Human L2 Regulates Leukocyte Transmigration at a Unique Step Between Those Regulated by PECAM and CD99

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Inflammation is the innate response to tissue damage and infection. Transendothelial migration (TEM), arguably the most important step of inflammation, is the process by which leukocytes leave the bloodstream to enter inflamed tissues. PECAM and CD99 are adhesion molecules that are essential for the initiation and completion of TEM, respectively. Identification of the proteins involved and their mechanisms are imperative for resolving uncontrolled TEM, which is the cause of chronic inflammatory diseases such as cancer, arthritis and multiple sclerosis. CD99L2 (L2) is a type-1 transmembrane protein required for TEM *in vivo*. Mice deficient in L2 have reduced leukocyte recruitment to sites of inflammation. Intravital microscopy and the acute dermatitis model show that L2 is required for leukocyte extravasation across the endothelium specifically and not leukocyte adhesion or rolling. L2 function in inflammation has been extensively studied in mice. The relevance of L2 in human inflammation remains unknown.

To characterize the role of L2 in human inflammation, we used *in vitro* TEM assays with primary human endothelial cells and leukocytes. Inhibition of L2 function using shRNAs or antibodies against L2 reduces TEM of monocytes and neutrophils to ~20-30%. TEM is restored upon crosslinking or re-expression of L2. Blockade of either leukocytes or endothelial cells blocks equivalently to blocking both. TEM is significantly reduced in the presence of soluble L2-Fc. These data are consistent with a homophilic mechanism of interaction. Our data also show that L2 functions at a step in TEM between steps regulated by PECAM and CD99. Similar to PECAM and CD99, L2 promotes transmigration by recruiting the lateral border recycling compartment to the site of TEM, specifically downstream of PECAM initiation. Ongoing studies are focused on identifying signaling pathways used by L2 to promote TEM and validating them in vivo using intravital confocal microscopy to study TEM in real time.