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

Female BALB/cJ mouse

- Th2- biaised
- 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

The BALB/c mouse model for the assessment of sensitizing properties of purified proteins and whole foods

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) : αs1, αs2, β and κ-casein

  - whey proteins (ca.7 g/L) : β-lactoglobulin (BLG, ca. 3.5 g/L), α-lactalbumin, lactoferrin

BALB/c mouse model of sensitization to CM proteins

i.g. / i.p. administration of purified food allergens or whole foods in presence of Th2 adjuvant

Female BALB/cJ mouse (Th2-biaised)

i.g. / i.n. administration of the same allergen or a structurally modified form or a related allergen

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

Serums

0 6 12 18 24 30

oral administration of cow's milk
+ cholera toxin

Specific IgEs to milk proteins

BLG
CAS
BSA
a-Lac
Lf

D 21 D 28 D 35

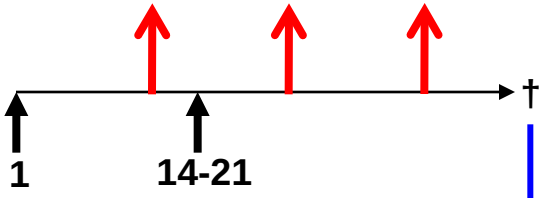
Cytokine production
by reactivated spleen cells

IFNγ
IL-5
IL-4
0 10 20
pg/mL

Adel Patient et al., Allergy 2005

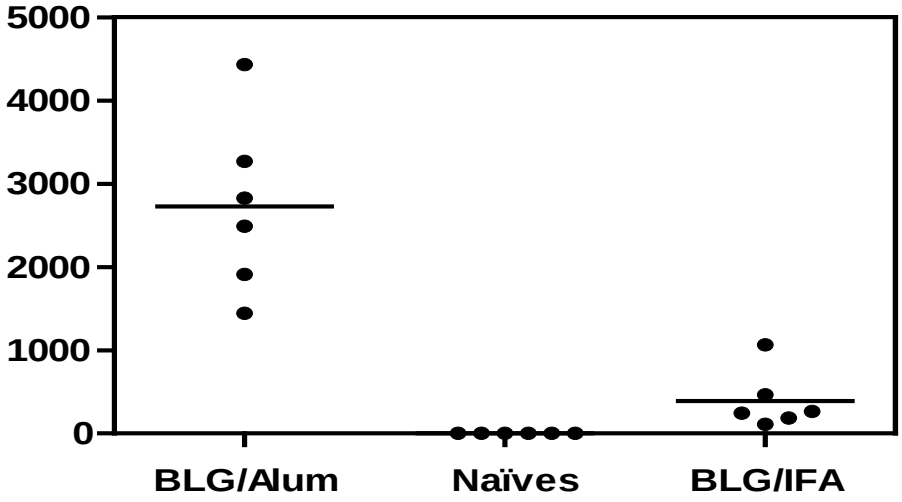
BALB/c mouse model for sensitization to cow's milk BLG

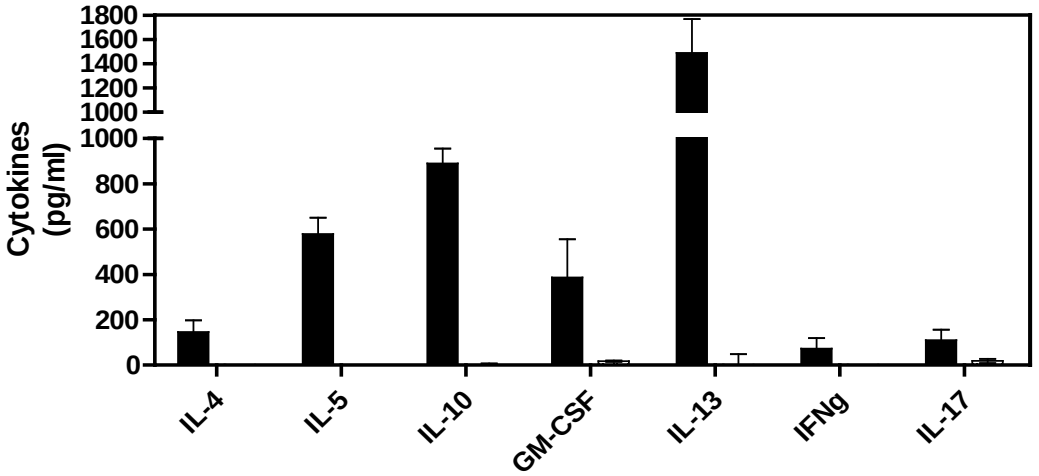
Serums



i.p. administrations of BLG
+ alum or IFA

Specific anti BLG IgEs (ng/mL)

