Editorial

Is the role of lung innate immune molecules, SP-A1 and SP-A2, and of the alveolar macrophage being overlooked in COVID-19 diverse outcomes?

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Lung innate immunity, a non-specific first line of host defense, plays a key role in maintaining a healthy lung as hundreds of thousands of irritants, bacteria, viruses, pollen, and other insults get inhaled on a daily basis. It is when an imbalance in this system occurs that undesirable downstream consequences may happen, leading to disease with a varying degree of severity. In this Editorial we first provide a brief review of the literature of the genetics of the human innate immune molecules, SP-A1 and SP-A2, and their impact on the alveolar macrophage and the bronchoalveolar lavage at baseline and in response to ozone-induced oxidative stress, as well as on lung function, and survival after infection. Next, we discuss the potential differential role of SP-A variants on different COVID-19 patient subgroups.

The innate host defense molecules, human pulmonary surfactant proteins (SP)-A1 and SP-A2, have been identified with extensive genetic and epigenetic variability[1-3]. Preclinical studies including animal, ex vivo, and cell culture experiments have resulted in a considerable body of information to indicate that SP-A1 and SP-A2 differentially affect the function and regulation of the alveolar macrophage, the sentinel cell of innate immunity, as well as the regulation of the type II epithelial cells, either at baseline conditions or in response to various insults, such as infection and ozone-induced oxidative stress. If survival were to be taken as the ultimate readout, it has been shown that mice that carry and express a different SP-A1 or SP-A2 variant, exhibit significantly different survival rates after *Klebsiella pneumoniae* infection[4], and the impact of these variants on lung mechanics also differs[5]. Beyond preclinical animal studies, in humans, one variant that resulted in better survival in animals, also correlated with significantly better survival in lung transplant patients during the first year post-transplantation, which is the most critical time for these patients[6]. Moreover, SP-A variants have been associated with disease susceptibility in a large number of pulmonary diseases[7-10], indicating their potential importance in determining lung disease susceptibility and/or severity.

SP-A1 and SP-A2 variants differentially affect the bronchoalveolar lavage

(BAL) proteome in response to infection and/or in the presence or absence of ozone-induced oxidative stress (OxS)[11], as well as they differentially affect the function and regulation of the alveolar macrophages under various conditions, in terms of inflammatory cytokine expression[12,13], phagocytic index[14,15], proteome[16,17], miRNome[18,19], gene expression[20], toponome[21], and other. Because of the differential SP-A1- and SP-A2-mediated impact on BAL and alveolar macrophages, it is reasonable to postulate that the microenvironment in the hypophase (i.e. the fluid lining the alveolus and surrounding the AM) differs, especially in response to infection and/or OxS. Although differences in the alveolar macrophage proteome and toponome have been observed under baseline conditions, these may not be critical for the health or survival of these mice, as health and life span of these animals is similar regardless as to which SP-A variant they carry. However, animals with these baseline or "resting" SP-A1- and SP-A2-mediated differences in the alveolar space and/or the alveolar cells in response to infection and/or OxS exhibit significantly different outcomes in a multitude of readouts, as noted above, including survival after infection[4]. This indicates that the SP-A variant-dependent baseline differences, in the face of an insult (i.e. bacteria, OxS, co-infecting pathogens in COVID-19 or SARS-CoV-2, or other), may be critical in determining disease susceptibility or severity as these baseline differences in response to a challenge may be magnified, synergistically or additively, or be nullified.

