

## **Abstract**

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are both low molecular weight lysophospholipid (LPL) ligands which are recognized by the Edg family of G protein-coupled receptors. In endothelial cells, these two ligands activate Edg receptors resulting in cell proliferation and cell migration. We examined the impact of lysophospholipids on the expression of intercellular adhesion molecule-1 (ICAM-1), and on the production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), key regulators of leukocyte recruitment in human umbilical cord vein endothelial cells (HUVECs). Incubation with both LPA and S1P enhanced ICAM-1 mRNA and protein expressions in HUVECs were in a dose- and time-dependent manner. Maximal expression appeared at 8 h postligand treatment, as detected by flow cytometry and Western blotting. Furthermore, prior treatment of HUVECs with pertussis toxin, a specific inhibitor of  $G_i$ , or PDTC, an inhibitor of the  $\text{NF}\kappa\text{B}$  pathway, prevented the enhanced effect of LPA- and S1P-induced ICAM-1 expression. However, pretreatment of HUVECs with C3, an inhibitor of rho, had no effect on LPL-enhanced ICAM-1 expression. In a static cell-cell adhesion assay system, pretreatment of LPL enhanced the adhesion between HUVECs and U937 cells, a human mononucleated cell line. The enhanced adhesion effect could be prevented by preincubation with a functional blocking antibody against human ICAM-1. On the other hand, LPA and S1P enhanced IL-8 and MCP-1 mRNA expression and protein secretion in a dose- and time-dependent fashion. Maximal mRNA expression appeared at 16 h postligand treatment. By chemical inhibitors prior treatment, we found both LPA and S1P enhanced IL-8 and MCP-1 expression through a  $G_i$ -, rho- and  $\text{NF}\kappa\text{B}$ -dependent mechanism. In a chemotaxis assay

system, results shows that LPL treatments do enhance chemotactic activity of endothelial cell and monocyte recruitment subsequently mediate through up-regulating IL-8 and MCP-1 protein secretion. These results suggest that LPLs released by activated platelets might enhance ICAM-1-dependent adhesion of monocytes to endothelial cells and IL-8- and MCP-1-dependent chemoattraction of monocytes toward endothelium, thus facilitating wound-healing and inflammation processes.