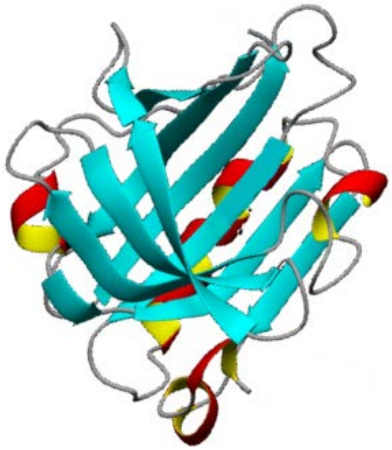




Cytokine production by
reactivated spleen cells

Adel Patient et al., JIM 2000

Specific IgE response to cow's milk BLG in Balb/c mouse



Native BLG

```
1                                                  50
LIVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRVYVEELKPTP
                                                  100
EGDLEILLQKWENDECAQKKIIAEKTKIPAVFKIDALNENKVLVLDTDYK
                                                  150
KYLLFCMENSAEPEQSLVCQCLVRTPEVDDEALEKFDKALKALPMHIRLS
            162
FNPTQLEEQCHI
```

denatured BLG

	Alum	IFA
Protein	BLGn > BLGd	BLGd > BLGn
Peptide	11-26 21-40 41-60	11-26 21-40 41-60 102-124 120-135