COVID-19 patients exhibit a wide spectrum of disease severity from extremely mild to extremely severe presentation of the disease. It remains to be determined whether and/or how the genetic variants of innate immune molecules, such as SP-A1 and SP-A2, shown previously to differentially affect function and regulation of the alveolar macrophage, could play a differential role against this viral infection and perhaps explain, in part, the variable outcome in terms of disease severity. Although the role of genetics of innate immune molecules on COVID-19 has not been addressed, as a prelude to future experimentation we consider three scenarios. 1) **Is it possible** that individuals with no other underlying disease and a certain SP-A1/SP-A2 genotype experience mild (or severe) symptoms in response to SARS-CoV-2 infection? Based on the significant amount of information available from preclinical studies this is highly likely. SP-A variants may differentially provide the first line of defense against the virus via perhaps its interaction with the alveolar macrophage and/or the regulation of the inflammatory response and/or via its regulation of the type II epithelial cell, the cell infected by SARS-CoV-2. 2) **Is it possible** that the genetics of innate immune molecules, SP-A1 and SP-A2, play a role in host defense against SARS-CoV-2 and/or in the host defense of co-infection with non-SARS-CoV-2 pathogens? About 26% of COVID-19 patients are also infected with other pathogens such as respiratory syncytial virus (RSV)[22]. SP-A has been shown to enhance RSV clearance in mice[23] and recently a functional trimeric SP-A fragment has been shown to reduce RSV infection[24]. Moreover, an association has been observed between SP-A variants and RSV susceptibility[25]. Thus, the available data indicate that SP-A variants are likely to provide differential host defense against potential co-infecting pathogens and/or differentially modulate the inflammatory response in response to virus as shown in preclinical studies for other insults. 3) **Is it possible** that individuals with additional major disease burden, where inflammation and the OxS level in the alveolar microenvironment are high, could fare better in response to SARS-CoV-2 infection, if they carry a given SP-A1/SP-A2 genotype? This remains to be determined. Preclinical studies indicate that OxS differentially affects the oxidation level and function of SP-A variants and that the higher the level of SP-A oxidation the lower its activity[15]. SP-A oxidation also impairs its ability to interact with the macrophage[26]. Preclinical studies also indicate that SP-A is more susceptible to oxidation in response to ozone-induced OxS compared to the total protein present in the BAL[27], raising the question of whether SP-A may serve as a "sacrificial antioxidant" (i.e. it eliminates ROS via its own oxidation to protect the function of proteins with perhaps more critical function). However, controlling and/or maintaining a redox balance, via antioxidant therapy regimens, may not only benefit/protect the functional activity of molecules and cells, such as SP-A and the alveolar macrophage, respectively, but may mitigate the negative effects of reactive oxidant species (ROS) and improve overall health. In fact, certain plant polyphenols considered to be strong antioxidants, protect SP-A from oxidation[28] and antioxidant supplementation seem to protect from the negative effects of ozone[29,30]. A recent clinical study implicated the alveolar macrophage and innate immunity in COVID-19 with promising therapeutic results[31]. In this study patients were treated with a Bruton tyrosine kinase (BTK) inhibitor to inhibit the BTK-mediated signaling in the alveolar macrophage, and this treatment mitigated the "hyperinflammmatory response" present in

these patients. Based on preclinical studies it is of interest to speculate whether the magnitude of this "hyperinflammatory response" varies as a function of SP-A genotype in the given microenvironment.

Children appear to be considerably less affected by COVID-19 than adults. SARS-CoV-2 uses the angiotensin converting enzyme 2 (ACE2) receptor to enter epithelial cells. A recent study showed that the nasal epithelial cells in children exhibit low, age-dependent activity of ACE2, the younger the children the lower of ACE2 activity[32]. It was postulated that this may be a reason that children are spared for the most part from COVID-19. Of interest, SP-A is also expressed in nasal epithelial cells[33], however, currently nothing is known about the role of SP-A genetics in nasal disease. It is possible that the low ACE2 expression combined with a specific SP-A genotype provide a strong protection in most children from SARS-CoV-2 infection.

In summary, preclinical studies have given us a wealth of information on the differential impact of genetic SP-A1/SP-A2 variants on lung host defense and human studies have shown associations with disease susceptibility of a wide spectrum of pulmonary diseases. The latter Is not surprising since these molecules provide the first line of defense and also contribute to surfactant-related functions[34]. However, there is still a lot to learn, especially in humans. This pandemic may provide an opportunity for focused research on the role of the genetics of SP-A1/SP-A2 innate immune molecules on COVID-19 disease severity. For example, SP-A1 and SP-A2 animal models could be used to study progression and severity of disease in response to different SP-A variants/genotypes. SP-A markers (i.e. SNPs or other) associated with low and high risk in different groups of patients, could be identified to help with clinical management and/or treatment. SP-A1 and SP-A2 variants are differentially regulated[35-42], but virtually nothing is known how this regulation may be affected by infection or other insults. Such information is important if one were to think of therapeutic regimens for maintaining strong innate immunity in the face of infection and other underlying diseases.

