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Title: Immunomodulatory effects of the rye grass pollen group 5 allergen on respiratory epithelial cells

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Body: Introduction: Several important aeroallergens are known to modulate respiratory epithelial function (RE), suggesting an immunological role. Rye grass is a significant contributor to pollen allergy including the ribonuclease Group 5 allergen but the effects of its biochemical activity on RE is unknown. Methods: Recombinant rye grass pollen allergen rLol p 5 and its N- and C-terminal domains were cloned, sequenced and expressed in E. coli. Physicochemical and modeling studies were conducted using Phyre^2, SDS-PAGE and HPLC. Antigenicity and ribonuclease activity were determined using immunoblot and enzyme assays, respectively. RE modulation was evaluated using the A549 cell line and IL-8 ELISA. Results: The protein sequence of the cloned Lol p 5 gene was similar to the previously reported Lol p 5a-c, with greatest homology to 5a. Modelling studies showed rLol p 5 was structurally similar to the previously reported Group 5 structure. HPLC showed the mature protein comprised two peaks with mol. wts of 13K (26%) and 86K (74%), as did the N- and C-terminal domains. In contrast, SDS-PAGE revealed proteins of the expected sizes. Western blot assay showed that each expressed protein was immunoreactive and enzymatically active, and all caused A549 cells to release IL-8 in a dose-dependent manner, and unrelated to LPS contamination (<10 ng/mL). Conclusions: The mature protein and both domains demonstrated ribonuclease activity, appeared to exist as oligomers and caused IL-8 release. The mechanism involved in chemokine release remains to be established.