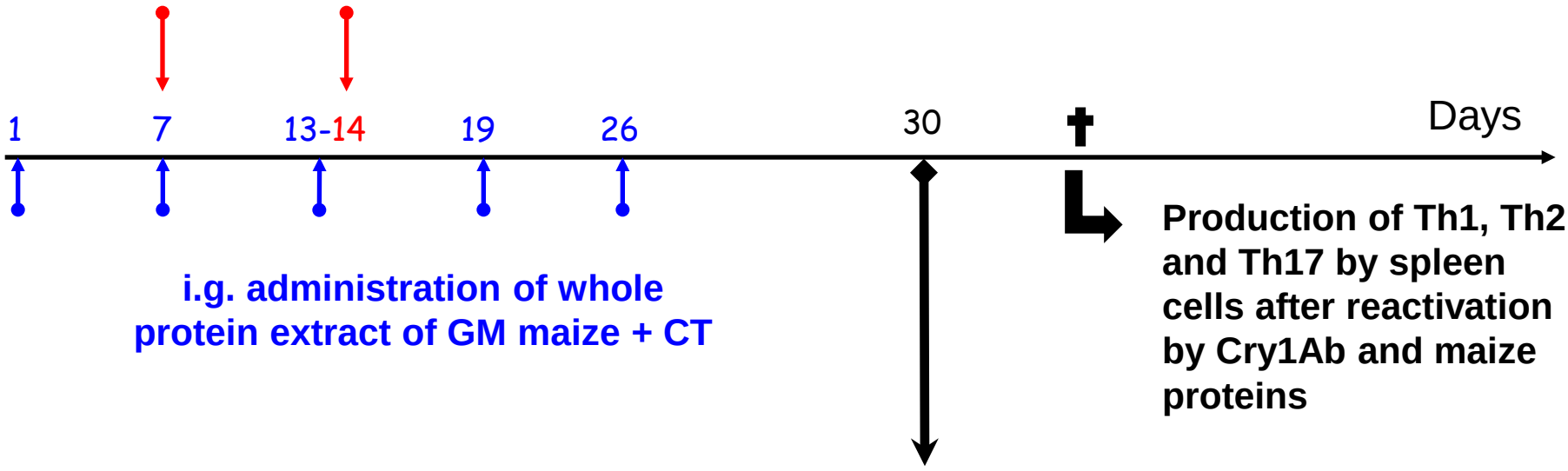
Adel-Patient et al., JIM 2000; CEA 2003

BALB/cJ mouse model of sensitization to CM proteins

- 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

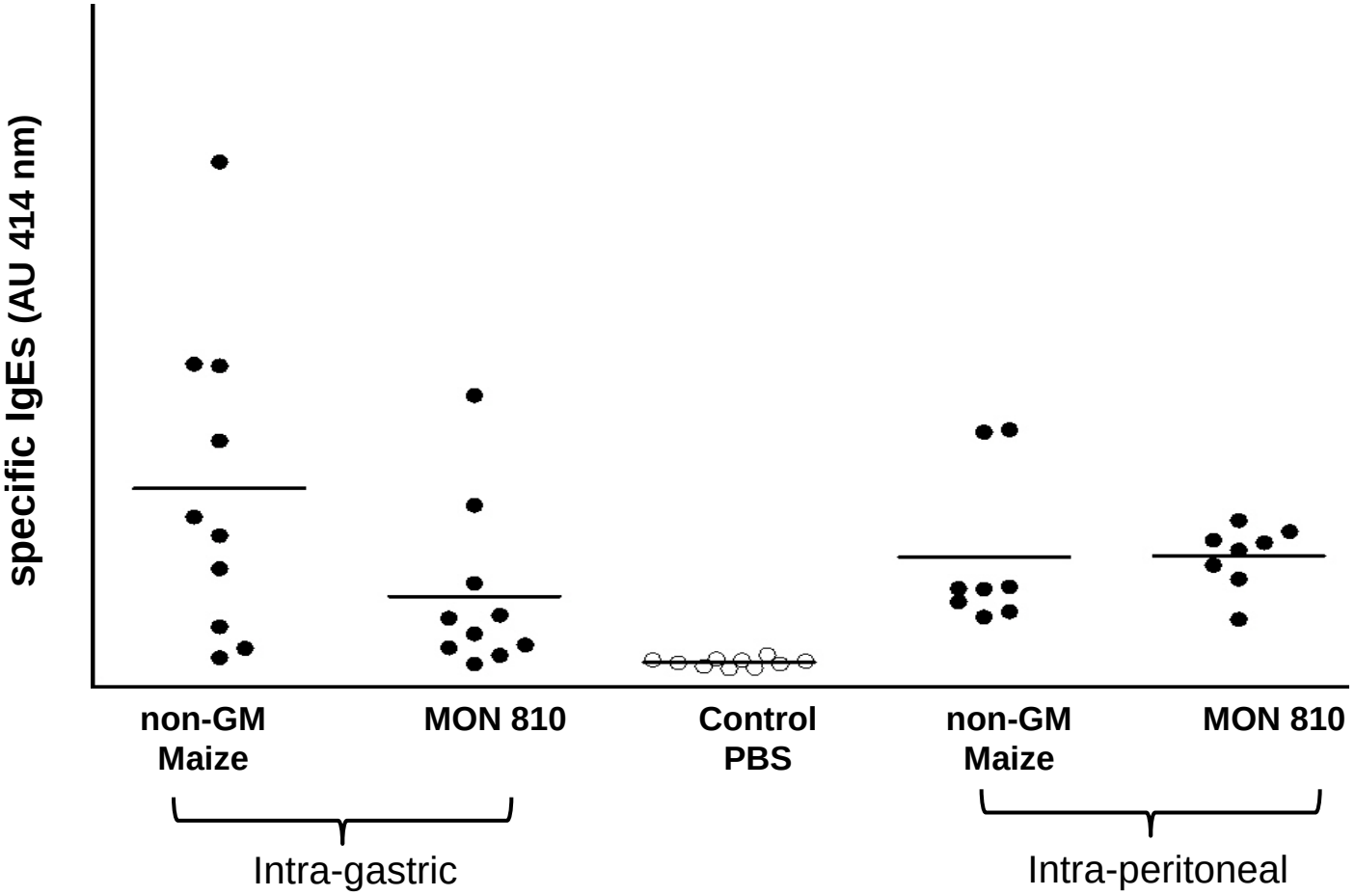
BALB/cJ mouse model for the assessment of possible sensitizing potential of novel proteins and GM foods

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



Adel-Patient et al., PLoS ONE 2011

BALB/c mouse model for the ssessment of possible sensitizing properties of GM maize MON 810 (Cry1Ab) *vs* non GM counterpart