CONFLICT OF INTEREST

None.

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REFERENCES

1. Floros J, Wang G, Mikerov AN. Genetic complexity of the human innate host defense molecules, surfactant protein A1 (SP-A1) and SP-A2--impact on function. Crit Rev Eukaryot Gene Expr 2009; 19:125-37.
2. Floros J, Wang G, Lin Z. Genetic diversity of human SP-A, a molecule with innate host defense and surfactant-related functions; characteristics, primary function, and significance. Curr Pharmacogenomics 2005; 3:87-95.
3. Floros J, Hoover RR. Genetics of the hydrophilic surfactant proteins A and D. Biochim Biophys Acta 1998; 1408:312-22. S0925-4439(98)00077-5 [pii];10.1016/s0925-4439(98)00077-5 [doi].
4. Thorenoor N, Umstead TM, Zhang X, Phelps DS, Floros J. Survival of Surfactant Protein-A1 and SP-A2 Transgenic Mice After Klebsiella pneumoniae Infection, Exhibits Sex-, Gene-, and Variant Specific Differences; Treatment With Surfactant Protein Improves Survival. Front Immunol 2018; 9:2404. 10.3389/fimmu.2018.02404 [doi].
5. Thorenoor N, Zhang X, Umstead TM, Scott HE, Phelps DS, Floros J. Differential effects of innate immune variants of surfactant protein-A1 (SFTPA1) and SP-A2 (SFTPA2) in airway function after Klebsiella pneumoniae infection and sex differences. Respir Res 2018; 19:23. 10.1186/s12931-018-0723-1 [doi];10.1186/s12931-018-0723-1 [pii].
6. D'Ovidio F, Floros J, Aramini B, et al. Donor surfactant protein A2 polymorphism and lung transplant survival. Eur Respir J 2020; 55.13993003.00618-2019 [pii];10.1183/13993003.00618-2019 [doi].
7. Silveyra P, Floros J. Genetic variant associations of human SP-A and SP-D with acute and chronic lung injury. Front Biosci (Landmark Ed) 2012; 17:407-29. 3935 [pii];10.2741/3935 [doi].
8. Floros J, Thomas NJ. Genetic variations of surfactant proteins and lung injury. In Surfactant Pathog. Treat. Lung Dis. Edited by Nakos G, Papathanasiou A. Kerala, India: Research Signpost; 2009:25-48.
9. Gandhi CK, Chen C, Wu R, et al. Association of SNP-SNP interactions of surfactant protein genes with pediatric acute respiratory failure. Journal of Clinical Medicine 2020; 9:1183.
10. Lin Z, Thorenoor N, Wu R, et al. Genetic Association of Pulmonary Surfactant Protein Genes, SFTPA1, SFTPA2, SFTPB, SFTPC, and SFTPD with cystic fibrosis. Front Immunol 2018; 9:2256.10.3389/fimmu.2018.02256 [doi].
11. Wang G, Umstead TM, Hu S, Mikerov AN, Phelps DS, Floros J. Differential Effects of Human SP-A1 and SP-A2 on the BAL Proteome and Signaling Pathways in Response to Klebsiella pneumoniae and Ozone Exposure. Front Immunol 2019; 10:561.10.3389/fimmu.2019.00561 [doi].
12. Wang G, Phelps DS, Umstead TM, Floros J. Human SP-A protein variants derived from one or both genes stimulate TNF-alpha production in the THP-1 cell line. Am J Physiol Lung Cell Mol Physiol 2000, 278:L946-54. 10.1152/ajplung.2000.278.5.L946 [doi].

