Protective role of Nox1 oxidase against influenza A virus-induced lung inflammation

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Introduction: The Nox2 isoform of the NADPH oxidase family of superoxide-generating enzymes promotes the acute lung injury and airways inflammation caused by influenza A virus infection (Snelgrove *et al.*, 2006; Vlahos *et al.*, 2011). However, airway epithelial cells and lung endothelial cells also express the Nox1 isoform of NADPH oxidase (Carnesecchi *et al.*, 2009) placing this enzyme at key sites to regulate influenza A virus-induced lung inflammation.

Aim: To investigate the potential regulation of the lung inflammation caused by influenza A viral infection by the Nox1 oxidase *in vivo* in mice.

Methods: Age-matched male WT (C57BL/6) and Nox1-deficient mice (*i.e.* $Nox1^{-/y}$) were infected intranasally with the moderately pathogenic HkX-31 (H3N2, 1×10^4 PFU) influenza A virus for analysis of bodyweight, airways inflammation (bronchoalveolar lavage fluid (BALF) cell counting), oxidative stress (L-O12 chemiluminescence and 3-nitrotyrosine staining for superoxide and peroxynitrite production, respectively), viral titers (plaque assay), lung histopathology (H & E staining), cytokine/chemokine expression (Q-PCR) and T lymphocyte subsets including $CD8^+$, $CD4^+$ and $CD25^+CD4^+FoxP3^+$ (*i.e.* T regulatory cells - Tregs; flow cytometry).

Results: HkX-31 virus infection of $Nox1^{-/y}$ mice resulted in a significantly greater: loss of bodyweight; BALF neutrophilia, peri-bronchial and alveolar inflammation; BALF inflammatory cell superoxide production and peri- bronchial, epithelial and endothelial oxidative stress; and expression of pro-inflammatory cytokines including CCl2, CCl3, CXCl2, IL-1, IL-6 and TNF- when compared to WT control mice. Also, expression of the anti- inflammatory cytokine IL-10 was lower in $Nox1^{-/y}$ mice. Lung viral titers, and the degree of airways infiltration of active (*i.e.* $CD69^+$ and $CD44^+$) $CD8^+$ and $CD4^+$ T lymphocytes, and of Tregs were similar between influenza infected WT and $Nox1^{-/y}$ mice.

Discussion: In summary, Nox1 oxidase has anti-inflammatory properties in the lungs and protects against influenza A virus infection in mice. Our findings imply that there are differential roles of Nox1 *versus* Nox2 oxidases in the regulation of inflammation caused by influenza A viruses.

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