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Reply: Protease Plays a Role in Ragweed Pollen–Induced Neutrophil Recruitment and Epithelial Barrier Disruption

From the Authors:

We appreciate Dr. Mabalirajan's insightful letter suggesting that pollen proteases might degrade epithelial tight junctions and that the sensing of pollen proteases by protease-activated receptor 2 (PAR2) may stimulate the PAR2/nuclear factor-κB (NF-κB)/IL-8 axis, possibly through cross-talk between Toll-like receptor 4 (TLR4) and PAR2 pathways. In our article, we reported that the repeated pollen-induced TLR4/C-X-C motif ligand (CXCL)/chemokine (C-X-C motif) receptor 2–dependent recruitment of neutrophils stimulates allergic sensitization and induces pollen-induced allergic airway inflammation (1).

Dr. Mabalirajan suggests that, in our study, pollen-derived proteases might have contributed to the pathogenesis of ragweed pollen extract–induced allergic inflammation by increasing epithelial permeability by directly degrading epithelial tight junction proteins. The most direct evidence supporting his hypothesis comes from a recent report that Hop J pollen extract from Japanese Hop increases the epithelial cell permeability and PAR2 levels associated with the degradation of occludin (2). However, not all pollens have protease activity. Indeed, we reported previously that ragweed pollen extract, the extract that was examined in our current study (1), lacks protease activity and instead, has potent intrinsic pollen nicotinamide adenine dinucleotide phosphate oxidase activity (3). This nicotinamide adenine dinucleotide phosphate oxidase activity induces oxidative stress in airway epithelial cells independent of protease activity (3) and vigorously stimulates allergic airway inflammation (3). Similar to the findings of our study, the findings of another study were that timothy grass pollen extract lacks significant protease activity and does not alter the epithelial tight junctions in human bronchial epithelial cells (4). Taken together, these studies suggest that the contribution of proteases in pollen extracts to epithelial tight junctions is dependent on the type of pollen.

The second point Dr. Mabalirajan makes is that the sensing of pollen proteases by PAR2 may stimulate the PAR2/NF-κB /IL-8 axis in epithelial cells. In support of his hypothesis is the fact that PAR2 is expressed in airway epithelial cells (5), PAR2-deficient mice demonstrate greatly reduced house dust mite (HDM)–evoked allergic lung inflammation compared with wild-type mice (6, 7), and the stimulation of PAR2 by the administration of PAR2 activating peptide Ser-Leu-Ile-Gly-Arg-Leu-amide trifluoroacetate salt enhances ovalbumin-induced airway hyperresponsiveness and inflammation (8). However, the administration of the same PAR2-activating peptide Ser-Leu-Ile-Gly-Arg-Leu-amide trifluoroacetate salt in pollen Par j 1 (the major allergen in *Parietaria judaica* pollen)–sensitized animals vigorously inhibits allergic airway inflammation and airway hyperresponsiveness (9). Thus, the role of PAR2 in pollen-induced allergic inflammation may be different from that of HDM and ovalbumin.

The third point Dr. Mabalirajan makes is that pollens may stimulate an interaction between PAR2 and TLR4 and may initiate CXCL8-mediated neutrophil recruitment. This hypothesis is supported by earlier reports that these receptors may interact with each other and facilitate signaling (10, 11). However, although pollen extract from Japanese Hop increases epithelial cell PAR2 levels (2) and plant cysteine protease, bromelain, ficin, and papain or HDM allergens activate PAR2 (12–14), there is no direct evidence that pollens cleave and activate PAR2 (15). One new development in our model that we have reported recently is that myeloid differentiation protein 2 (MD2) is a crucial receptor for ragweed pollen extract to stimulate TLR4-dependent NF-κB activation and CXCL8 secretion (16). Because ragweed pollen extract binds to and stimulates MD2 to initiate TLR4-dependent NF-κB activation, CXCL secretion, allergic sensitization, and allergic airway inflammation (16), additional direct experiments will be required to demonstrate that ragweed pollen extract stimulates an interaction between PAR2 and MD2. At this time, we believe that the allergenic potential of certain pollens like ragweed is dependent on their ability to generate oxidative stress and to stimulate MD2/TLR4/CXCL/chemokine (C-X-C motif)

receptor 2–dependent recruitment of neutrophils by a process that is independent of their intrinsic protease activity or their ability to induce damage to epithelial barrier proteins (3). ■

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