(SS02) Cross-reactivity of pollen allergy

Date: August 25
Place: Room 5233 (oral), Room 6302 (poster)
Organizers: Terumi Midoro-Horiuti,Yasuto Kondo & Reiko Kishikawa
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Purpose: Plants from related species have cross-reactive allergens. Some of the pollen allergens induce oral allergy syndrome (OAS) among pollen allergen sensitized subjects when ingesting food which contain cross-reactive allergens. We will discuss recent findings on cross-reactivities among pollen allergens including OAS.

Oral Presentation

Aug. 25 [PM2] Room: 5233

Chair: Terumi Midoro-Horiuti

14:30-14:50 **The molecular basis for birch pollen related oral allergy syndrome (OAS)** SS02-O01 (490)

Michael D Spangfort, Jens Holm, Lars Harder Christensen, Mercedes Ferreras, Henrik Ipsen, Peter Würtzen, Michael Gajhede, Jørgen Larsen, Kaare Lund

14:50-15:10 **Birch pollen related OAS in Japan** SS02-O02 (473)

Hideaki Shirasaki

Chair: Yasuto Kondo

15:10-15:30 **Cross-reactivity due to pathogenesis-related (PR)-protein** SS02-O03 (335)

Terumi Midoro-Horiuti

15:30-15:50 **Structural basis for cedar pollen related OAS** SS02-O04 (155)

Randall M. Goldblum, G. Silky Sharma, Jay H. Van Bavel, Terumi Midoro-Horiuti

Aug. 25 [PM3] Room: 5233

Chair: Reiko Kishikawa

16:20-16:40 **New method for assessment of cross-reactivity** SS02-O05 (250)

Yasuto Kondo, Kenichi Tanaka, Norihiko Naruse, Satoko Suzuki, Noriko Hirata, Kazuo Yamawaki, Yuriko Ohkubo, Chisato Inuo, Yoichi Nakajima, Ikuya Tsuge, Atsuo Urisu

Poster Presentation

Aug. 25 [PM1] Room: 6302

13:30-14:30 **Pollen grains characteristics over the State of Kuwait from October 2009 to August 2011** SS02-P01 (205)

Mohamed Ismail Ibrahim, Ali El Dossari

SS02-O01 (490)
The molecular basis for birch pollen related oral allergy syndrome (OAS)

Michael D Spangfort[1], Jens Holm[2], Lars Harder Christensen[2], Mercedes Ferreras[2], Henrik Ipsen[2], Peter Würtzen[2], Michael Gajhede[3], Jørgen Larsen[2], Kaare Lund[2]

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Birch allergic patients often experience OAS upon ingestion of vegetables and fruits, most prominently apple. The underlying cause for OAS is cross- reactivity of IgE antibodies towards proteins that are homologous to the major birch-pollen allergen Bet v 1. We examined the structural similarity of Bet v 1 and the homologous allergen from apple, Mal d1, and demonstrate how increasing and decreasing surface similarity affects the cross-reactivity of IgE antibodies towards variants of the two allergens. The three-dimensional structure of Bet v 1 has been determined by X-ray diffraction and NMR. The structure shows three regions predicted to harbor antibody epitopes shared with Mal d 1 as well as with homologous proteins from related trees eg hazel. In addition, the contact-amino acid residues of a monoclonal anti-Bet v 1 antibody (BV16) were identified by resolving the three-dimensional structure of a Bet v 1-antibody (Fab-fragment) complex. BV16 recognizes a structural epitope of Bet v 1 and binds Bet v 1 with high affinity while binding to Mal d 1 is undetectable. However, recombinant variants of Mal d 1 where surface-exposed amino-acid residues within the area homologous to the BV16 epitope of Bet v 1 were substituted with the corresponding residues of Bet v 1 bound the BV16 antibody with the same affinity as Bet v 1. This demonstrates that conformational epitopes can be grafted onto a homologous scaffold-molecule without loss of functionality. Clinical symptoms of AOS caused by exposure to apple rely on cross-linking of receptor-bound IgE antibodies on the surface of effector cells (mast cells and basophils), and require the presence of at least two non-overlapping IgE epitopes. Given that the IgE repertoire of birch pollen allergic patients are specific for Bet v 1 and reactivity towards Mal d 1 caused by cross-reactivity, only a minor part of patient's IgE repertoire is expected to be responsible for AOS. Using well-defined and affinity-engineered recombinant IgE antibodies we recently examined the mechanistic basis for IgE-allergen mediated effector cell activation, and we demonstrated that only two different IgE specificities, the minimal theoretical number, are required for efficient activation. Furthermore, the maximal level of effector cell activation only requirds that one antibody of the specific IgE-repertoire is of high affinity, even with the total number of different IgE antibodies being as low as two, indicating that AOS experienced by birch allergics may depend on a very low number of cross-reacting IgE antibodies.

