Lipopolysaccharide-induced Monocyte Retention in the Lungs of Rabbits

Role of Cell Stiffness and the CD11/CD18 Leukocyte Adhesion Complex

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The marginating pool of blood monocytes normally present in the pulmonary microvasculature is markedly increased during endotoxemia in the rabbit. We have also shown that lipopolysaccharide (LPS) enhances monocyte adherence in vitro and retention in the lung in vivo long before its effects on endothelial cell adhesion molecules. Because the mean pulmonary capillary diameter (5.5 μm) is smaller than that of the monocyte (8.5 μm), we hypothesized that LPS induces monocyte retention by increasing stiffness and thereby diminishing the ability of monocytes to deform and transit the narrow pulmonary capillary.

Human and rabbit monocytes were isolated under LPS-free conditions by elutriation (>90 percent pure). Human monocyte assays included the following: net F-actin assembly and reorganization (cytofluorograph analysis and fluorescent microscopy of NBD-phallacidin-monocytes), monocyte stiffness (using a “cell poker”), monocyte adherence to endothelial cell monolayers, and monocyte retention in capillary-sized pores (via a filtration system). Scintigraphy of rabbit monocytes labelled with 111In was used to assess LPS-induced lung retention in vivo. The LPS induced a concentration-dependent (10 to 1,000 ng/ml) increase in monocyte stiffness (100 to 150 percent above baseline) and increased monocyte retention in 6.5 and 8 μm pores (100 to 125 percent above baseline) compared to control monocytes within 10 min after exposure to LPS, a time well before LPS stimulates monocyte adherence to surfaces in vitro (>20 min required). We questioned if LPS-induced stiffness and retention were dependent on F-actin assembly. Lipopolysaccharide induced an increase in net actin assembly which paralleled the time course and dose response of increased stiffness and retention, and LPS induced monocytes to reorganize actin within minutes. Furthermore, LPS-induced stiffness and retention of monocytes was inhibited by pretreatment with 5 μM cytochalasin D (CD), which disrupts actin assembly. To determine whether these mechanisms, identified in vitro, were active in vivo, we monitored LPS-induced monocyte lung retention by scintigraphy in rabbits. Monocytes pretreated 5 min with 10 ng/ml LPS prior to IV infusion were significantly retained in the lungs over time compared to saline solution-treated control monocytes. The CD (5 μM) pretreatment of LPS-treated monocytes (10 ng/ml) led to lung retention similar to that of unstimulated control monocytes. Antibodies to the common β chain of CD11/CD18 (60.3 and IB4), a monocyte cell surface adhesion glycoprotein, were used to determine if LPS-induced monocyte lung retention was CD18-dependent.

We established that LPS-induced monocyte adherence to endothelial cells in vitro was significantly attenuated (50 to 60 percent) by 60.3 or IB4 (40 μg/ml saturates monocytes per FACS analysis). The 111In-monocytes were pretreated with 60.3 or IB4 and infused into endotoxemic rabbits 20 min after IV infusion of 2 mg/kg with 60.3 (n = 5) or IB4 (n = 5), or into normal rabbits treated with antibody 2 mg/kg alone (n = 4). There was no significant difference in monocyte lung retention in endotoxemic animals (± antibody treatment) over the first hour, however, antibody treatment led to a 80 to 90 percent inhibition of LPS-induced monocyte lung retention (per scintigraphy and organ counts) by 2 to 6 h after monocyte infusion. Murine IgG had no effect. Monocytes not retained in the lungs were recovered in the blood, liver, and spleen.

These data suggest that the initial retention of monocytes in the lung during endotoxemia is governed by alterations in cytoskeletal actin assembly/organization which increase monocyte stiffness, and later by CD18-dependent adhesive interactions between monocytes and the pulmonary vasculature.

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