Aging and Smoking--Two Roads to Emphysema

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Background: Emphysema is a chronic lung disease characterized by airspace destruction, clinically observed most frequently in older patients with a history of smoking. A series of mouse studies have begun to suggest a previously unappreciated role for toll-like receptor 4 (TLR4) in emphysema. Mice deficient in TLR4, the innate immune system’s receptor for lipopolysaccharide (LPS), develop emphysema as they age without exposure to cigarette smoke. These mice also demonstrate upregulation of a novel pro-oxidant NADPH oxidase 3 (Nox3), as well as the endolysosomal protease Cathepsin E (CatE). In support of our mouse findings, recent reports have found that in humans, TLR4 levels are lower in smokers than nonsmokers, and lower in patients with more severe COPD.

Specific Aim: To delineate a role for TLR4, Nox3, and CatE in the development of emphysema in mice and humans as a function of cigarette smoke exposure and age

Hypothesis: Cigarette smoke exposure, additively or synergistically with aging, leads to downregulation of TLR4, upregulation of Nox3 and CatE, and the phenotypic changes of emphysema.

Methods: Wild-type mice were exposed to daily cigarette smoke from ages 3-6 months. Lung volumes (a surrogate for emphysema) and lung tissue levels of Nox3 and CatE mRNA and protein were measured. Peripheral blood mononuclear cells (PBCs) of people of different ages were challenged with LPS and changes in tumor necrosis factor alpha (TNF-α), a product of TLR4 stimulation, were measured as an assessment of TLR4 function. Serum was assayed for CatE protein levels. Lung sections from nonsmokers without COPD and former smokers with COPD were stained for CatE and Nox3 protein expression.

Results: Wild-type mice exposed to cigarette smoke exhibited increased levels of Nox3 and CatE mRNA, and greater lung volumes, when compared to nonsmoking mice. Lung sections from former smokers with COPD demonstrated markedly increased expression of both Nox3 and CatE. In people, PBCs obtained from those over the age of 65 exhibited less increment in TNF-α levels after LPS stimulation compared to that of younger patients, suggesting that TLR4 function may be decreased in the elderly. An unexpected, yet interesting, contribution of gender was also discovered in both mice and humans: in wild-type mice, for the same amount of cigarette smoke exposure, female mice had greater lung volumes, which is consistent with clinical observations that for equivalent smoking histories, women experience more severe COPD than men. A gender difference in CatE levels was also observed with men expressing higher levels of CatE than women.

Conclusions: In smoking wild-type mice and nonsmoking TLR4 deficient mice, lung levels of Nox3 and CatE are elevated, and lung volumes are increased, suggesting TLR4 deficiency may mimic, at least in part, smoke-induced emphysema. Our studies show that aging alone is also associated with decreased TLR4 activity. Taken together, these data suggest a common pathway by which smoking and aging cause emphysema: downregulation of TLR4 expression and/or activity, upregulation of Nox3 and CatE, increased oxidative and proteolytic damage, with resultant airspace destruction. The unexpected gender effect on lung volumes and CatE expression is interesting and suggest the presence of distinct gender-related pathways for COPD but further investigation is required.