Mechanistic Insights into Macrophages Regulation of Neutrophil Transendothelial Migration in Inflamed Mucosa

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Introduction: Rapid neutrophil (PMN) mobilization to sites of insult is critical for host defense and requires crossing of the vascular wall. PMN transendothelial migration (TEM) involves several well-studied sequential adhesive interactions with vascular ECs, however what initiates or terminates this process is not well-understood. **Methods and results:** Our findings identified a new mechanism where gut interstitial macrophages (Mϕ), which are rapidly recruited towards the vascular wall in response to inflammatory cues, were found to locally prime endothelial cells (ECs) responses to regulate PMN TEM. Using real-time intravital microscopy (IVM) on lipopolysaccharides (LPS)-inflamed intestines in anesthetized CX3CR1-EGFP macrophage-reporter mice, complemented by whole-mount tissue imaging we demonstrate that macrophage presence was critical for the induction of PMN-ECs adhesive interactions and subsequent PMN recruitment and accumulation in the intestinal mucosa. Anti CSFR-1 antibody-based macrophage depletion in the lamina propria and at the vessel wall significantly reduced PMN adhesion and TEM in inflamed intestines. We further observed that macrophages at the vessel wall localized specifically to regions of high ICAM-1 intensity and their removal resulted in elimination of the ICAM-1 “hot spots”, overall lowering the ECs ICAM-1 expression. Mechanistically, using murine/human ECs-macrophage and PMN co-cultures we established that activated macrophages elevate PMN adhesion and TEM via TNFα–dependent upregulation of ECs ICAM-1. Antibody-mediated neutralization of TNFα in macrophage co-cultures with ECs and/or PMNs suppressed ICAM-1 upregulation, decreasing PMN TEM. Further *in vivo* imaging studies of inflamed gut revealed high TNFα expression in macrophages and specific expression of TNFα receptor type II (TNFR II) but not type I in intestinal ECs. Inhibition of intestinal Mϕ-TNFα or ECs-TNFR II reduced intestinal PMN TEM. The use of bone marrow chimeras with TNFα knockout Mϕs further confirmed the novel role of Mϕ-TNFα in regulating ECs adhesion molecule expression and PMN TEM. **Conclusions:** As such, our findings identify new, clinically relevant mechanism by which macrophages regulate PMN trafficking in inflamed mucosa.