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)

Internal Medicine

University of Cincinnati

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

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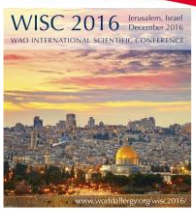

Conflict of Interests

- Medical director for FHR immunosurveillance program

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

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

Bernstein JA, Sarlo K, Houba R. Enzymes IN: Bernstein DI, Malo J-L, Chan Yeung M, Bernstein IL. Eds. Asthma in The Workplace 4th Edition. Informa. 2013.

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

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.

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.

Brisman J. Occup Environ Med 2002;59:498–502.
Houba R, et al. J Allergy Clin Immunol 1997;99: 286–92.

Plant derived enzymes

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

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Microbial derived enzymes

- Bacterial organisms - household cleaning products and detergents
  - Bacillus
  - *Pseudomonas*

  and
- Fungal organisms – food industry
  - *Aspergillus*
  - *Streptomyces*
  - *Rhizopus*
  - *Trichoderma*

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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

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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

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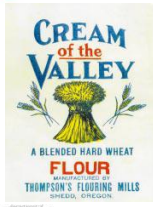

Table 1 Allergens associated with baker's asthma and rhinitis

Cereal flours	Wheat, Rye, Barley, Oats, Rice, Maize
Non-cereal flours	Buckwheat, Soybean flour
Additives: Enzymes	α-Amylase, Cellulase, Xylanase, Papain, other proteases, Glucose oxidase, Almonds, hazelnuts, Carmine red
Nuts, Colour, Spices, Egg powder, Milk powder, Insects	Flour beetle (Tribolium confusum), Flour moth (Ephestia kuehniella), Cockroach (Blattella spp), Granary weevil (Sitophilus granarius), Alternaria, Aspergillus
Moulds, Sesame seeds	

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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

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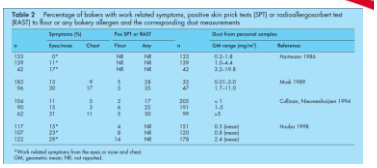

Table 2 Percentage of bakers with work related symptoms, positive skin prick tests (SPT) or radioallergosorbent test (RAST) to flour or any bakery allergen and the corresponding dust measurements

	Symptoms (%)		Pos SPT or RAST			Dust from personal samples	
n	Eyes/nose	Chest	Flour	Any	n	GM range (mg/m³)	Reference
133	0*		NR	NR	133	0.3–1.8	Hartmann 1986
139	11*		NR	NR	139	1.0–4.4	
42	17*		NR	NR	42	3.3–19.8	
183	13	9	5	28	33	0.01–3.0	Mosk 1989
98	30	17	5	33	47	1.7–11.0	
104	11	5	2	17	205	< 1	Calkoen, Nieuwenhuijsen 1996
90	15	3	6	25	191	1–5	
62	31	11	5	35	99	>5	
117	15*		4	NR	151	0.5 (mean)	Houba 1998
107	23*		8	NR	120	0.8 (mean)	
122	29*		14	NR	178	2.4 (mean)	

*Work related symptoms from the eyes or nose and chest.
GM, geometric mean; NR, not reported.

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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 (inhalation versus ingestion);
3) To predict potential cross-reactions with allergens from plant foods or pollens;
4) To investigate changes in allergenic capacity in cereal (wheat)-derived foodstuffs;
5) To engineer allergen variants with modified allergenic properties (ie, lower IgE-binding potency).

Internal Medicine *Solorzdo G, et.al. J Investig Allergol Clin Immunol* 2011; Vol. 21(2): 81-92. University of Cincinnati

IgE mediated mechanisms

- 81 bakers with occupational symptoms were evaluated using commercial tests to wheat allergens
  - Skin prick testing
  - Specific IgE
  - ISAC (Immuno Solid-phase Allergen Chip) microarray
  - Dot blotting to 6 wheat allergens (Tri a39, Tri a Trx, Tri a GST, Tri a 32, Tri a 12 and Tri a DH)

Internal Medicine Olivieri M, et.al. Occup Environ Med 2013; 70:617-622. University of Cincinnati

Results

- Positive wheat SPT in 29 bakers with occupational asthma
- Wheat sIgE was found in 51 workers and was associated with OA and rhinoconjunctivitis
- 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
  - When Tri a 32, Tri a GST and Tri a 30 were analyzed together this association with dermatitis was even stronger

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

Internal Medicine Olivieri M, et.al. Occup Environ Med 2013; 70:617-622. University of Cincinnati

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 sIgE sensitized patients without occupational exposure were used as controls
- Diagnostic efficiency of IgE testing of single allergens and combination allergens were assessed for their ability to discriminate between subjects with baker's allergy and controls.