- **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.**

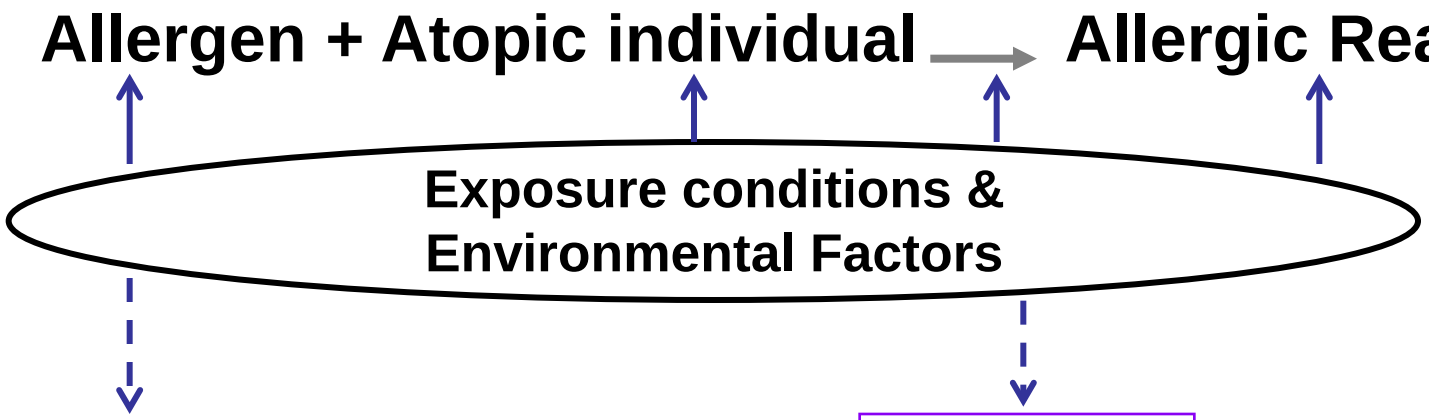
Adel-Patient et al., PLoS ONE 2011

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

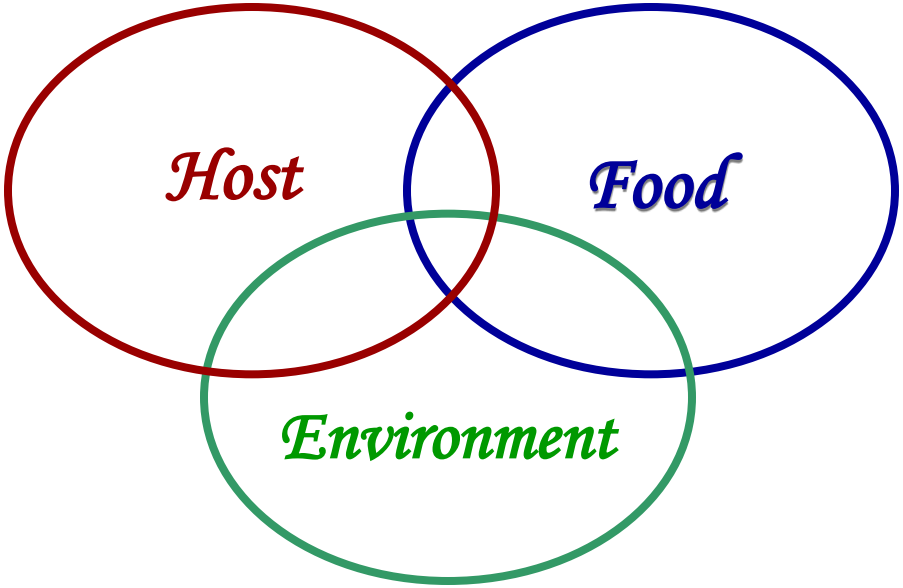
Sensitization to food/food proteins : background

Allergic sensitization to food proteins is considered as resulting from an impaired development of oral tolerance or a breakdown in an existing oral tolerance

Multifactorial aspect of sensitization to foods



- 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

Native BLG

1 50
LIVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRVYVEELKPTP
100
EGDLEILLQKWENDECAQKKIIAEKTKIPAVFKIDALNENKVLVLDTDYK
150
KYLLFCMENSAEPEQSLVCQCLVRTPEVDDEALEKFDKALKALPMHIRLS
162
FNPTQLEEQCHI

denatured BLG

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

		Early mediators		Late phase				
	Elicitation	LT	PGD2	IL-4	IL-5	Eosino BAL	Eosino Lung tissue	BHR
Sensitization Native BLG	Native BLG	+	-	+	+	+	+	-
	Denaturated BLG	-	+	-	+	-	+	+

