**Session:** Beyond IgE - Mediated Mechanisms in the Workplace

**Wednesday, 14 October 2015: 03:30 PM - 05:00 PM, Coex Convention Center, Room 201 (Floor 2)**

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**Baker’s Asthma: A Model of Interacting between Innate and Th2 Immune Responses**

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**Conflict of Interests**

- Medical director for FHR immunosurveillance program

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**Learning Objectives**

- Discuss background and general properties of enzymes in the workplace
- Describe what is Baker’s asthma
- Define the mechanisms of action pertaining to Baker’s asthma

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**Enzymes**

- Proteins used as biocatalysts to reduce or replace the use of chemicals in a variety of processes
- Used in multiple, repeated processes in a variety of industries
  - Cleaning
  - Food processing
  - Animal feed
  - Fuel alcohol
  - Textile
  - Paper
  - Pharmaceuticals

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**History of Commercial Use of Enzymes**

- First enzyme introduced commercially in the USA and England was "Alcalase™" in 1967
- Alcalase was isolated from *Bacillus subtilis* through a submerged fermentation process,
  - Used in soap detergents
- Within 3 years, 80% of all soap detergents sold in the USA contained enzymes

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**Health Effects**

- Flindt and Pepys reported the first cases of respiratory symptoms in detergent workers after inhalation exposure to Alcalase and Maxatase® B. subtilis derived powdered enzymes.
  - 20 workers with respiratory symptoms had positive wheal and flare skin test responses to enzymes prepared from *B. subtilis* spore extracts
  - Strong indication that enzymes were allergenic and that susceptible exposed workers were at increased risk for sensitization leading to asthma.

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**Health effects (cont.)**

- Subsequently strong correlations in multiples studies have been found between respiratory symptoms, enzyme exposure, positive skin test reactions to enzymes, and specific bronchial inhalation challenges to support enzyme induced occupational asthma and other respiratory diseases
Risk Factors For Sensitization and Asthma

- Not entirely clear
  - Magnitude of exposure
  - Atopy
  - Smoking?
- Houba et al. found IgE sensitization found in 1.4%, 12.8%, and 30.4% among workers in low-, medium-, and high-exposure areas, respectively.

Plant derived enzymes

- Papain – pharmaceutical, cosmetic and food industries
- Chymopapain – dissolution of herniated lumbar discs
- Emphyrase – anti-inflammatory drugs
- Pepsin – liquors, cheeses and cereals
- Bromelain – pharmaceuticals
- Pectinases – food industry
- Phytase and β-glucanase – animal feed industry
- Lipase – egg industry

Microbial derived enzymes

- Bacterial organisms - household cleaning products and detergents
  - Bacillus
  - Pseudomonas
- Fungal organisms – food industry
  - Aspergillus
  - Streptomyces
  - Trichoderma

Microbial derived enzymes (cont.)

- α-Amylase enzymes derived from Aspergillus oryzae
  - Added to baking flour in order to compensate for the low natural content of amylases and carbohydrates fermentable by yeast
  - Improves the rising of dough and the quality of the bread

Baker’s asthma

- One of the most common causes of occupational asthma in certain parts of the world
  - Flour dust allergens
  - Enzymes
  - Other food products
  - Insects

Baker’s asthma

- Newer enzymes
  - Amylase/trypsin inhibitors
  - Xylanase
  - Cellulase and hemicellulase
  - Amyloglucosidase
  - Glucose oxidase
  - Phospholipase
  - Asparaginase (reduces acrylamide formation in products at high baking temperatures)

Incidence of Baker’s Asthma

- From the SWORD and SHIELD registered data base annual incidence for:
  - UK was 290–450 cases per million between 1989 to 1994
  - Sweden was 800 in 1984–86
  - Finland was 4000 in 1990
- Incidence and prevalence rates vary between studies
Wheat Allergens as Molecular Tools for Diagnosis of Bakers Asthma: Why are they Important?

1) To establish potential links between sensitization profiles and clinical symptoms, geographical areas, or age;
2) To compare molecules involved in different routes of sensitization (inhaled versus ingestion);
3) To predict potential cross-reactions with allergens from plant foods or pollen;
4) To investigate changes in allergic capacity in cereal (wheat)-derived foods/substrates;
5) To engineer allergen variants with modified allergenic properties (i.e., lower IgE-binding potency).

IgE mediated mechanisms

- 81 bakers with occupational symptoms were evaluated using commercial tests to wheat allergens
  - Skin prick testing
  - Specific IgG
  - ISAC (Immuno Solid-phase Allergen Chip) microarray
  - Dot blotting to 6 wheat allergens (Tri a 19, Tri a 27, Tri a GST, Tri a 32, Tri a 12 and Tri a DH)

Results

- Positive wheat SPT in 29 bakers with occupational asthma
- Positive IgE to wheat or rye flour for 18 wheat allergen components and to rye flour for 18 wheat allergen components
- Wheat dot blotted allergen was found in 22 bakers
- Tri a 32 and Tri a GST were positive in 13 and 3 bakers, respectively and were both associated with work related dermatitis

Conclusions: Specific wheat allergens may be associated with specific occupational allergic phenotypes

Component analysis of sIgE recombinant wheat flour proteins

- Sera from 101 bakers with wheat flour allergy was tested using 19 recombinant wheat flour proteins and 2 cross reactive carbohydrate determinants by CAP-FEIA
- Sera of 29 pollen and wheat sensitized patients without occupational exposure were used as controls
- Diagnostic efficiency of sIgE testing of single allergens and combination allergens were assessed for their ability to discriminate between subjects with baker’s allergy and controls.

