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Paradigms and perspectives

Does pollen exposure influence innate immunity to SARS-CoV-2 in allergy or asthma?

Saeideh Hajighasemi, MSc,^a Kirsten Spann, PhD,^a and Janet M. Davies, PhD^{a,b} *Brisbane, Australia*

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The vulnerability of patients with asthma encountering severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still a matter of debate. A growing body of evidence indicates a reduced risk of both SARS-CoV-2 infection and coronavirus disease 2019 (COVID-19) severity associated with atopy.¹ However, how allergen exposure per se, and pollen exposure in particular, might influence SARS-CoV-2-induced inflammation and its clinical outcomes remains unknown.

In vitro allergen exposure can attenuate antiviral response to some respiratory viruses.² Separately, an ecologic study including 31 countries indicated that pollen exposure may increase the risk of SARS-CoV-2 infection.³ Because this ecologic study included only periods when COVID-19 and pollen exposure coincided and was conducted during the exponential surge in COVID cases, its findings may not be generalizable. Nonetheless, with increasing pollen exposure associated with climate change, the interplay between pollen and respiratory viruses, including SARS-CoV-2, warrants consideration. It is postulated that pollen exposure, independent of allergic status, has a direct effect on antiviral defense within the airway epithelium and therefore enhances susceptibility to SARS-CoV-2 infection.

Upper airway epithelial cells are the first to be exposed to inhaled bioaerosols. Pollen grains are complex biologic particles that carry an array of biomolecules including allergens, proteases, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and pollen-associated lipid mediators (PALMs) (Fig 1). These biomolecules induce airway inflammation via multiple routes⁴ to drive allergic sensitization by classical T_H2 cell pathways, but they may also interact with virus particles to increase infectivity and alter antiviral responses in both pollen-sensitized individuals and allergy-free individuals.

Pollen and respiratory viruses induce distinct as well as some overlapping inflammatory markers in airway epithelial cells, although whether coexposure would result in cumulatively

enhanced reactions is uncertain. For example, in the human bronchial epithelial cell line BEAS-2B, exposure to seemingly high amounts (10 mg) of whole birch (*Betula pendula*) pollen grain (WBPB), which carry an array of the aforementioned components, upregulated a high number of inflammatory genes, including *IL6*, *IL-1β*, *CXCL2*, and *IL-24*.⁵ SARS-CoV-2 also induces a number of cytokines, namely, IL-6, IL-1β, and IL-18, via activation of nuclear factor-κB and mitogen-activated protein kinase (MAPK).⁶ However, the balance of effects of coexposure to pollen grains and SARS-CoV-2 on airway inflammation is yet to be determined.

HOW POLLEN COMPONENTS MIGHT INTERACT WITH AIRWAY EPITHELIUM TO INFLUENCE SARS-COV-2 INFECTION

Pollen proteases

The impact of house dust mite allergen protease on epithelial tight junction integrity is well appreciated. Proteases, including aminopeptidases and serine and cysteine proteases, have been found within the pollen coat of some allergenic plants (eg, Bermuda grass and ragweed pollen).⁷ However, the presence and effect of pollen proteases are not well documented because of the predominant use of aqueous pollen extracts (which may lack some pollen coat proteins) in experimental studies. Some studies do indicate that pollen proteases might disrupt epithelial integrity and barrier function,⁴ so it can be postulated that this might increase permeability to allow pollen, and respiratory viruses in general, to enter subepithelial layers, where dendritic cells (DCs) reside (Fig 1).

It can be hypothesized that pollen proteases might enhance virus invasion of the subepithelium by promoting cleavage of the SARS-CoV-2 spike protein to expose the S2 fusion peptide, which mediates virus-cell fusion. In these ways, pollen coexposure could enhance SARS-CoV-2 cellular infectivity, but such mechanisms have not yet been studied (Fig 2).

NADPH oxidase

NADPH oxidases found in pollen extracts from grasses, weeds, and trees can increase allergic inflammation by the induction of reactive oxygen species in the epithelium, which leads to induction of oxidized glutathione or 4-hydroxynonenal and activation of p38 MAPK to stimulate IL-8 and recruit neutrophils. Ragweed pollen NADPH oxidases also enhance mucin production and accumulation of inflammatory cells. In addition, ragweed pollen grains increase intracellular levels of reactive oxygen species in human monocyte-derived DCs via their NADPH oxidases to induce IL-8, IL-6, and TNF-α and stimulate maturation and activation of DCs⁴ (Fig 1).