13. Wang G, Umstead TM, Phelps DS, Al-Mondhiry H, Floros J. The effect of ozone exposure on the ability of human surfactant protein a variants to stimulate cytokine production. Environ Health Perspect 2002; 110:79-84. sc271_5_1835 [pii];10.1289/ehp.0211079 [doi].
14. Mikerov AN, Wang G, Umstead TM, et al. Surfactant protein A2 (SP-A2) variants expressed in CHO cells stimulate phagocytosis of Pseudomonas aeruginosa more than do SP-A1 variants. Infect Immun 2007; 75:1403-12. IAI.01341-06 [pii];10.1128/IAI.01341-06 [doi].
15. Mikerov AN, Umstead TM, Gan X, et al. Impact of ozone exposure on the phagocytic activity of human surfactant protein A (SP-A) and SP-A variants. Am J Physiol Lung Cell Mol Physiol 2008; 294:L121-30. 00288.2007 [pii];10.1152/ajplung.00288.2007 [doi].
16. Phelps DS, Umstead TM, Silveyra P, Hu S, Wang G, Floros J. Differences in the alveolar macrophage proteome in transgenic mice expressing human SP-A1 and SP-A2. J Proteom Genom Res 2013; 1:2-26. 10.14302/issn.2326-0793.jpgr-12-207 [doi].
17. Phelps DS, Umstead TM, Floros J. Sex differences in the acute in vivo effects of different human SP-A variants on the mouse alveolar macrophage proteome. J Proteomics 2014; 108:427-44. S1874-3919(14)00310-8 [pii];10.1016/j.jprot.2014.06.007 [doi].
18. Noutsios GT, Thorenoor N, Zhang X, et al. SP-A2 contributes to miRNA-mediated sex differences in response to oxidative stress: pro-inflammatory, anti-apoptotic, and anti-oxidant pathways are involved. Biol Sex Differ 2017; 8:37. 10.1186/s13293-017-0158-2 [doi];10.1186/s13293-017-0158-2 [pii].
19. Thorenoor N, Kawasawa YI, Gandhi CK, Zhang X, Floros J. Differential Impact of Co-expressed SP-A1/SP-A2 Protein on AM miRNome; Sex Differences. Front Immunol 2019; 10:1960. 10.3389/fimmu.2019.01960 [doi].
20. Thorenoor N, Kawasawa YI, Ghandi CK, Floros J. Sex-specific regulation of gene expression networks by surfactant protein-A (SP-A) variants in alveolar macrophages in response to Klebsiella pneumoniae. Front Immunol 2020, in press:
21. Phelps DS, Chinchilli VM, Weisz J, Shearer D, Zhang X, Floros J. Using toponomics to characterize phenotypic diversity in alveolar macrophages from male mice treated with exogenous SP-A1. Biomark Res 2020; 8:5. 10.1186/s40364-019-0181-z [doi];181 [pii].
22. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. JAMA 2020; 2764787[pii];10.1001/jama.2020.6266 [doi].
23. LeVine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfhagen T. Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. J Clin Invest 1999; 103:1015-1021.
24. Watson A, Kronqvist N, Spalluto CM, et al. Novel expression of a functional trimeric fragment of human SP-A with efficacy in neutralisation of RSV. Immunobiology 2017; 222:111-8. S0171-2985(16)30423-5 [pii];10.1016/j.imbio.2016.10.015 [doi].
25. Thomas NJ, Diangelo S, Hess JC, et al. Transmission of surfactant protein variants and haplotypes in children hospitalized with respiratory syncytial virus. Pediatr Res 2009; 66:70-73. 10.1203/PDR.0b013e3181a1d768 [doi].
26. Oosting RS, Van Iwaarden JF, Van Bree L, Verhoef J, van Golde LM, Haagsman HP. Exposure of surfactant protein A to ozone in vitro and in vivo impairs its interactions with alveolar cells. Am J Physiol 1992; 262:L63-8.
27. Haque R, Umstead TM, Ponnuru P, et al. Role of surfactant protein-A (SP-A) in lung injury in response to acute ozone exposure of SP-A deficient mice. Toxicol Appl Pharmacol 2007; 220:72-82. S0041-008X(06)00469-8 [pii];10.1016/j.taap.2006.12.017 [doi].