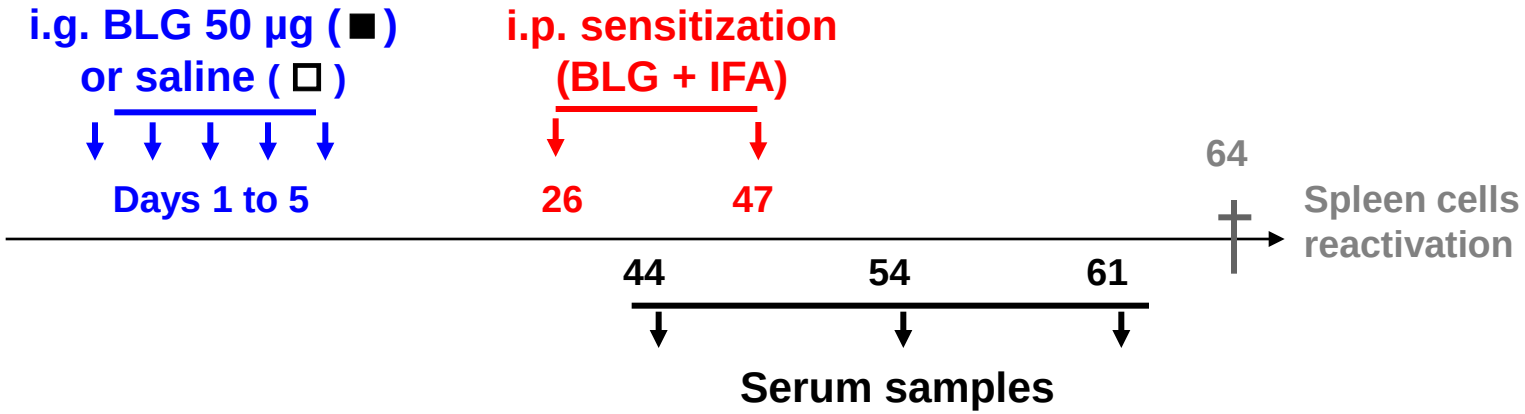
Adel Patient et al., CEA 2003

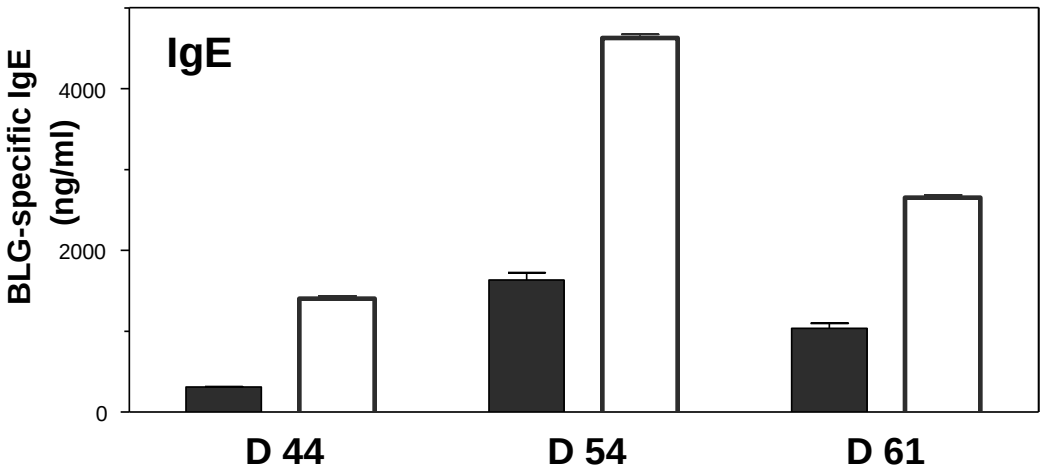
The Balb/c mouse model as a tool for studying factors that influence the sensitizing properties of proteins