Oxidative stress generated by pollen NADPH oxidase may increase proinflammatory cytokine production via activation of the NLR family, pyrin domain containing 3 (NLRP3)

From ^athe Centre for Immunology and Infection Control, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, and ^bthe Office of Research, Metro North Hospital and Health Service, Brisbane.

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Corresponding author: Janet M. Davies, PhD, Centre for Immunology and Infection Control, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology at QIMR Berghofer, Bancroft Building, 300 Herston Road, Brisbane, QLD 4006, Australia. E-mail: j36.davies@qut.edu.au. Or: Saeideh Hajighasemi, MSc, Centre for Immunology and Infection Control, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology at QIMR Berghofer, Bancroft Building, 300 Herston Road, Brisbane, QLD 4006, Australia. E-mail: saeideh.hajighasemi@hdr.qut.edu.au.

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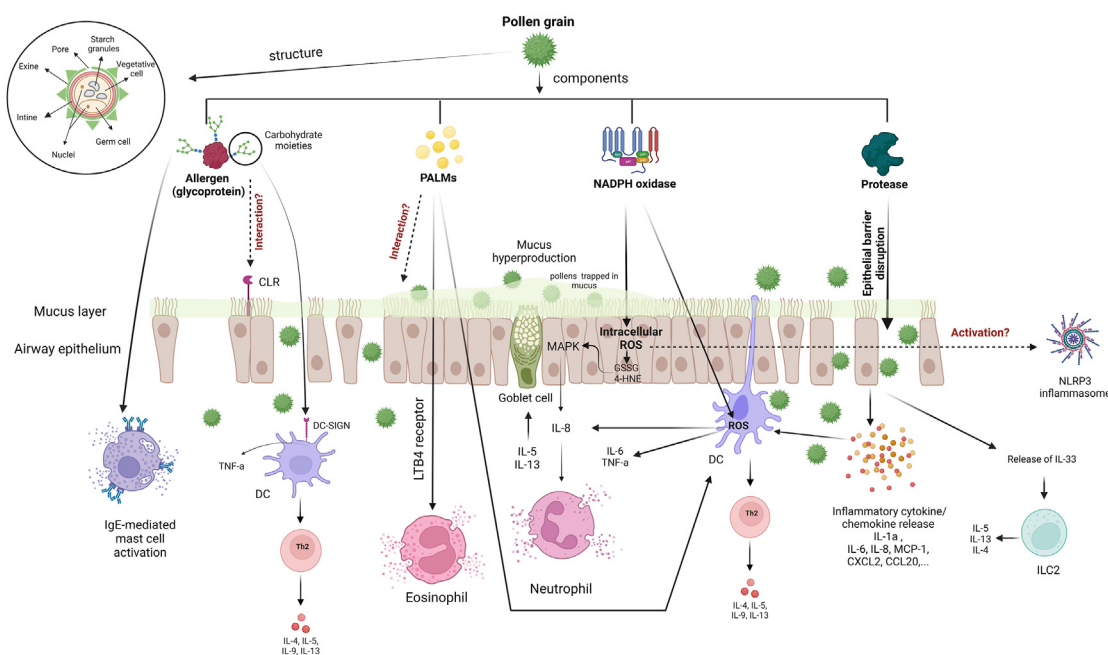


FIG 1. Pollen structure, components, and interaction with cells of airway epithelium and immune system. A pollen grain has an outer (exine) and inner (intine) coat encompassing 1 vegetative cell and 1 germ cell. The vegetative cell contains starch granules that carry allergenic proteins. The allergens are also found in other parts of the pollen grain. In addition to allergens, pollen grains carry proteases, NADPH oxidase, and PALMs. Each of these components can stimulate airway and/or innate immune cells to evoke an immune response. The known interactions are shown in solid lines and putative interactions are shown in dashed lines. *CLR*, C-type lectin receptor; *GSSG*, oxidized glutathione; *4-HNE*, 4-hydroxynonenal; *ILC2*, group 2 innate lymphoid cell; *NLRP3*, NLR family pyrin domain-containing 3; *ROS*, reactive oxygen species. Figure created with BioRender.

