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Isolating the influences of fluid dynamics on selectin-mediated particle rolling at venular junctional regions

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ABSTRACT

The objective of this study was to isolate the impact of hydrodynamics on selectin-mediated cell rolling in branched microvessels. Significant advancements have been made in furthering the understanding of complex interactions between biochemical and physical factors in the inflammatory cascade in simplified planar geometries. However, few studies have sought to quantify the effects of branched configurations and to isolate the effects of associated fluid forces. Experimental techniques were developed to perform in vitro adhesion experiments in Y-shaped micro-slides. The micro-slides were coated with P-selectin and microspheres coated with Sialyl-Lewis^x were observed as they rolled in the chambers at different wall shear stresses. Study results revealed that microsphere rolling velocities and rolling flux were lowest in regions closest to the apex of a junctional region and were dependent on both branch angle and wall shear stress. The regions closest to the junctional region were shown to have low bulk flow velocities and shear stresses using computational fluid dynamics (CFD) modeling. Collectively, the study demonstrates that despite the presence of a uniform coating of P-selectin, hydrodynamic factors associated with the chamber geometry yield non-uniform effects on particle behavior. These findings could explain why cells have been observed to preferentially adhere or transmigrate near junctional regions. Future characterization of inflammatory processes in microvascular network configurations is therefore crucial for furthering our fundamental understanding of inflammation.

1. Introduction

Accumulating evidence suggests that inflammation plays an important role in numerous disease states ranging from cardiovascular disease to cancer. As a result, there is an important need to understand the factors, both biochemical and hydrodynamic, that influence the inflammatory response in vivo. To fully characterize multifaceted factors impacting leukocyte rolling, both biochemical as well as hydrodynamic mechanisms should be considered. An essential first step in understanding the relative contribution of each mechanism is to isolate one of the mechanisms of interest. The current study therefore seeks to isolate hydrodynamic effects on selectin-mediated rolling in branched geometries.

Neutrophil recruitment, rolling, and adhesion are important steps in the inflammatory cascade since these processes ultimately lead to transmigration and phagocytosis and degranulation of pathogens during a normal inflammatory response (Matharu et al., 2006). Studies have suggested a potential sensitivity of the inflammatory response to the local fluid environment (Matharu et al., 2006; Kim and Sarelius, 2004), that adherent leukocytes generate significant wall shear stress gradients (Chapman and Cokelet, 1996, 1997, 1998), and that fluid shear forces are known to stimulate biochemical responses by the vascular endothelium (Kim and Sarelius, 2003). Previous studies have suggested that cellular deformation, endothelial fiber alignment, and microvillus tethering, all related to the local fluid environment, are also significant (Jadhav et al., 2005; Pawar et al., 2008; Park et al., 2002; Sun and Munn, 2005). However, what has not been established is the linkage between these fluid forces, neutrophil margination, rolling, and adhesion, and the geometric configurations in which these processes occur. Understanding the structural consequences of venular networks may potentially reveal new causes of pathogenesis, alter the effects of different therapies, and reveal structural abnormalities that are causing aberrant inflammatory responses.

Post-capillary venular networks have highly branched, tortuous structures that produce significantly different flow patterns than arteriolar networks and it is well established that neutrophil recruitment, adhesion, and transmigration occur more often in venules than in arterioles. However, the vast majority of studies done to date to quantify

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leukocyte margination and adhesion have been done in parallel plate flow chambers or straight, unbranched tubes (Matharu et al., 2006; Chapman and Cokelet, 1996, 1997, 1998; King and Hammer, 2001a, 2001b, 2003; Ley, 1993; Kunkel et al., 1998). Only a limited number of studies have looked at the effects of branched geometries and none have systematically studied the isolated fluid forces associated with branched geometries. Therefore, we posit that our current study will be able to provide a more comprehensive picture of the inflammatory response by quantifying the impact of fluid forces on selectin-mediated rolling in branched geometries using in vitro experimentation in branched micro-slides.

1.1. Biochemical influences on leukocyte adhesion

Previous studies have sought to characterize how the preferential expression of adhesion molecules impacts the distribution of adherent leukocytes on the vascular endothelium. In 1991, Ley and Gaehtgens (1991) proposed that the venular endothelium appears to be specialized to support leukocyte adhesion and that during inflammation, there is a preferential expression of various adhesion molecules. Over the next decade, the adhesion molecules were extensively studied and were shown to be important biochemical mechanisms influencing leukocyte rolling and adhesion (Ley and Gaehtgens, 1991; Ley et al., 1993; Ley, 1993; Lamberti et al., 2014). Several studies specifically looked at how the expression of adhesion molecules contributes to the spatial distribution of adherent leukocytes. For example, Kim and Sarelius (2004) suggested that non-uniform patterns of P-selectin expression contribute to leukocyte interactions with the endothelium. A later study by Wojciechowski and Sarelius (2005) suggested that most adherent leukocytes in blood-perfused venules are located at or near endothelial junctions. Sumagin and Sarelius (2007) further suggest that the spatial distribution and expression levels of adhesion molecules in the microcirculation determine the timing and placement of leukocyte interactions. Mundhekar et al. (2006) reported similar findings that suggest both rolling velocity and arrest frequency for neutrophils are governed by adhesion molecule interactions at endothelial cell junctions. All of the previously mentioned studies highlight the important role of the adhesion molecules on neutrophil interactions with the endothelium and suggest how non-uniform distributions of adhesion molecules contribute to the distribution of adherent leukocytes. However, these studies do not account for the intrinsic hydrodynamics that may be affecting the distribution of adherent leukocytes or the effects on rolling behavior.

