

**Title:**

In vivo imaging of leukocyte-endothelium interactions in septic lung injury

**Abstract:**

Excessive accumulation of leukocytes in the lung represents a key feature in the development of an acute lung injury (ALI). Numerous studies demonstrated that leukocyte rolling is a compulsory precondition for the firm adhesion in organs. However, it seems that the recruitment process of leukocytes in the lung is more complex. Increasing evidence suggests that recruitment of leukocytes in pulmonary arterioles and venules is mediated by adhesion molecules and leukocyte trapping in pulmonary capillaries is due to mechanical forces. Moreover, platelets were shown to be involved in the pulmonary recruitment of leukocytes by shedding CD40L into systemic circulation. However, the detailed mechanisms of leukocyte recruitment within the different parts of the pulmonary microcirculation and the detailed role of platelets remain elusive.

We utilized a model of intravital fluorescence microscopy to analyze mechanisms of leukocyte recruitment in the pulmonary microcirculation in ALI. We found that local and systemic inflammation were characterized by different patterns of leukocyte recruitment in the lung. Under local inflammatory conditions pulmonary venules were the predominant site of leukocyte recruitment. By contrast, systemic inflammation caused an enhanced leukocyte recruitment in pulmonary capillaries.

Analysis of PSGL-1-mediated leukocyte rolling in lung arterioles and venules in abdominal sepsis revealed that the importance of rolling varies in different parts of the pulmonary microcirculation. While the inhibition of PSGL-1-dependent rolling reduced leukocyte adhesion in arterioles, we could not observe any reduction in leukocyte adhesion in pulmonary venules.

Inhibition of the integrins CD11a and CD11b significantly reduced leukocyte adhesion in all parts of the pulmonary microcirculation, indicating that CD11a and CD11b not only mediate firm leukocyte adhesion in pulmonary arterioles and venules, but also are critical in supporting leukocyte trapping in pulmonary capillaries.

Abdominal sepsis caused a significant increase in the formation of platelet-leukocyte aggregates, facilitating the size-dependent leukocyte trapping in pulmonary capillaries. Moreover, we could show that the inhibition of matrix-metalloproteinases reduced shedding of CD40L from platelets and subsequent pulmonary leukocyte recruitment in abdominal sepsis.

These results identify different mechanisms of leukocyte recruitment within different parts of the pulmonary microcirculation in acute lung injury, which might help to develop new therapeutic strategies.