inflammasome, as some pollen types stimulate IL-1 β and IL-18³ (Fig 1). Although this mechanism for pollen exposure needs validation with further research, activation of NLRP3 inflammasomes by SARS-CoV-2 or other respiratory viruses⁶ may be enhanced during pollen coexposure.³

PALMs

Pollen grains contain PALMs capable of stimulating a variety of innate immune cells, eliciting a T_H2 cell–dominant immune response and suppressing T_H1 cell–mediated immunity⁴ (Fig 1). Bioactive lipids such as eicosanoid-like components, including leukotriene B4 (LTB4)-like and prostaglandin E₂-like substances, are released from pollen grains after contact with the humid mucosal environment. These hydroxy fatty acids exhibit both chemoattraction and activation activity on eosinophils mediated by LTB4 receptors, independent of allergic status.⁴

Interestingly, elevated levels of inflammatory lipid mediators, predominantly prostaglandins and leukotrienes (including LTB4), have been found in bronchoalveolar lavage fluids from patients with severe COVID-19.⁸ The elevation of inflammatory lipid mediator levels might be another avenue by which coexposure to pollen and SARS-CoV-2 could enhance airway inflammation, but this has not yet been studied.

THE ANTIVIRAL MUCOSAL IMMUNE RESPONSE FOLLOWING POLLEN EXPOSURE

In addition to potentially enhancing inflammation, pollen exposure might dampen the antiviral response. For example,

aqueous extract of timothy grass (*Phleum pratense*) pollen significantly attenuated the IFN- β and IFN- $\lambda_{1,2}$ response to human rhinovirus 16 infection of human primary bronchial epithelial cells by suppressing interferon response factor-7 and melanoma differentiation-associated protein 5, leading to enhanced viral replication. Furthermore, exposure of human nasal epithelial cells to either WBPG or aqueous birch pollen extract reduced human rhinovirus 16–induced IFN- λ_1 .² Intriguingly, this effect was independent of allergic status. In a murine model of RSV infection, ragweed (*Ambrosia artemisiifolia*) pollen extract attenuated antiviral responses *in vivo*.² Because angiotensin-converting enzyme 2 (ACE-2) is an interferon-stimulated gene upregulated in response to IFN- α and IFN- γ ,⁹ it can be hypothesized that dampening of the interferon response by pollen reduces viral-cell surface attachment. Notably, the type 2 cytokine IL-13, which is induced by allergens such as pollen, reduces ACE-2 expression.¹

In contrast to the hypothesized role(s) of pollen in enhancing SARS-CoV-2 infection by facilitating penetration into the sub-epithelium, enhancing inflammation or dampening interferon response, some components in pollen might inhibit viral infection. WBPG upregulates serpin family B member 3 (SERPINB3), which is an inhibitor of serine and sometimes cysteine proteases,¹⁰ in WBPG-exposed BEAS-2B cells.⁵ Furthermore, SERPINB3 inhibits cathepsin, and possibly TMPRSS2, which are involved in priming S protein during SARS-CoV-2 entry.⁶ Whether SERPINB3 inhibits pollen proteases as well is unknown. SERPINB3 is postulated to be involved in the antiviral response to SARS-CoV-2, and it might inhibit SARS-CoV-2 main protein (Mpro), a cysteine protease that produces proteins necessary for viral replication.¹⁰