28. Stagos D, Umstead TM, Phelps DS, et al. Inhibition of ozone-induced SP-A oxidation by plant polyphenols. Free Radic Res 2007; 41:357-66. 773163500 [pii];10.1080/10715760601064714 [doi].
29. Samet JM, Hatch GE, Horstman D, et al. Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 2001; 164:819-25. 10.1164/ajrccm.164.5.2008003 [doi].
30. Steck-Scott S, Arab L, Craft NE, Samet JM. Plasma and lung macrophage responsiveness to carotenoid supplementation and ozone exposure in humans. Eur J Clin Nutr 2004; 58:1571-9. 10.1038/sj.ejcn.1601988 [doi];1601988 [pii].
31. Roschewski M, Lionakis MS, Sharman JP, et al. Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. Sci Immunol 2020; 5.5/48/eabd0110 [pii];10.1126/sciimmunol.abd0110 [doi].
32. Bunyavanich S, Do A, Vicencio A. Nasal Gene Expression of Angiotensin-Converting Enzyme 2 in Children and Adults. JAMA 2020; 2766524[pii];10.1001/jama.2020.8707 [doi].
33. Noutsios GT, Willis AL, Ledford JG, Chang EH. Novel role of surfactant protein A in bacterial sinusitis. Int Forum Allergy Rhinol 2017; 7:897-903. 10.1002/alr.21985 [doi].
34. Lopez-Rodriguez E, Pascual A, Arroyo R, Floros J, Perez-Gil J. Human Pulmonary Surfactant Protein SP-A1 Provides Maximal Efficiency of Lung Interfacial Films. Biophys J 2016; 111:524-36. S0006-3495(16)30471-4 [pii];10.1016/j.bpj.2016.06.025 [doi].
35. Karinch AM, Floros J. Translation in vivo of 5′ untranslated-region splice variants of human surfactant protein-A. Biochem J 1995; 307(Pt 2):327-30. 10.1042/bj3070327 [doi].
36. Karinch AM, Deiter G, Ballard PL, Floros J. Regulation of expression of human SP-A1 and SP-A2 genes in fetal lung explant culture. Biochim Biophys Acta 1998; 1398:192-202. S0167-4781(98)00047-5 [pii];10.1016/s0167-4781(98)00047-5 [doi].
37. Wang G, Guo X, Floros J. Human SP-A 3′-UTR variants mediate differential gene expression in basal levels and in response to dexamethasone. Am J Physiol Lung Cell Mol Physiol 2003; 284:L738-48. 10.1152/ajplung.00375.2002 [doi];00375.2002 [pii].
38. Wang G, Guo X, Floros J. Differences in the translation efficiency and mRNA stability mediated by 5′-UTR splice variants of human SP-A1 and SP-A2 genes. Am J Physiol Lung Cell Mol Physiol 2005; 289:L497-L508. 00100.2005 [pii];10.1152/ajplung.00100.2005 [doi].
39. Wang G, Guo X, Silveyra P, Kimball SR, Floros J. Cap-independent translation of human SP-A 5′-UTR variants: a double-loop structure and cis-element contribution. Am J Physiol Lung

Cell Mol Physiol 2009; 296:L635-47. 90508.2008 [pii];10.1152/ajplung.90508.2008 [doi].
40. Noutsios GT, Silveyra P, Bhatti F, Floros J. Exon B of human surfactant protein A2 mRNA, alone or within its surrounding sequences, interacts with 14-3-3; role of cis-elements and secondary structure. Am J Physiol Lung Cell Mol Physiol 2013; 304:L722-35. ajplung.00324.2012 [pii];10.1152/ajplung.00324.2012 [doi].
41. Noutsios GT, Ghattas P, Bennett S, Floros J. 14-3-3 isoforms bind directly exon B of the 5′-UTR of human surfactant protein A2 mRNA. Am J Physiol Lung Cell Mol Physiol 2015; 309:L147-57. ajplung.00088.2015 [pii];10.1152/ajplung.00088.2015 [doi].
42. Aramini B, Geraghty P, Lederer DJ, et al. Surfactant protein A and D polymorphisms and methylprednisolone pharmacogenetics in donor lungs. J Thorac Cardiovasc Surg 2019; 157:2109-17. S0022-5223(19)30052-2 [pii];10.1016/j.jtcvs.2018.12.098 [doi].