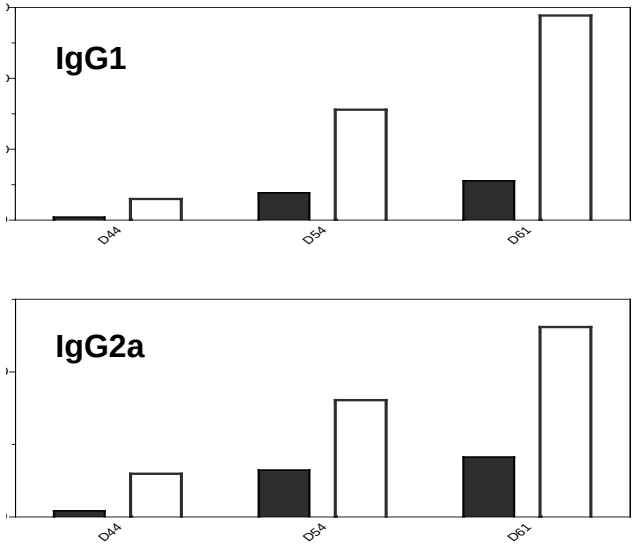
- Influence of the structure of the protein
- **Influence of the dose of exposure**
  - ➲ **sensitizing *vs.* tolerizing properties of BLG**
  - ➲ **impact on sensitizing properties of other CMPs**
- Influence of the mode and route of administration
- Influence of the environment, e.g. microbial environment of the allergen