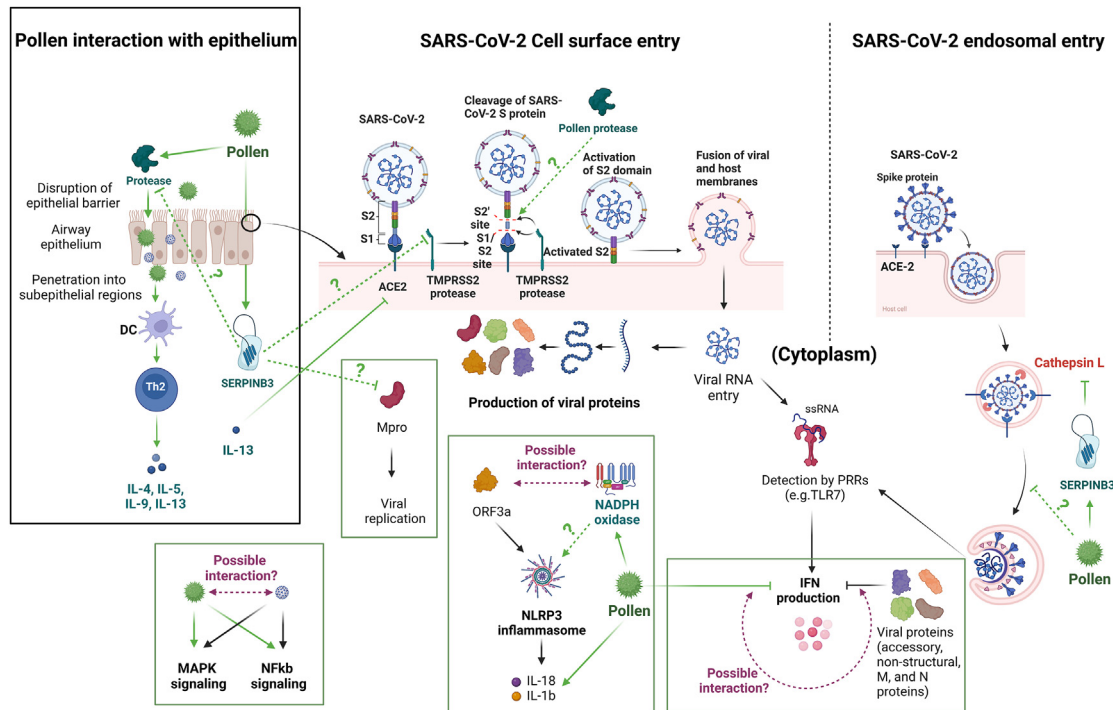


FIG 2. Putative interactions between pollen and SARS-CoV-2. The known interactions are shown in solid lines, and the possible, but not proved, interactions are shown in dashed lines. Green arrows indicate pollen effects, and black arrows are indicative of SARS-CoV-2 effects. Pollen may block endosomal viral entry via SERPINB3 (a cathepsin L inhibitor that might also be a TMPRSS2 inhibitor) and also reduce viral cell surface entry through downregulation of angiotensin-converting enzyme 2 (ACE-2) by inhibiting interferon (IFN) production and inducing IL-13. Pollen might instead enhance SARS-CoV-2 entry, possibly through its protease activity on the S2 subunit. SERPINB3 induced by pollen might inhibit the main protein (Mpro) to block viral replication. Whether SERPINB3 inhibits pollen proteases is not clear. The inhibition of IFN response by both pollen and viral proteins might allow the virus to evade immune surveillances. Viral open reading frame 3a (ORF3a) activates NLRP3 inflammasome to induce IL-18 and IL-1β, and some pollen types also induce IL-18 and IL-1β; however, whether pollen activate the NLR family, pyrin domain containing 3 (NLRP3) inflammasome via reactive oxygen species (ROS) produced by their NADPH oxidase is not clear. Both pollen and SARS-CoV-2 stimulate MAPK and nuclear factor-κB (NF-κB) signaling pathways, but whether they interact is unknown. PRR, Pattern recognition receptor; TLR7, Toll-like receptor 7. Figure created with BioRender.

A summary of the multiple putative and complex interactions between pollen and SARS-CoV-2 at the respiratory epithelial layer is presented in Fig 2. The specificity, or universality, of the effects of pollen components from different taxa (eg, birch, ragweed, or grasses), as well as the influence of dose and timing of these pollen exposures, need to be further delineated in the context of primary human epithelial cells from healthy donors, donors with allergy, and/or asthmatic donors rather than in particular cell lines of limited provenance and lineages. There remain many gaps in knowledge about how particular pollen components, not only allergens per se, induce airway inflammation in health and disease. Little is known about variability in concentration of pollen oxidases, proteases, and lipid mediators between batches of the same pollen or between pollens of different plants, but such factors will undoubtedly influence mucosal responses, which will need to be determined empirically. Moreover, the ways in which pollen interacts with antiviral pathways, and how pollen exposure may influence viral exacerbation of asthma, remain to be elucidated.

Further investigations are needed to deepen insights into respiratory mucosal responses to pollen molecules in healthy nonatopic individuals, as well as in patients with respiratory allergy and/or asthma. In addition to atopy, there appear to be

direct roles for exposure to pollen allergen sources in the modulation of antiviral immunity and viral infection. Deciphering the putative mechanisms of interactions between the various pollen components and specific respiratory viruses, which may influence inflammatory and antiviral responses in the airway, should also be a priority.

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