1.2. Hydrodynamic influences on leukocyte adhesion

Studies that have been done to look hydrodynamic effects were often carried out using dilute cell suspensions in flow chambers, such as parallel plate chambers, capillary tubes, or radial flow chambers. Only a limited number of studies have been done that focus specifically on hydrodynamic effects on leukocyte behavior in complex, network geometries (Schmid-Schönbein et al., 1980; Blixt et al., 1985; Vejlens, 1938; Bagge and Karlsson, 1980; Perkkio et al., 1988). Studies in T-junctions, such as those done by Bagge and Karlsson (1980) or Blixt et al. (1985) provided general qualitative observations about the relative locations and concentrations of cells passing through a junctional region, but did not explicitly characterize cell rolling velocities or rolling fluxes or other effects due to the hydrodynamics associated with different geometrical configurations. More recent studies looked at where cells tend to adhere in different types of branch configurations (Tousi et al., 2010), but did not quantify specific effects of geometry on selectin-mediated rolling, particularly rolling velocities and rolling fluxes. Lamkin-Kennard et al. (2005) showed that regardless of geometric configuration, neutrophils have a tendency to adhere at the junction points between two converging vessels. Lamberti et al. (2014) and Smith et al. (2014) found similar distributions using synthetic microvascular networks. However, the precise mechanisms leading to the distributions of cells were not studied. Lamberti et al. (2015) also showed that adhesion increases with increasing branch angle, but did not quantify how branching affects rolling velocities or rolling fluxes.

Lamberti et al. (2014) and Sumagin et al. (2009) sought to quantify the effects of adhesion molecules on adhesion in branched vessels. Lamberti et al. (2014) studied the combined effects of adhesion efficiency associated with ICAM-1 and E-selectin with different antibody ratios in branched geometries. Both factors were shown to impact adhesion efficiency, but the isolated effects of hydrodynamics were not included. Sumagin et al. (2009) showed that ICAM-1 expression and neutrophil adhesion occur differently in straight vessel segments than in branched segments. Results from the study also suggested that even if ICAM-1 is fully upregulated in straight venular segments vs. venular junctional regions, recruitment and adhesion occur more prominently in the junctional regions. The study by Sumagin et al. (2009) suggests that geometry has important effects on the inflammatory response and another mechanism, perhaps hydrodynamics, may be contributing to the observed cellular distributions when ICAM-1 is fully upregulated.

1.3. Leukocyte studies in microfabricated devices with branched geometries

Several investigators have taken steps to microfabricate flow devices that more closely mimic geometries found in vivo. In 1993, Cokelet et al. (1993) fabricated microvascular channels using photolithography on microscope slides. Frame and Sarelius (1995) also created semi-circular microvascular channels on glass. In 2004, Lu et al. (2004) developed a microfluidics based variable shear device with a network- like structure to study cell detachment forces. These devices were closer in structure to the true configuration of a vessel in vivo, but do not fully represent the complex geometries found in vivo. Recently, Prabhakarpandian et al. (2008, 2011) and Tousi et al. (2010) microfabricated devices to mimic a microvascular network using PDMS (polydimethylsiloxane). The network configuration was obtained from the digitization of in vivo microvascular topology and channels in the PDMS had a rectangular cross section. Microspheres were perfused over an antibody coated network and locations with higher levels of adhesion were identified. Tousi et al. (2010) suggested that flow patterns near bifurcations/junctions, and not the presence of cellular aspects of the system, were the main controlling factor leading to preferential adhesion patterns of leukocytes near bifurcations. Rosano et al. (2009) performed a similar study in which bovine aortic endothelial cells (BAEC) were cultured in the channels and microspheres were perfused over the channels with and without stimulation from TNF-α. Smith et al. (2014) and Lamberti et al. (2014, 2015) showed how changing flow patterns, branch angle, and shear rate contribute to an increase adhesion percentage near bifurcations. Results from all studies showed that microparticle adhesion was non-uniform in the network and spatially localized near bifurcations. These results highlight the potential significance of studying the inflammatory response in branches and networks, but did not specifically include isolated hydrodynamic effects on cell rolling. The current study seeks to improve on these previous studies by isolating how the fluid forces associated with branched geometries impact selectin-mediated rolling in branched microchannels.

2. Materials and methods

Experimental techniques for studying rolling behavior were developed to perform in vitro adhesion experiments in microfabricated junctional regions using commercially available IBIDI, Inc. (München, Germany) Y-shaped micro-slides. The experimental techniques were developed specifically to quantify hydrodynamic effects due to branch configuration on microsphere rolling velocities and rolling fluxes. The Y-shaped IBIDI micro-slides were coated with soluble, recombinant P-selectin and microspheres coated with Sialyl-Lewis^x (sL^x) were rolled in