Influence of the dose of exposure on sensitizing properties

Induction of tolerance by the allergen / modified allergen



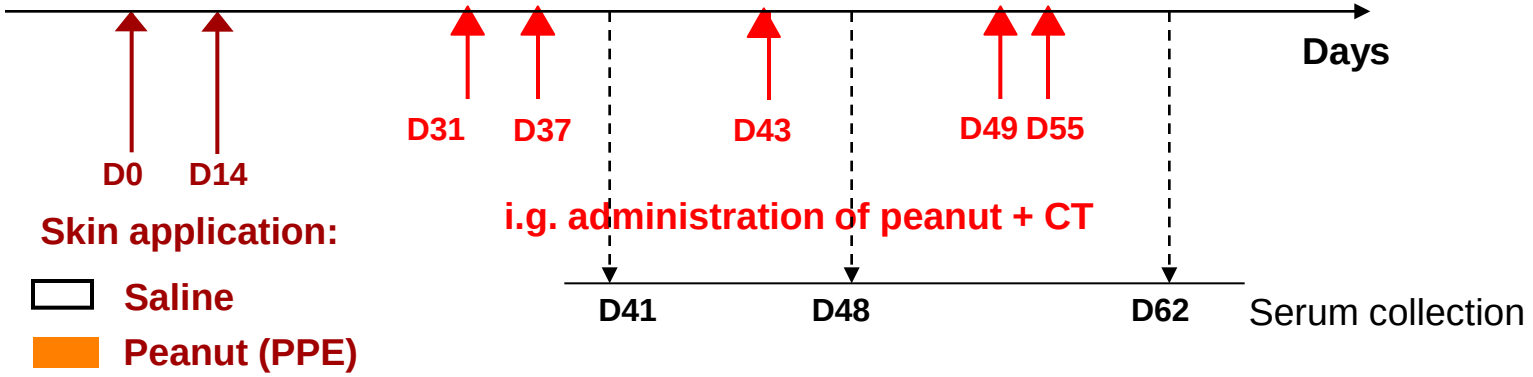


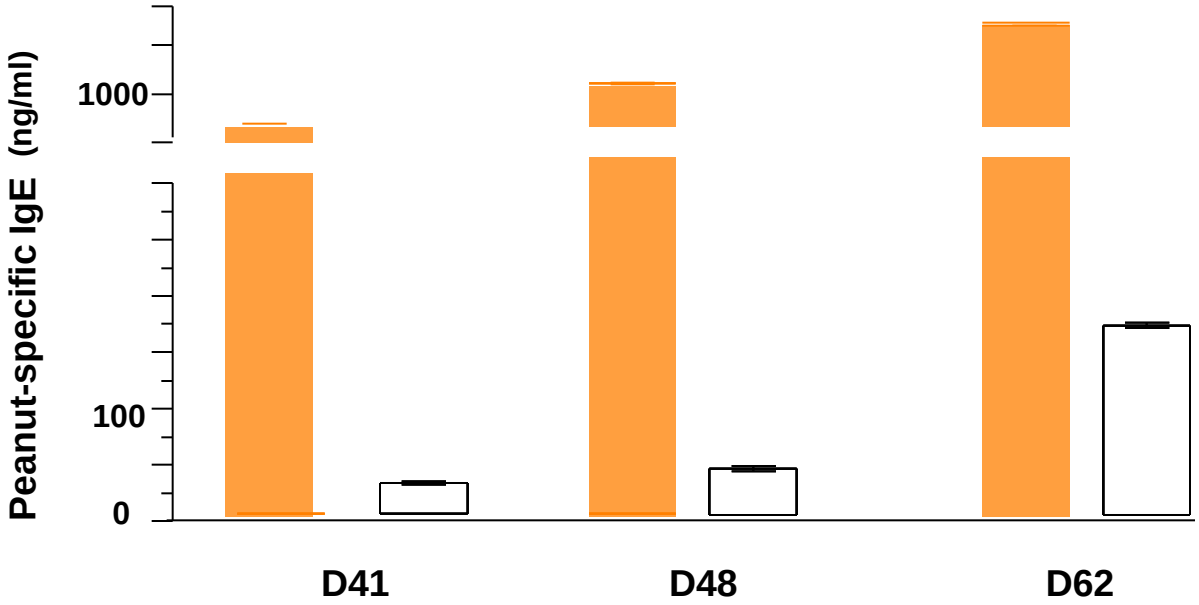


A systemic tolerance can be totally or partially induced by administration of low doses of BLG via i.g. route

Adel-Patient et al., Allergy 2005; 2011

Influence of the exposure/route of administration





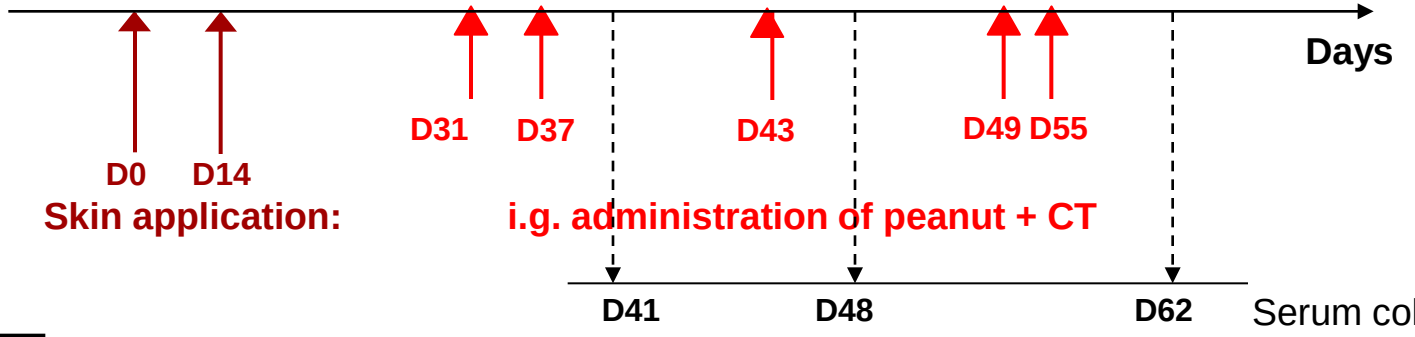
Brief exposure via intact skin potentiates the gastrointestinal sensitizing properties of peanut allergens