Keywords: Allergen cross-reactivity, epitope-grafting, allergen structure, recombinant IgE, effector cell activation.

SS02-O02 (473)
Birch pollen related OAS in Japan

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Pollen-allergic patients frequently present oral allergy syndrome (OAS) after ingestion of several kinds of plant-derived foods. The majority of these reactions are caused by cross-reactive structures that are present in birch pollen. Birch pollen is a major cause of pollinosis in northern Japan and is responsible for cross-reactive oral allergies to fruits, nuts, and vegetables. IgE reactivity to individual birch pollen allergens has been shown to differ between populations of birch pollen-allergic patients

living in different countries. We examined the IgE profiles to recombinant birch pollen allergens in birch-sensitive patients living in Sapporo. Of 40 sera with positive CAP results for natural birch pollen extract, 39 (97.5%) had specific IgE towards rBet v 1; 6 (15%) contained specific IgE against rBet v 2. rBet v 4 reactivity was documented in only one subject (2.5%). These results suggest that the specific IgE reactivity profiles to birch pollen allergen in birch-sensitive patients in Sapporo correspond to those in Scandinavia, possibly due to the heavy birch pollen exposure in this area. Moreover, we evaluated relationship between IgE profiles and food which caused OAS symptoms. Rose-family fruit-apples, peaches, cherries, pears, plums, and strawberries-often caused OAS regardless of positive or negative rBet v 2 CAP and were associated with rBet v 1. In contrast, more of those who were rBet v 2 CAP-positive had OAS to non-rose-family fruit-melon and watermelon-than those Bet v 2-negative. In northern Japan, Bet v 1 is associated with OAS due to Rosaceae fruits and Bet v 2 with OAS due to non-rose-family fruit.

Keywords: birch, OAS, pollinosis, Bet v1, Bet v2.

SS02-O03 (335)

Cross-reactivity due to pathogenesis-related (PR)-protein

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Higher plants protect themselves against various stresses through patterned, physiologic alterations or "defense response." Plant proteins produced as plant defenses are called "defense-related proteins." One group of defense-related proteins produced in response to pathogens is termed pathogenesis-related (PR) proteins. PR proteins are characterized by certain common chemical properties, such as low molecular weight, stability at low pH and resistance to proteases. Production of PR proteins is induced in plants by stress, including fungal and bacterial infections, drought, flooding, freezing temperature, ozone, ultraviolet B light (UV-B) and mechanical injuries. Many PR proteins from plants are allergenic. PR proteins represent an increasingly important group of plant-derived allergens. Members of PR protein groups 2, 3, 4, 5, 8, 10, and 14 have demonstrated allergenicity. PR2-, 3-, 4-, and 8-homologous allergens are represented by the latex allergens. Cross-reactivity of PR3-homologues latex allergen, Hev b 6.02, with some fruit (avocado, turnip, chestnut and banana) allergens may be a reflection of the representation of homologous PR proteins among varied plants. The expression of one of the representative PR5-homologous cedar pollen allergens, Jun a 3, is highly variable across years and geographic areas, possibly because of variable induction of this PR-protein by environmental factors. PR10-homologous birch pollen allergen, Bet v 1, is structurally similar to and cross-reacts with PR10 proteins from fruits (eg, Mal d 1 from apple) which cause oral allergy syndrome (OAS). PR14 allergens (eg, Zea m 14 from maize) consist of lipid transfer proteins (LTP) found in grains and fruits (apricot, peach, apple, weed and soybean) and are inducers of anaphylaxis. PR-homologous allergens are pervasive in nature. Similarity in the amino acid sequences among members of PR proteins may be responsible for cross-reactivity among allergens from diverse plants. Induced expression of PR homologous allergens by environmental factors may explain varying degrees of allergenicity. Man-made environmental pollutants may also alter the expression of some PR protein allergens.

Keywords: allergy, birch, food, latex, pollen.

