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International Conference on Glycobiology- Mass spectrometry based identification of novel allergens from sunflower pollen: A common sensitizer to rhinitis patients

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Abstract:

Allergy is a burning problem in today's world. Allergy is caused by several environmental antigens or allergens present in pollen grains, fungal spores, dust mites, etc. Atmospheric pollen grains are the major cause of inhalative allergies, affecting 15-30% of world population. Allergens are mainly immunogenic proteins or glycoproteins regarded as a foreign substance by the human immune system. Therefore, it is necessary to identify and characterize allergenic proteins from severe allergy causing pollen grains for proper diagnosis and treatment of allergy sufferers. Helianthus annuus pollen grains are present in large proportion in the aerosol of Kolkata, a mega-city of India. Sunflower is a common flowering plant grown all over the world for its immense economic and ornamental value. Allergic rhinitis caused by sunflower pollen grains is reported from different countries including India. Present study aims to identify and characterizes the sunflower pollen allergens using immuno-proteomic tools and emphasizes on purification of a novel pectate lyase allergen. Allergenic potential of sunflower pollen grains was investigated by several clinical and immunoproteomic studies followed by mass spectrometry based identification of allergens. Homology driven search of MS/MS data of these IgE-reactive proteins identified seven previously unreported allergens from sunflower pollen. Hierarchical clustering of 2D IgE-immunoblot with patient sera revealed pectate lyase as a major sunflower pollen allergen, which is a glycoprotein. In the present study, pectate lyase has been purified from sunflower pollen for the first time. Purified pectate lyase was further characterized by enzyme assay, immunoblot and mass spectrometry. Other studies such as cross reactivity, epitope mapping, glycan profiling of this allergen will open new avenues to improve the current tools of component resolved diagnosis and immunotherapy of pollen allergy.

Experimental design, materials and methods

Using bottom-up proteomic approach we have previously identified sunflower pollen allergens. The experimental workflow was designed to identify allergenic pollen proteins of sunflower. First, clinico-immunological tests were performed to understand the prevalence of sensitivity towards sunflower pollen among the atopic population. Sera from selected

sunflower positive patients were used as the probe to detect the IgE-reactive proteins from twodimensional electrophoretic separated proteome of sunflower pollen. Finally, these allergens were identified by mass-spectrometry.

2.1. Pollen sampling and protein extraction

Pure pollen grains of H. annuus were collected from mature anthers of the fresh flowers growing around the city during their peak flowering period (April to the first week of July of 2012–2014). Total pollen protein was extracted from 1 g of pollen in 20 ml of 0.1 M phosphate buffer (PB), pH 7.2 and used for serological studies. For proteomic analysis, total protein was extracted using trichloro-acetic acid (TCA)-acetone protocol following the method described earlier.

2.2. Patient selection and clinical studies

Pollen allergic patients, visiting Mediland Diagnostics, Kolkata were tested with antigenic extract of sunflower pollen grains. Patients with positive cutaneous response against sunflower pollen antigen were selected, and 5 ml of peripheral blood were collected for immunological studies. The patient group in our study was represented by 20 individuals sensitive to sunflower pollen, while the control group included six non-atopic subjects. The total IgE, specific IgE and released histamine in these sera were quantified. This study protocol was approved by the human ethics committee of Bose Institute and Mediland Diagnostic Clinic, Kolkata. Informed written consents were obtained from patients and non-allergic volunteers for participation in the study. In the case of minors, informed written consents were obtained from their guardians.

2.3. 2 Dimensional (2D) gel electrophoresis and 2D immunoblot

Note : This work is partly presented at International Conference on Glycobiology (September 21-22, 2017| HOUSTON, USA)

2D electrophoresis was performed following the protocol earlier described with minor modification. Around 120 mg of protein was reconstituted in 125 µl rehydration buffer containing 0.75% IPG (pH 3–10 linear) buffer (v/v) (GE Healthcare), 25 mM DTT and traces of bromophenol blue. The sample was applied to 7 cm IPG strip (pH 3–10 Linear) in a re-swelling tray and left overnight at room temperature for rehydration. Separation of proteins in the first dimension was carried out in Ettan PGphor 3 isoelectric focusing system (GE Lifescience) as per manufacturer's instructions. The second dimension separation was performed in miniVE Vertical Electrophoresis System (GE Healthcare).

2.4. Sample preparation for mass spectrometry

For mass spectrometry (MALDI TOF/TOF and LC-ESI qTOF), spots from 2D gel corresponding to the IgE reactive spots on 2D blot, were excised and subjected to in-gel trypsin digestion following the protocol as described by Shevchenko et al. [4] with slight modifications. Briefly, the gel pieces were destained with ethanol in 50 mM ammonium bicarbonate (pH 8.0) (1:1 v/v) and Acetonitrile (ACN).

Reduction and alkylation was done with 10 mM DTT and 55 mM iodoacetamide respectively. Digestion was carried out in 12.5 ng/ml modified sequencing grade Trypsin Gold (Promega) at 37 °C for 16 h.Tryptic fragments were eluted from gel pieces by vigorous vortexing in extraction buffer containing 3% TFA and 30% ACN. The final volume of the sample was reduced up to 10 times in Speed Vac Thermo Fischer). Approximately, 1.5 µl of peptide digests were mixed with 5 volumes of 0.5 mg/ml α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (Bruker Daltonics), spotted on MTP 384 ground steel target plate (Bruker Daltonics) and air dried. For LC-ESI experiments the solvent was completely evaporated and the peptides were dissolved in a suitable volume of 2% ACN containing 0.1% formic acid. This reconstituted sample was then mixed with solvent A used for loading onto LCcolumn.

2.5. MALDI-TOF/TOF analysis

Mass spectra of trypsin-digested proteins were obtained in Autoflex II MALDI-TOF/TOF (Bruker Daltonics). Mass spectra were recorded in linear mode equipped with a pulsed N2 laser (λ¼337 nm, 50 Hz) at 54% power in positive ion mode. After MS spectra acquisition, the instrument was switched to LIFT mode. The MS/MS spectra of top ten peptides with the highest intensity were recorded by fragmentation of these peptides using LID (laser induced dissociation). MS/MS spectra were acquired with a minimum of 4000 and a maximum of 8000 laser shots using the instrument calibration file. Spectra baseline subtraction, smoothing and centroiding were performed in Flex Analysis software v3.0 (Bruker Daltonics).

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