The BALB/c mouse model of allergy for the assessment of sensitizing properties of proteins and foods and their alteration by environmental conditions

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The BALB/c mouse

- Th2-biased
- Used to assess the allergenic potency of a novel protein by comparison with that of known food proteins (Dearman and Kimber, 2001)
  - i.p. administration of purified proteins without adjuvant
  - IgE responses reflect allergenic potency as observed in humans:
    - PA (peanut) > OVA (egg) > BSA (milk) > non allergenic proteins
Cow’s milk (CM) allergy

- affects about 3.5 - 7% of children
- mainly type I, IgE mediated hypersensitivity
- polysensitization to several proteins (± 35 g protein/L):
  - whole casein (ca.27 g/L) : $\alpha$s1, $\alpha$s2, $\beta$ and $\kappa$-casein
  - whey proteins (ca.7 g/L) : $\beta$-lactoglobulin (BLG, ca. 3.5 g/L), $\alpha$-lactalbumin, lactoferrin
i.g. / i.p. administration of purified food allergens or whole foods in presence of Th2 adjuvant

Female BALB/cJ mouse (Th2-biaised)

Experimental Sensitization:

- Analysis of the antibody responses (IgE, IgG1 and IgG2a) to:
  - the different proteins of the food and
  - the different epitopes of purified allergens
- Analysis of the cytokines produced after allergen specific reactivation of spleen cells

Confirmation: experimental elicitation

- Analyse the early phase of the reaction (LT, PG, Histamine)
- Analyse the mediators and biomarkers (cytokines, eosinophilia) & symptoms of the late phase of the allergic reaction
BALB/c mouse model for sensitization to whole cow’s milk

oral administration of cow’s milk + cholera toxin

Cytokine production by reactivated spleen cells

Specific IgEs to milk proteins

- BLG
- a-Lac
- CAS
- BSa
- Lf

Serums

D 21

D 28

D 35

pg/mL

Adel Patient et al., Allergy 2005
BALB/c mouse model for sensitization to cow’s milk BLG

Specific anti BLG IgEs (ng/mL)

Cytokines production by reactivated spleen cells

Adel Patient et al., JIM 2000
Specific IgE response to cow’s milk BLG in Balb/c mouse

Native BLG

Protein
Alum
BLGn > BLGd

Peptide
11-26
21-40
41-60

denatured BLG

IFA
BLGd > BLGn

11-26
21-40
41-60
102-124
120-135

Adel-Patient et al., JIM 2000; CEA 2003
Sensitization (i.p or i.g.) with whole milk results in the production of IgE specific of the same proteins as in allergic humans.

In the BLG model: recognition of the same epitopes as those identified in humans.
BALB/cJ mouse model for the assessment of possible sensitizing potential of novel proteins and GM foods

i.p. administration of whole protein extract of IR-GM or non GM maize + IFA
(Cry1Ab concentration = 0.0013 % of protein content)

i.g. administration of whole protein extract of GM maize + CT

Production of Th1, Th2 and Th17 by spleen cells after reactivation by Cry1Ab and maize proteins

Serum: antibody response to Cry1Ab and maize proteins

Adel-Patient et al., PLoS ONE 2011
No humoral or cellular response to Cry 1 Ab

No differences in the specific IgE and cellular responses to maize proteins between GM and non GM maize.
Allergic sensitization to food proteins is considered as resulting from an impaired development of oral tolerance or a breakdown in an existing oral tolerance.

