Immune receptor- and transcription factor- stimulatory effects of bacteria and endotoxin in sea spray aerosols on human cells

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Frequent, low-level exposure to marine microorganisms and bioactive compounds in sea spray aerosols (SSA) has been suggested to confer various health benefits (Moore 2015; Asselman *et al.*, 2019; Van Acker *et al.*, 2020). Few studies to date have, however, examined the potential immunostimulatory consequences of SSA exposure. In this study, we investigated the immunostimulatory activity of SSA by exposing human reporter cells to SSA samples with total bacterial counts ranging from 3.0 x 10^3 to 2.4 x 10^6 cells/m^3 air and endotoxin levels from 7 to 2,191 EU/m^3 air. The induction of immune receptors toll-like receptor 4 (TLR4) in HEK-Blue hTLR4 cells and TLR2/6 in HEK-Blue hTLR2/6 cells, as well as the induction of the transcription factor nuclear factor kappa B (NF-κB) and interferon regulatory factors (IRF) in THP1-Dual monocytes were measured. We observed that all tested SSA samples activated TLR4, TLR2/6, NF-κB, and IRF, which correlated dose-dependently with the total bacterial counts, endotoxin levels, or both.

Next, we examined immunostimulation by investigating how exposure to SSA modulate the activation of TLR4, TLR2/6, NF-κB and IRF to subsequent challenges with potent agonists. Human reporter cells were pre-exposed for 30 minutes to SSA samples or negative controls, followed by 24 hours exposure to *Escherichia coli* lipopolysaccharide (LPS) (for HEK-Blue hTLR4 cells and THP1-Dual monocytes) or the synthetic diacylated lipopeptide Pam2CSK4 (for HEK-Blue hTLR2/6 cells). We observed that SSA exhibited a dual modulation effect on both TLR4 activation and TLR2/6 activation. Specifically, SSA samples inhibited TLR4 activation induced by *E. coli* LPS at low bacterial and endotoxin levels, but enhanced it synergistically at high levels. In contrast, SSA samples inhibited TLR2/6 activation induced by Pam2CSK4 at high bacterial and endotoxin levels, while enhancing it synergistically at low levels. Regarding on the modulation on NF-κB and IRF activation induced by *E. coli* LPS, SSA showed a neutral effect at low bacterial and endotoxin levels, while exerted a synergistic effect at high levels.

To further elucidate the potential mechanisms underlying the immunostimulatory effects of SSA, we measured the bacterial communities in SSA samples. This was done using full-length Nanopore sequencing of the 16S rRNA gene. We are now using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved states (PICRUSt2) algorithm, referencing the Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaCyc databases, to analyze which specific bacteria or their predicted metabolic functions contribute to the differential immunostimulatory effects observed.

This study sheds the first light on the immunostimulatory consequences of SSA and its underlying mechanisms. Our findings not only provide new insights into coastal health benefits, but also highlight the need for further research to deepen our understanding on the health implications of SSA exposure.

References

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Keywords
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