Leptin in Fibroproliferative Acute Respiratory Distress Syndrome

Not Just a Satiety Factor

The acute respiratory distress syndrome (ARDS) is a devastating form of acute lung injury that occurs in critically ill patients. Early epidemiological studies identified several at-risk diagnoses that were associated with an increased susceptibility for the development of ARDS, including sepsis, major trauma, massive transfusion, and the aspiration of gastric contents (1, 2). However, only 20 to 40% of patients with these at-risk diagnoses eventually develop ARDS. Therefore, other factors including pre-existing co-morbid conditions may influence the vulnerability of a critically ill patient to develop ARDS.

Diabetes mellitus is one pre-existing disorder that has biologically plausible reasons to alter the pathogenesis of ARDS. Three different clinical studies have reported an association between a history of diabetes and a decreased risk of developing ARDS with very consistent odds ratios ranging from 0.33 to 0.58, even after adjustment for several important confounding variables, including age, sex, and severity of illness (3–5). Combining the 961 patients enrolled into these observational studies, the incidence of ARDS was 26.3% (66/251) in patients with diabetes and 38.3% (272/710) in those patients without diabetes ($P < 0.0005$; odds ratio, 0.57; 95% confidence interval, 0.41–0.79). Though hyperglycemia is a common occurrence in diabetic patients, these studies did not provide conclusive evidence that the mechanism by which diabetes diminishes the risk of developing ARDS is related to the effects of hyperglycemia alone.

The protective effects of diabetes on the development of acute lung injury have also been reproduced in various animal models. After exposure to intratracheal endotoxin, type I diabetic rats demonstrated less lung injury, reduced concentrations of tumor necrosis factor, interleukin-1, and decreased neutrophils in the bronchoalveolar lavage fluid (6, 7). Similarly, type II diabetic rats exhibited less protein leakage in the lung after intratracheal exposure to lipopolysaccharide (LPS) (8).

It is likely that there are multiple mechanisms by which diabetes attenuates the susceptibility to develop ARDS. For example, type II diabetes is associated with a variety of immunomodulatory conditions, including insulin resistance, obesity, hyperleptinemia, and dyslipidemia. In addition, patients with diabetes receive specific medications that alter the systemic inflammatory response including insulin, peroxisome proliferator-activated receptor- γ (PPAR- γ), agonists, and metformin. Recently, leptin has emerged as a potentially important mediator of the pathogenesis of multiple lung diseases. Leptin is a protein that is synthesized and secreted primarily by white adipose cells and acts on the brain to decrease hunger. Leptin is also an important mediator of the inflammatory response (9). Disorders associated with reduced leptin production, such as malnutrition, are associated with an increased susceptibility to infection. Conversely, increased secretion of leptin is associated with the production of proinflammatory pathogenic cytokines. For example, increasing evidence suggests that the proinflammatory effects of leptin may contribute to the higher incidence of asthma in the obese population (10). Leptin resistance is present in more

than 90% of obese patients with type II diabetes and is believed to result from receptor down-regulation. Besides adipose tissue, the leptin receptor is also present in other organs, including liver, pancreas, kidney, and importantly the lung.

In the study by Jain and colleagues in this issue of the *Journal*, the authors (pp. 1490–1498) make the intriguing observation that intratracheal bleomycin challenge in mice induces a substantial increase in leptin levels in BAL fluid, and mice with defective leptin receptor signaling (db/db) are resistant to the development of bleomycin-induced fibroproliferation (11). Treatment of human lung fibroblasts with leptin *in vitro* served to potentiate TGF- β -mediated expression of several profibrotic genes, including the autocrine induction of TGF- β itself. Leptin down-regulated both the expression and activity of PPAR- γ , and leptin-induced augmentation of TGF- β 1 transcriptional activity was negated in cells deficient in PPAR- γ activity (either by stable gene knockdown or use of PPAR- γ -specific inhibitors). These findings provide compelling evidence implicating leptin as an important cytokine mediator of fibrogenesis in experimental acute lung injury. Although this study is the first to identify leptin as a potential co-factor in lung fibroproliferative responses, mice deficient in leptin (ob/ob) or with defective leptin receptor signaling (db/db) have recently been shown to be protected against toxin-induced hepatic injury and fibrosis (12). Similarly, leptin inhibited PPAR- γ expression in hepatic stellate cells. This effect was mediated by leptin-induced extracellular signal-regulated kinase (ERK) activation and expression of the transcription factor Egr-1 (13). Finally, Jain and associates have extended their findings to the bedside by reporting increased leptin concentrations in bronchoalveolar lavage fluid of patients with ARDS that was positively correlated with the bronchoalveolar lavage TGF- β 1 levels. In the subgroup of patients with ARDS with a normal body mass index (and presumably intact leptin signaling), higher BAL levels of leptin were associated with both fewer ventilator- and ICU-free days, and a higher mortality.