Internal Medicine Sander I, et.al. JACI 2015; 135: 1529-37. University of Cincinnati

Results

- 91% of bakers had sIgE levels ≥.1kUA/L to at least one of 21 allergens
  - Highest frequencies for Tri a 27 (thiol 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 IgE

Internal Medicine Sander I, et.al. JACI 2015; 135: 1529-37. University of Cincinnati

Conclusions

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

Internal Medicine Sander I, et.al. JACI 2015; 135: 1529-37. University of Cincinnati

Using wheat component allergens to predict different wheat allergy phenotypes

- Background: Specific IgE to gliadin was proposed as a marker for wheat dependent exercise induced anaphylaxis and Tri a 14 was found to induce IgE response in baker's asthma
- Rationale: To evaluate whether wheat components could discriminate between wheat allergy phenotypes
- Methods: 29 patients with wheat-induced anaphylaxis and/or urticaria (n = 21, Group I) and baker's asthma (n = 8, Group II) were enrolled.
- Results: Prevalence of serum specific IgE to Tri a 14 was higher in Group II (25%) than Group I (4.8%) whereas serum specific IgE to gliadin was significantly higher in Group I (70%) vs. Group II (12.5%).
- Conclusions: A Tri a 14/gliadin may be a potential marker for predicting baker's asthma.

Internal Medicine Nam Y-H, et.al. J Korean Med Sci 2013; 28: 1697-1699. University of Cincinnati

Immunopathogenesis: What do we know about Baker's asthma

- Disruption of nasal and bronchoepithelial 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 proteinases 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)

Internal Medicine Porter PC, et al. Biochim Biophys Acta 2011; 1810: 1059–65. University of Cincinnati

Proteinase 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 Toll like receptors (the antigen will dictate Th1 vs. Th2 response)

Porter PC, et.al. Biochim Biophys Acta. 2011; 1810(11): 1059–1065.

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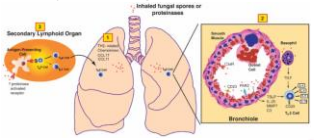
Pathogen associated molecular patterns (PAMPS)

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



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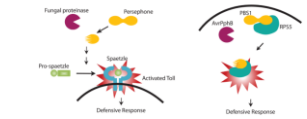
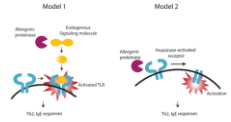
Proteinase-activated host defense reactions of insects and plants



Insect Model: Proteinase activation of enzymatic cascade involving the endogenous proteinase Persephone terminates in the cleavage of Pro-spaetzle to yield spaetzle, the final common ligand forToll. Activation of Toll induces a broad anti-microbial defensive response against fungi, bacteria and other organisms.
Plant Model: Bacterial proteinases such as AvrPphB can activate plant proteins such as PBS1 and RPS5 to induce a defense response against bacterial invasion.

Porter PC, et.al. Biochim Biophys Acta. 2011 November ; 1810(11): 1059–1065.

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.

Porter PC, et.al. Biochim Biophys Acta. 2011; 1810(11): 1059–1065.

Exemplary proteinases linked to human and experimental asthma and the major organisms producing them.

Proteinase	Source	Link to asthma	Mechanism of action
Subtilisin	*Bacillus* spp.	Induces human asthma	Unknown
Der p 1	*Dermatophagoides pteronyssinus* (common dust mite)	Most common human allergen; induces experimental asthma	Cleaves of CD23[51], CD25[52]; alters IL-4/IFN cytokine balance[74]; induction of allergic cytokine secretion[51, 75]
Fel d 1	*Felis domesticus* (domestic cat)	Common allergen	Unknown
Bromelain	*Ananas comosus* (pineapple)	Induces human asthma	Unknown
Papain	*Carica papaya* (papaya fruit)	Induces human and experimental asthma	Promotes basophil and airway epithelial activation; production of TSLP[28, 45, 60]
Aspergillo pepsin I	*Aspergillus* spp.	Induces experimental asthma	Activation of dendritic cells [60]; induction of IL-25, TSLP, and allergy-related chemokines[37, 42, 43]
Proteinase 2A	*Alternaria*	Induces experimental asthma	Unknown

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Potential therapies for proteinase-dependent allergic disease.

Potential Approach	Mechanism of action
Serine proteinase inhibitors (Serpins)	Inhibit activity of inhaled or in situ-generated fungal proteinases
Neutralization of proteinase-activated receptors (TLR, PAR2, CD23, CAR), soluble substrates (C3) or proteinase-induced allergy-promoting factors (CCL17, CCL11, TSLP, IL-25)	Disrupts key signaling pathways potentially underlying allergic inflammation.
Anti-fungal antibiotics (e.g., itraconazole)	Resolves the airway infection that may serve as a source of in situ proteinase that promotes disease.

Porter PC, et.al. Biochim Biophys Acta. 2011 November ; 1810(11): 1059–1065.

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

Brisman J. Occup Environ Med 2002;59:498–502.

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

Brisman J. Occup Environ Med 2002;59:498–502.

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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