Lipopolysaccharide-Induced Neutrophil Gene Expression Under In Vivo or In Vitro Conditions*

John Arcaroli, MS; John Kupfner, BS; Ho-Kee Yum, MD; Robert Shenkar, PhD; Jong Sung Park, PhD; and Edward Abraham, MD

*From the Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO.

Abstraction: LPS = lipopolysaccharide

Endotoxemia is a frequent precipitating etiology for acute lung injury. Acute lung injury is characterized by the accumulation of activated neutrophils in the lungs. Although neutrophils can be stimulated in vitro by exposure to lipopolysaccharide (LPS), it is likely that in vivo interactions with other pulmonary cell populations modulate the activation patterns in neutrophils migrating to the lungs after endotoxemia.

To study the effects of LPS on neutrophil activation in vivo, lung neutrophils were isolated from male Balb/c mice 1 h after LPS administration (1 mg/kg intraperitoneally). For in vitro studies, bone marrow neutrophils were isolated and stimulated with LPS (100 ng/mL) for 1 h. MN11KsubA and MN11KsubB chips were used to determine messenger RNA expression patterns after LPS exposure in vivo and in vitro.

In lung neutrophils, which were isolated from mice 1 h after endotoxin administration, there was a twofold or greater increase in 423 genes, and there were 401 genes that demonstrated twofold or greater decreases in expression. In contrast, after culturing neutrophils with LPS in vitro for 1 h, there were only eight genes that showed twofold or greater up-regulation and none that were down-regulated. All of the genes that showed increased expression in vitro were increased in vivo, but to a greater extent. Genes that were up-regulated in vitro and in vivo included both proinflammatory cytokines and mediators, as well as antiapoptotic proteins. Among the genes up-regulated in vitro and in vivo were the following: KC (fold increase in vitro/in vivo) (6.7/79); tumor necrosis factor (5.1/28); serum amyloid A (4.55/25); prostaglandin synthase (3.8/57); A20 (3/111); glycophosphate ZP3 (3/92); putative B-chemokine receptor (2.9/171); and macrophage inflammatory protein-2 (2.6/124.4).

Patterns of gene expression in neutrophils are markedly different after in vitro and in vivo exposure to endotoxin. The present results show that whereas LPS alone can activate neutrophils, such LPS-induced activation is much greater in neutrophils accumulating in the lungs in vivo. Such results indicate that neutrophil activation patterns in response to LPS are modulated in vivo by the exposure to pulmonary cell populations and additional regulatory factors. Similarly, the in vivo responses of neutrophils to LPS are incompletely delineated by in vitro studies.