Inhibitory effects of leptin on PPAR- γ expression and activity are of considerable biological relevance. PPAR- γ is a member of the nuclear hormone receptor superfamily that is required for adipogenesis and insulin sensitivity. In addition, this ligand-activated transcription factor can exert anti-inflammatory effects by inhibiting NF- κ B and mitogen-activated protein kinase (MAPK)-dependent signal transduction pathways. PPAR- γ also functions as a key inhibitor of both smad-dependent and smad-independent TGF- β effects in fibrogenesis, including suppression of TGF- β expression, collagen synthesis, myofibroblast differentiation, and epithelial to mesenchymal transition (EMT) (14–16). Activation of PPAR- γ using various thiazolidinediones has been shown to markedly suppress fibrotic responses *in vivo*, including bleomycin-induced fibrosis (14). PPAR- γ effects are largely dependent on level of expression rather than availability of activating ligand (16). Therefore, leptin-mediated suppression will necessarily interfere with PPAR- γ -dependent regulation of lung remodeling processes.

The *in vitro* evidence supporting profibrotic effects of leptin is compelling. However, a causal link between leptin and fibrogenesis has not been previously demonstrated in critically ill or otherwise healthy patients with type II diabetes. The existing epidemiological evidence has only revealed an association be-

tween diabetes and a decreased susceptibility to develop ARDS (3–5). These studies did not identify an association between diabetes and improved outcomes in patients with ARDS (3, 5). In addition to leptin resistance, the db/db mice display a plethora of metabolic abnormalities, including hyperglycemia, hyperinsulinemia, insulin resistance, and obesity. This constellation of metabolic derangements mimic those found in patients with type II diabetes. As readily acknowledged by the authors, several of these abnormalities can substantially influence both the magnitude of lung injury and the subsequent fibroproliferative response. For instance, hyperglycemia has been shown to either promote or suppress inflammation and tissue injury depending upon the experimental context (7). Similarly, insulin exerts immunomodulatory effects that extend beyond glycemic control. Specific effects of insulin include but are not limited to suppression of NF- κ B-mediated inflammatory cytokine and adhesion molecule expression, inhibition of injurious free fatty acid release, and regulation of apoptosis responses (7). The clinical observation that patients with other conditions associated with leptin resistance, such as obesity, are not protected from acute lung injury or its consequences support the notion that leptin alone is unlikely to explain the lower incidence of ARDS in diabetics. Pharmacologic inhibition of leptin during bleomycin-induced acute lung injury would go a long way toward defining leptin-specific effects independent of confounding metabolic abnormalities present in ob/ob or db/db mice.

The experimental approach taken in the study by Jain and colleagues is comprehensive. However, several unanswered questions remain. The authors have previously shown that leptin-resistant mice were protected from hyperoxia-induced lung inflammation, lung injury, and mortality (17). For that reason, it is surprising that no differences in inflammatory cell influx and cytokine production were observed in db/db mice after bleomycin challenge. Moreover, factors that induce leptin expression in acute lung injury have not been identified. Likely candidates include the proximal cytokines IL-1 β and TNF- α , or cellular stressors such as hypoxia (18). Finally, more research is needed to define molecular signals involved in crosstalk between leptin and PPAR- γ . Regardless, this study has identified a possible role for leptin in regulating key reparative responses in the lung, further validating the paradigm that this unique cytokine is not just a satiety factor. Whether or not leptin represents a target for immunomodulatory therapy in inflammatory and fibroproliferative diseases of the lung requires further study.

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Could You Please Pass the Salt?

Airway surface liquid (ASL) has been the focus of intense investigation in recent years. The ASL is the layer covering the apical membrane of the airway epithelium and its epithelia. Electrolyte transporters and channels, most importantly the

cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial sodium channel (ENaC) (1), regulate its height ($\sim 7 \mu\text{m}$) and water volume. An increasing body of evidence supports the hypothesis that attributes lung disease in