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Title: Pseudomonas aeruginosa causes endoplasmic reticulum (ER) stress in bronchial epithelial cells via its virulence factors pyocyanin (PCN) and alkaline protease (AP)

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Body: P. aeruginosa infection significantly increases morbidity and mortality of cystic fibrosis and COPD. This has been ascribed to a variety of virulence factors, including secreted PCN and AP. Efficient ER functioning is crucial for cellular homeostasis, while an excess of unfolded proteins leads to “ER stress” and activation of the “unfolded protein response” (UPR). Bacterial infection and TLR activation trigger the UPR, most likely through increased biosynthesis of secreted inflammatory mediators. We hypothesized that PCN and AP secreted by P. aeruginosa increase UPR signaling in bronchial epithelial cells. Conditioned medium from cultures of P. aeruginosa strain PAO1 activated the UPR, as evidenced by an increase of splicing of XBP1 mRNA (12-fold; $p<0.001$), and increases of CHOP mRNA (13-fold; $p=0.02$) and GADD34 mRNA (16-fold; $p<0.05$), which were completely abolished when inhibiting p38 MAPK. In contrast, stimulation of bronchial epithelial cells with conditioned medium generated in the presence of iron to reduce PCN production, failed to induce GADD34 mRNA. In addition, purified PCN also increased splicing of XBP1 mRNA and the expression of CHOP and GADD34 mRNA (all $p<0.001$), which remarkably was independent of p38 MAPK. Culture medium derived from a mutant strain that failed to secrete AP induced less splicing of XBP1 mRNA and less up-regulation of CHOP mRNA compared to the wild-type strain. In summary, P. aeruginosa virulence factors activate the UPR, and specifically PCN and AP contribute to this phenomenon through different pathways. This study was supported by grant 3.2.08.032 from the Netherlands Asthma Foundation.