SS02-O04 (155)
Structural basis for cedar pollen related OAS

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Mountain cedar (*Juniperus ashei*, MC, Cupressaceae) pollen, like that from Japanese cedar trees, is a major cause of winter-time hypersensitivity in the central part of the US. Some patients with nasal hypersensitivity to other pollen allergens also have oral allergy syndrome (OAS), an IgE-mediated reaction of the lips, mouth and throat, after eating native fruits and vegetables. OAS is thought to be due to the patient's IgE anti-pollen antibodies that cross-react with proteins in these foods. OAS has not been reported in MC pollinosis, but OAS to tomatoes has been described in a few patients with Japanese cedar pollinosis. We performed a mail-out / telephone survey of 800 mountain cedar-sensitive patients in the Austin, TX area. After telephone screening, 28 patients were interviewed, skin tested and had serum collected for serological testing. Of the 28 cedar sensitive patients with suspected food allergies, 15 had the typical symptoms of OAS. Eleven of the 28 subjects had positive skin tests to tomato and 6 the 11 tomato sensitive patients also has positive skin tests to banana. Subjects with oral allergy syndrome to tomatoes and bananas tended to have stronger cutaneous and in vitro reactivity to cedar pollen. The intensities of the tomato and banana reactivities were correlated with the intensity of cedar reactivity. Sera from the 11 of the cedar-sensitive patients with positive skin tests to tomato, banana and apple were tested for IgE antibodies to these fruits by ImmunoCap. ImmunoCap inhibition assays with cedar and tomato extracts were performed on 7 sera with adequate IgE antibodies to one or more of the fruits. The results of the inhibition experiments demonstrated strong cross-reactivity between IgE antibodies to cedar pollen and the three fruits. We showed that the primary sensitization was to mountain cedar, since absorption with cedar pollen extract strongly inhibited reactivity to each of the fruits, but absorption with tomato extract did not significantly inhibit IgE binding to cedar extract. This is the first report of an OAS in mountain cedar pollinosis patients. Sensitivity to tomato, banana and apple should be considered in cedar-sensitive patients and confirmed if symptoms develop. Given the similarity in the structure of the major allergens MS and Japanese cedar, it is likely that a subset of Japanese patients with OAS to tomato may also react to bananas and potentially apples.

SS02-O05 (250)
New method for assessment of cross-reactivity

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Traditionally ELISA or RAST inhibition studies have been used to demonstrate cross-reactivity; however, the results may be inconsistent with clinical symptoms. Cross-reacting carbohydrate determinants (CCDs) have been known as primary cause of this phenomenon. We have developed a passive absorption test of basophil activation, which is a combination of an IgE competition test and a basophil activation test. Using this new test, the assessment of cross-reactivity is expected to be more consistent with clinical symptoms. Sera were obtained from five patients with pollinosis to Japanese cedar pollen (JCP) with positive IgE results for tomato fruit (TF) and bromelin (CCD) by Immuno-CAP analysis. Complaints of oral allergy syndromes to TF were obtained by interview. ELISA inhibition studies were performed using both extracts (0.1 mg/ml). The new method

consisted of a few steps. At first, patient sera were reacted with allergen-adsorbed (0, 0.01, 0.1, 1.0 mg/ml) CNBr-activated beads. After the beads were removed, the treated sera were reacted with IgE-stripped basophils from a non-allergic-healthy donor, for 1 h at 37°C. Lastly, these obtained basophils were incubated with each concentration of both extracts of JCP and TF at a concentration of 1mg/ml for 15 min, and CD63 expression was measured. For the results using ELISA inhibition, cross-reactivity between JCP and TF was seen in all patients. On the other hand, the results from the new method were different. CD63 expression was inhibited by the presences of both allergens in three patients; however, in two patients with relatively high-IgE to CCD compared with tomato, no CD63 expression was seen. The presence of expression and clinical symptoms were consistent. IgE competition between both antigens (JCP and TF) was demonstrated by both methods. Regarding the assessment of cross-reactivity, the new method was more consistent with clinical symptoms.

SS02-P01 (205)

Pollen grains characteristics over the State of Kuwait from October 2009 to August 2011

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Pollen grains in fallen dust were trapped using special sand traps cover the State of Kuwait during a period of two years from October 2009 to August 2011. Pollen grains concentration of 2009-2010 is higher in percentage than that of the year 2010-2011. It is also clear that pollen counts vary from season to season. Two pollen peaks are pronounced in spring (April-May) and autumn (October-Nov.). The first peak is higher and is due to the greater number of pollen grain species which flower during the spring season such as Chenopodiaceae, Gramineae (Poaceae), Cyperaceae, Leguminosae, and Plantaginaceae. The second peak in autumn (October-Nov.) is lower and is characterized by the abundance of Chenopodiaceae and Leguminosae. On the other hand, two troughs have been observed during summer (July-August) and winter (January-February) seasons due to summer drought, dust storm and winter wind and rainfall. The summer lows are characterized by higher counts than the winter lows. In autumn and winter, Chenopodiaceae dominates followed by Leguminosae. Chenopodiaceae and Leguminosae together comprise over 70% of the total pollen during autumn. The spring peaks are higher due to the greater number of Cyperaceae and Gramineae. The local flora is the main source of the pollen load and the concentration depends on the amount of vegetation around each station. Except for very low percentage (2-3%) of Pinaceae pollen recorded in the north and northwest of Kuwait which could be interpreted as being transported by the north-westerly wind. Pollen distribution maps are constructed for the most abundant pollen families.

Keywords: pollen grains concentrations, fallen dust, Kuwait.