Unlike the classical recruitment cascade which is initialed by selectin-mediated tethering and rolling, no rolling of PMNs is found in the inflammatory liver sinusoids, suggesting that selectins play null roles in PMN recruitment into sinusoids [20]. Since the absence of these traditional fast-kinetics adhesion molecules, it is hypothesized that PMN arrest occurs physically upon confined space and slow flow in the sinusoids [14,21,22]. In classical inflammatory cascade, LFA-1 and Mac-1, two β2 integrins expressed on PMNs, mediate PMN adhesion and crawling which are correlated to their respective molecular structures and distinct binding affinities [23,24]. However, the role of these adhesive molecules in the liver-specific PMN recruitment is diverse and not known clearly. On one hand, a body of evidences indicates that PMN migration in septic liver is independent on traditional adhesive molecules such as selectins, β2 integrins, intercellular adhesion molecule 1 (ICAM-1) and α4 integrins [13,14,18,20,22,25,26]. On the other hand, distinct adhesive molecules

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have unraveled liver-specific PMN recruitment under different inflammatory conditions. For example, Mac-1 dominates PMN recruitment in liver with local myxoma virus infection or fMLF stimulation, while the role of CD44-hyaluronan (HA) interactions is emerged with systemic LPS stimulation since Mac-1 expression is down-regulated by KC-secreted IL-10 [18,25,27]. Therefore, it is crucial to elucidate the respective contributions of those adhesive molecules in PMN recruitment in liver sinusoids under blood flow.

After being arrested on endothelial cells, PMNs need to crawl on the vessel wall to find proper emigration sites, cross the endothelium into the tissue, and finally execute their immune response functions. Although intraluminal crawling is a prerequisite for PMN trans-endothelial migration, the molecular mechanisms of PMN crawling have not been extensively investigated in contrast to those for cell adhesion and emigration processes. Only a few works indicate that Mac-1 and its endothelial ligands ICAM-1 and ICAM-2 are the major adhesive molecules involved in intraluminal crawling [28–30]. Specifically in liver sinusoids, PMN crawling percentage and velocity are down-regulated in ICAM-$1^{-/-}$ or Mac-$1^{-/-}$ mice during local fMLF stimulation or focal hepatic necrosis [25,31]. Understanding molecular mechanisms of PMN intraluminal crawling in the sinusoids is specially meaningful due to its featured biomechanical microenvironment of confined space and slow flow, which has been little known.

Here we applied a two-dimensional (2D) live-cell flow chamber assay to decipher the multistep process of PMN recruitment on LSEC monolayer under physiological flow [32]. Shear-resistant cell adhesion was quantified and flow-induced cell crawling was analyzed for fMLF-activated PMNs on TNF-α-stimulated LSECs. Related molecular regulations of LFA-1, Mac-1, and CD44 were determined. The presence of KCs in PMN recruitment in liver sinusoids were also discussed.

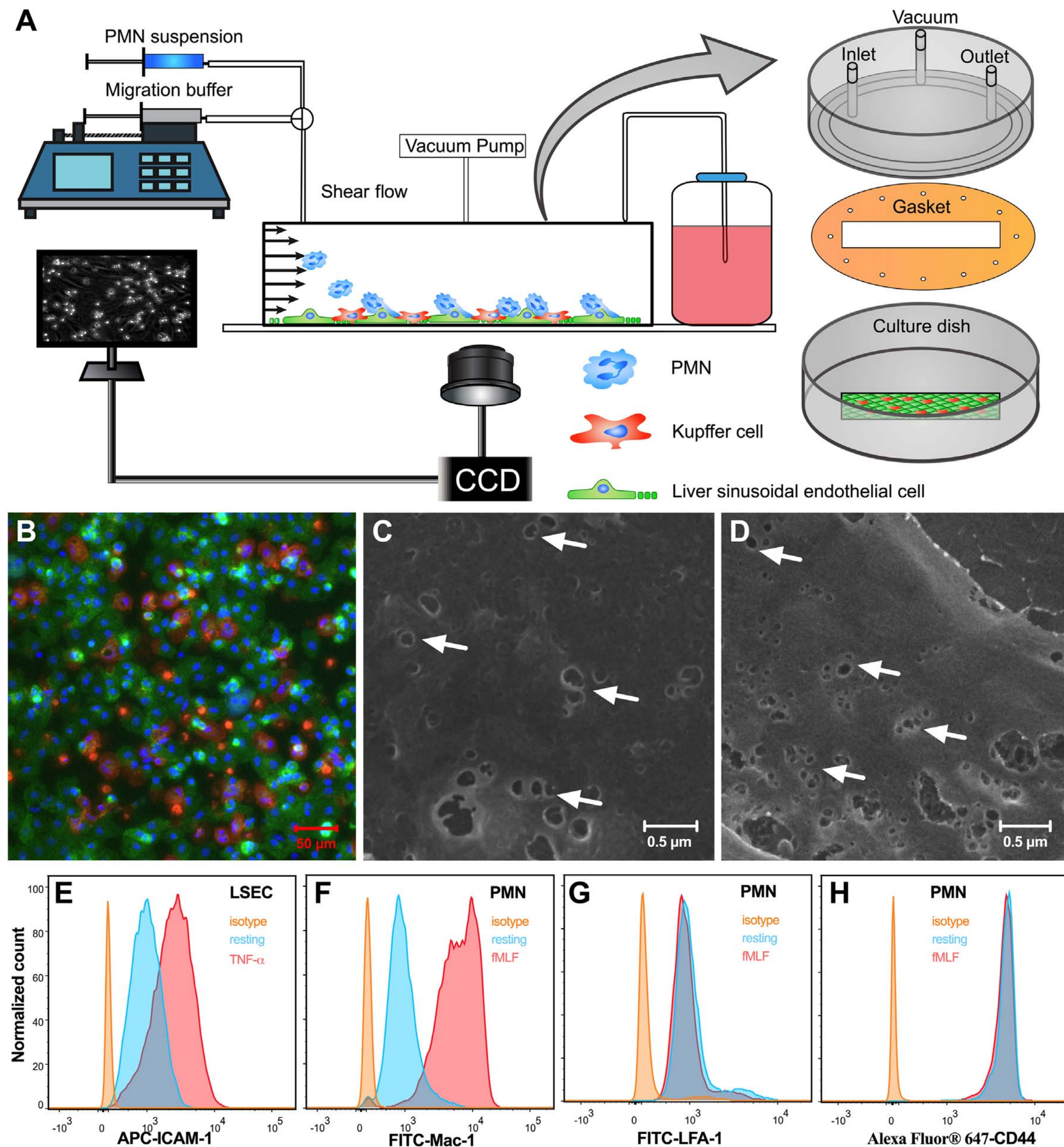

Fig. 1. Establishment of a 2D *in vitro* liver sinusoid model. (A) Schematic of the experimental system (not in scale). (B) Confocal images of murine LSECs (*green*; stained by FITC-conjugated anti-CD146 mAbs) cocultured with KCs (*red*; stained by PE-conjugated anti-F4/80 mAbs). Cell nuclei were stained by Hoechest 30332 (*blue*). (B-C) SEM images of LSECs cultured for 24 (C) and 72 h (D). *Arrows* indicate the fenestrae of cultured LSECs. (*E-H*) Flow cytometry analysis of ICAM-1s expressed on LSECs (E) and Mac-1s (F), LFA-1s (G) and CD44s (H) expressed on PMNs. Cells were incubated with respective fluorescein-conjugated primary mAbs or isotype-matched mAbs (control) in the absence or presence of TNF-α or fMLF stimulation.

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