Neutrophils do not require CXCR2 for LPS-induced migration into the lung

Jörg Reutershan1, 3, Margaret A. Morris1, Klaus Ley1, 2

1) Cardiovascular Research Center, 2) Biomedical Engineering, University of Virginia, Charlottesville, VA, and 3) Department of Anesthesiology and Intensive Care Medicine, University of Tübingen, Tübingen, Germany

Introduction

In acute lung injury (ALI), CXCR2 has been shown to play a crucial role in the migration of neutrophils (polymorphonuclear cells; PMN) into the lung.

In a model of LPS-induced ALI, we investigated which step of the transmigration is mediated by CXCR2 and whether CXCR2 expression on radiosensitive cells rather than on leukocytes is important.

Methods

In wildtype (wt) and CXCR2-/- mice, ALI was induced by LPS aerosol. At different times after LPS exposure, PMN counts were performed in the bronchoalveolar lavage fluid (BAL) and in the lung homogenate. To distinguish PMNs in the different lung compartments, an antibody to PMNs (GR-1, APC-labeled) was injected i.v., 5 minutes prior to harvesting the lung. This was to label all intravascular PMNs. The lung homogenate was then labeled with a second antibody to PMNs (7/4 or CD11b, FITC-labeled). This allowed us to distinguish between intravascular (GR-1, 7/4) and interstitial (GR-1, 7/4) PMNs (Fig. 1). Cytospins of BAL were performed to morphologically confirm the presence of PMNs.

We created chimeric mice by transplanting bone marrow from CXCR2-/- into irradiated wt mice. These chimeric mice express CXCR2-/- on radiosensitive cells but not on leukocytes.

In addition, in-vitro migration of wt and CXCR2-/- PMNs was studied in the different groups of mice, using a transmigration assay (Neuroprobe®).

Results

In wt, LPS-induced PMN migration into all lung compartments was found. In contrast, LPS-exposed BAL from CXCR2-/- showed predominantly alveolar macrophages (Fig. 2a).

CXCR2-/- PMN accumulation in the pulmonary vasculature was found, but cells did not advance into the interstitium (Fig. 2b). Accordingly, almost no PMNs were found in the alveolar airspace (Fig. 3).

Surprisingly, in the chimeric mice, LPS induced a significant PMN migration into both lung interstitium and alveolar airspace, at a level of about 60% of that found in wt (Fig. 3 and Fig. 4).

In-vitro, CXCR2-/- PMN were able to migrate to LPS-exposed BAL from both CXCR2-/- and wt (Fig. 5).

Conclusion

In LPS-induced ALI, CXCR2 is important for both transendothelial and transepithelial migration in the lung but not for the accumulation of PMN in the pulmonary vasculature.

CXCR2 expression on radiosensitive cells, e.g. endothelium or epithelium, does contribute to the PMN migration in the lung.