Results

- 91% of bakers had sIgE levels ≥ 0.1 kU/L to at least one of 21 allergens
- Highest frequencies for Tri a 27 (Halo reductase) and Tri a 28 (wheat dimeric α amylase inhibitor)
- Cross reactivity between grass pollen was found for 9 wheat allergen components and to rye flour for 18 wheat allergen components
- 5 wheat proteins (Tri a 27, 28, 29-02, 39 and 32) produced the highest AUC using ROC analyses which was still lower than wheat or flour specific IgG

Conclusions

- Wheat component testing is helpful for distinguishing between sensitization to occupational flour exposure and wheat sensitization
- For diagnosis of bakers’ asthma specific testing to wheat or rye flour is still mandatory due to increased sensitivity

Using wheat component allergens to predict different wheat allergy phenotypes

- Background: Specific IgG to gliadin encoded on a marker for wheat-dependent exercise induced anaphylaxis and Tri a 14 was found to induce IgG response in bakers’ asthma
- Rationale: To evaluate whether wheat components could discriminate between wheat allergy phenotypes
- Methods: 101 patients with wheat induced anaphylaxis and/or celiac disease (≥ 21) were included in this study, 56 patients were sensitized to Tri a 14
- Results: Presence of serum specific IgG to Tri a 14 was higher in Group I (70%) than Group II (4.8%) whereas serum specific IgG to gliadin was significantly higher in Group II (25%) than Group I (4.8%)
- Conclusions: A Tri a 14/Gliadin may be a potential marker for predicting baker’s asthma.

Immunopathogenesis: What do we know about Baker’s asthma

- Disruption of nasal and bronchopulmonary cell walls enhances systemic exposure to relevant enzyme proteins
- Proteolytic activity of these enzyme/allergens can act as adjuvants to enhance Th2 cell-dependent IgE mediated allergenicity
- Certain proteases may also induce disease by disrupting cell proteins such as toll-like receptors (TLR4)
- Immune responses resulting in lung disease demonstrated in several animal models (mice, guinea pigs and monkeys)

Protease Hypothesis

- Immune responses are triggered by exposure to specific danger signals
  - Induction of effector immunity resulting in active inflammation
  - Determination of the direction of immunity (Th1, Th2, Th17)
  - Mediated through TLR like receptors (the antigen will dictate Th1 vs. Th2 response)
Pathogen associated molecular patterns (PAMPS)

- Enzymes act as adjuvants capable of inducing Th2 responses through TLRs

Insect Model: Insect defensive mechanisms involve enzymatic cascade leading to the microbe killer Persephone serine protease. The enzyme Persephone (PP) induces a broad overview of enzyme activation, leading to a complex array of protease-activated host defense reactions.

Model 1: Insect model – Pro-Enzymes are activated via the endogenous proteinase Persephone (PP). The cascade results in the cleavage of Pro-spaetzle to yield spaetzle, the final common ligand for Toll. Activation of Toll induces a broad anti-microbial defensive response against fungi, bacteria and other organisms.

Plant Model: Plant model – Bacterial proteinases such as AvrPphB can activate plant proteins such as PBS1 and RPS5 to induce a defense response against bacterial invasion.

Mechanisms by which allergenic proteinases may induce allergic responses through Toll-like receptors

Model 1: Allergenic proteinases may induce enzymatic cascades leading to a common cleavage product that is capable of binding to one or more Toll-like Receptors (TLRs) and initiating essential allergic immune responses such as TH2 differentiation and IgE secretion.

Model 2: Allergenic proteinases may cleave and directly activate distinct immune receptors such as CD23, CD25, and PAR2 to induce allergic responses.

Clinical Key Points

- Baker’s asthma is often preceded by rhinitis, and skin involvement is often concomitant.
- Frequently there is atopy and sensitization to flour and/or enzymes (i.e., α-amylase).
- Mechanisms behind cases without overt allergy to bakery allergens are unknown.
- Risk is increased by high exposure to bakery dust.

Management

- Reduce exposure by dust control or relocation.
- Change of job to non-bakery work is often necessary.
- Long term use of respirators is usually not feasible in bakeries.

Prevention

- There is an exposure–response relation, meaning increased risks for baker’s asthma, rhinitis, and sensitisation by exposure to flour or enzyme.
- Today’s MELs for flour dust (≤3 mg/m3) probably do not protect against baker’s asthma.
- Dust control in bakeries includes adequate local exhaust ventilation and good work practice. General dilution ventilation has only marginal effect on dust levels.

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