Mutations Introduced into the Surfactant Protein-D Lectin Binding Domain Enhance Influenza-A Viral Binding, Neutralization, and Clearance

Kara E. Lewnard, Nikolaos M. Nikolaidis, Erika Crouch, Kevan L. Hartshorn, Francis X. McCormack
Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0564

Background and Purpose:

Surfactant protein D, a collagen containing lectin (collectin), is an innate immune protein of the alveolar lining fluid that forms the first line of defense against Influenza A Virus (IAV) by binding, aggregating and facilitating clearance of inhaled viral particles. SP-D binds to IAV by calcium-dependent interaction between its C-type lectin domains at the end of the extended collagen arms and the carbohydrates decorating the hemagglutinin proteins on the viral envelope. Recombinant trimeric SP-D fragments composed of only the C-terminal neck and carbohydrate recognition domains (NCRD) retain both binding and key functional activities of the full length collectin.

Hypothesis:

Our working hypothesis is that mutagenesis of the residues flanking the lectin site of human SP-D will enhance interactions with specific strains of IAV and result in a protein which will more effectively neutralize the virus both in vitro and in vivo.

Methods:

We examined the differential ability of wild-type and mutant SP-D NCRDs to inhibit hemagglutination, block epithelial infection and death, and reduce viral burden and mortality in mouse models of IAV infection. In addition, we examined the effect of cross-linking of the N-terminally S-tagged NCRDs with an S-protein horseradish peroxidase (HRP) conjugate to enhance SP-D interactions with specific strains of IAV.

Results:

We report that tandem D325A and R343V (D+R) mutations of the CRD confer increased hemagglutination inhibition (HAI) and neutralizing activity for collectin sensitive strains of IAV such as WSNHAnc-Asp225Gly (IAWSN), compared to the wild type NCRD. We also report that intratracheal (i.t.) co-administration of the D+ R double mutant with IAWSN in murine models reduces viral load and IAV-induced mortality. Furthermore, cross-linking the N-terminus of the NCRDs using an S-protein HRP conjugate enhances the valency of the NCRD for viral interactions and further increases HAI and neutralization activity for IAV by the mutant SP-D NCRDs. In vivo studies suggest that cross-linking the D325A/R343V NCRD by preadministration of the S-Protein conjugate reduces viral mortality caused by WSNHAnc-Asp225Gly to a greater extent than D325A/R343V NCRD alone.

Conclusions:

We have demonstrated that enhanced antiviral properties can be engineered into SP-D NCRDs. Our studies suggest that reengineered collectins have therapeutic potential for IAV infections.