The BALB/c mouse model for the assessment of the modulation/alteration of sensitizing properties of proteins and foods upon “environmental” conditions.
Multifactorial aspect of sensitization to foods

Allergen + Atopic individual → Allergic Reaction

Exposure conditions & Environmental Factors

- Structure
- Dose, frequency and route of administration
- Microbial environment
- Interactions with food matrix
- Influence of processing
- Impact of diet
The Balb/c mouse model as an integrative tool for studying factors that influence the sensitizing properties of proteins

- Influence of the structure of the protein
- Influence of the dose of exposure
- Influence of the mode and route of administration
- Influence of the environment of the allergen e.g. influence of immunological status of the gut mucosa and presence and composition of the gut microbiota
The Balb/c mouse model for studying the role of the structure of the allergen on the mechanisms of the allergic reaction

The structure of the protein used for sensitization and elicitation (native vs denatured) influences the activation pathways involved in the allergic reaction

<table>
<thead>
<tr>
<th>Sensitization</th>
<th>Native BLG</th>
<th>Denaturated BLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native BLG</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Denaturated BLG</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Early mediators

- LT
- PGD2

Late phase

- IL-4
- IL-5
- Eosino BAL
- Eosino Lung tissue
- BHR

Adel Patient et al., CEA 2003
The Balb/c mouse model as a tool for studying factors that influence the sensitizing properties of proteins

- Influence of the structure of the protein
- Influence of the dose of exposure
  - sensitizing vs. tolerizing properties of BLG
  - impact on sensitizing properties of other CMPs
- Influence of the mode and route of administration
- Influence of the environment, e.g. microbial environment of the allergen
A systemic tolerance can be totally or partially induced by administration of low doses of BLG via i.g. route

Adel-Patient et al., Allergy 2005; 2011
Brief exposure via intact skin potentiates the gastrointestinal sensitizing properties of peanut allergens
The Balb/c mouse model as a tool for studying factors that influence the sensitizing properties of proteins

- Influence of the structure of the protein
- Influence of the dose of exposure
- Influence of the mode and route of administration
- Influence of environmental conditions, e.g. microbial environment of the allergen
Brief exposure via intact skin potentiates the gastrointestinal sensitizing properties of peanut allergens.

Presence of GpG ISS decreases the sensitizing potency.

Adel-Patient et al., IAAI 2006
Administration of recombinant LAB producing BLG prevents sensitization by induction of a moderate Th1-type response:
- Decrease anti-BLG IgG1 antibody response
- Decrease IL5 and increase IFN-γ production by reactivated spleen cells

Administration of non recombinant LAB in presence or absence of BLG did not demonstrate significant preventive effect

Cortes-Perez et al., CVI 2007; IAAI 2009
Influence of the gut microbiota on the sensitizing properties of a food protein
Sensitizing properties of BLG in GF vs Cv Balb/c mouse

i.p. sensitization with IFA

anti-BLG IgG1

anti-BLG IgE

Days

sensitivity

Hazebrouck et al, IAAI 2009

i.p. sensitization without adjuvant

anti-BLG IgG1

anti-BLG IgE

GF BLG, GF PBS, CV BLG, CV PBS

Morin et al, MNFR 2011
A sensitization can be achieved by ip or ig administration of pure BLG or CM + adjuvant that mimics the immune response of CM allergic humans. The same with peanut.

But a systemic tolerance to BLG can also be induced by ig administration of low doses of BLG

A brief exposure to PN via intact skin results in a intense potentiation of the subsequent gastro-intestinal sensitization,

Application of immunostimulatory sequences from bacterial DNA with PN induces a Th1 specific immune response counterbalancing the Th2 one.

The use of recombinant lactic acid bacteria as delivery vector of BLG prevents from a subsequent sensitization by induction of a Th1 - type response

Presence of an established gut microbiota may decrease the sensitizing potency of a protein and delay the sensitization process in the Cv vs. GM BALB/c mouse
Conclusion

- Sensitizing properties of proteins result from intrinsic structural and physicochemical characteristics that interact with the host genetics and physiology and with environmental conditions.

- To study those interactions, BALB/C mouse provides an useful integrative model which may reflect some aspects of the situations observed in allergic humans and allows to investigate the underlying mechanisms.

- Among other factors, the dose, route and mode of administration, the presentation and the environment of the protein influence the polarization (i.e. Th1 vs Th2 vs Th17 vs Treg response) and intensity of the immune response and thus modulate the sensitizing potency of proteins.
Thank you for your attention

INRA Food Allergy Team