Adel-Patient et al., IAAI 2006

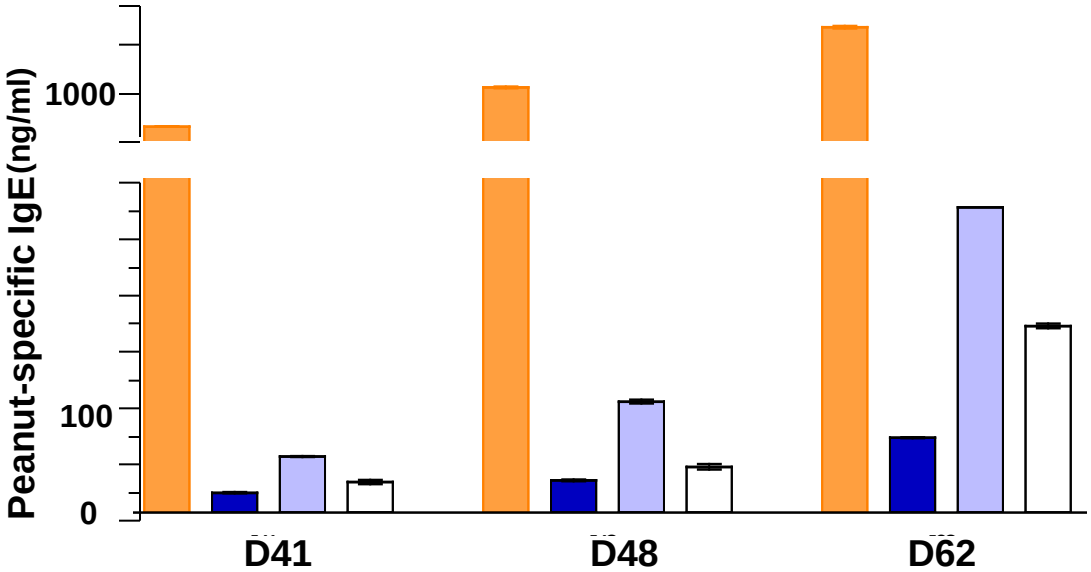
The Balb/c mouse model as a tool for studying factors that influence the sensitizing properties of proteins

- 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**

Influence of the microbial environment of the protein



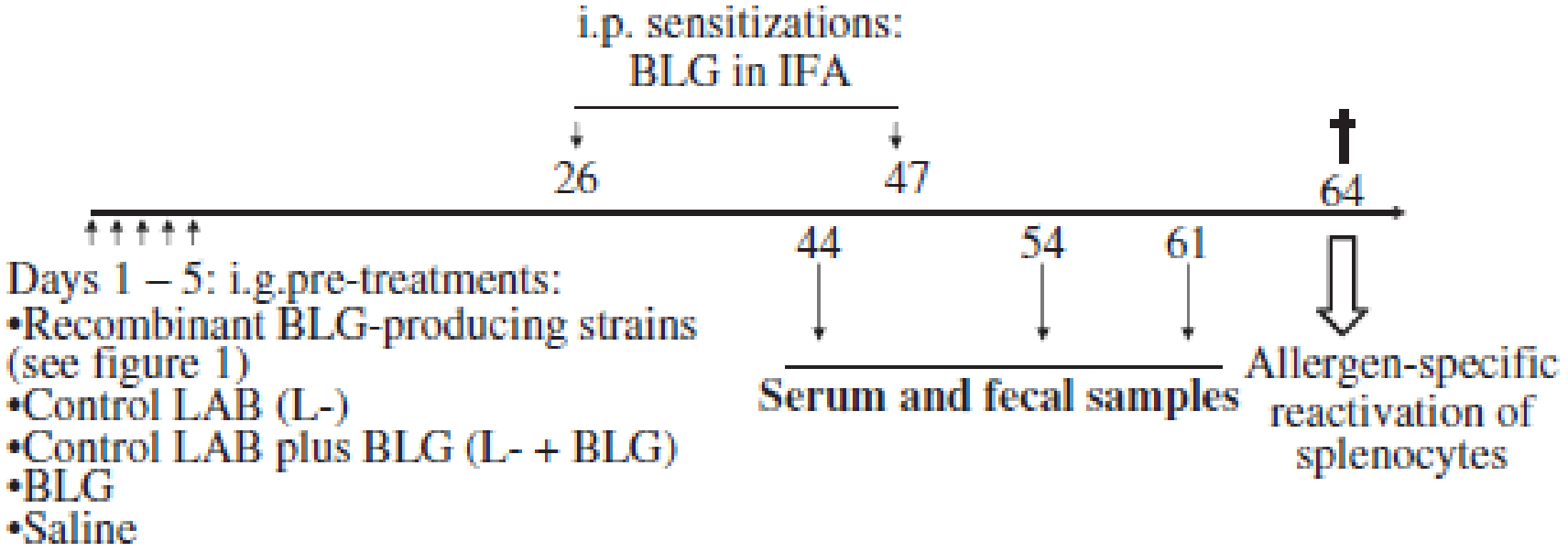
Saline
Peanut
Peanut + CpG
non ISS DNA (control)



- 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

Influence of the microbial environment of the protein. *In situ* delivery by lactic acid bacteria

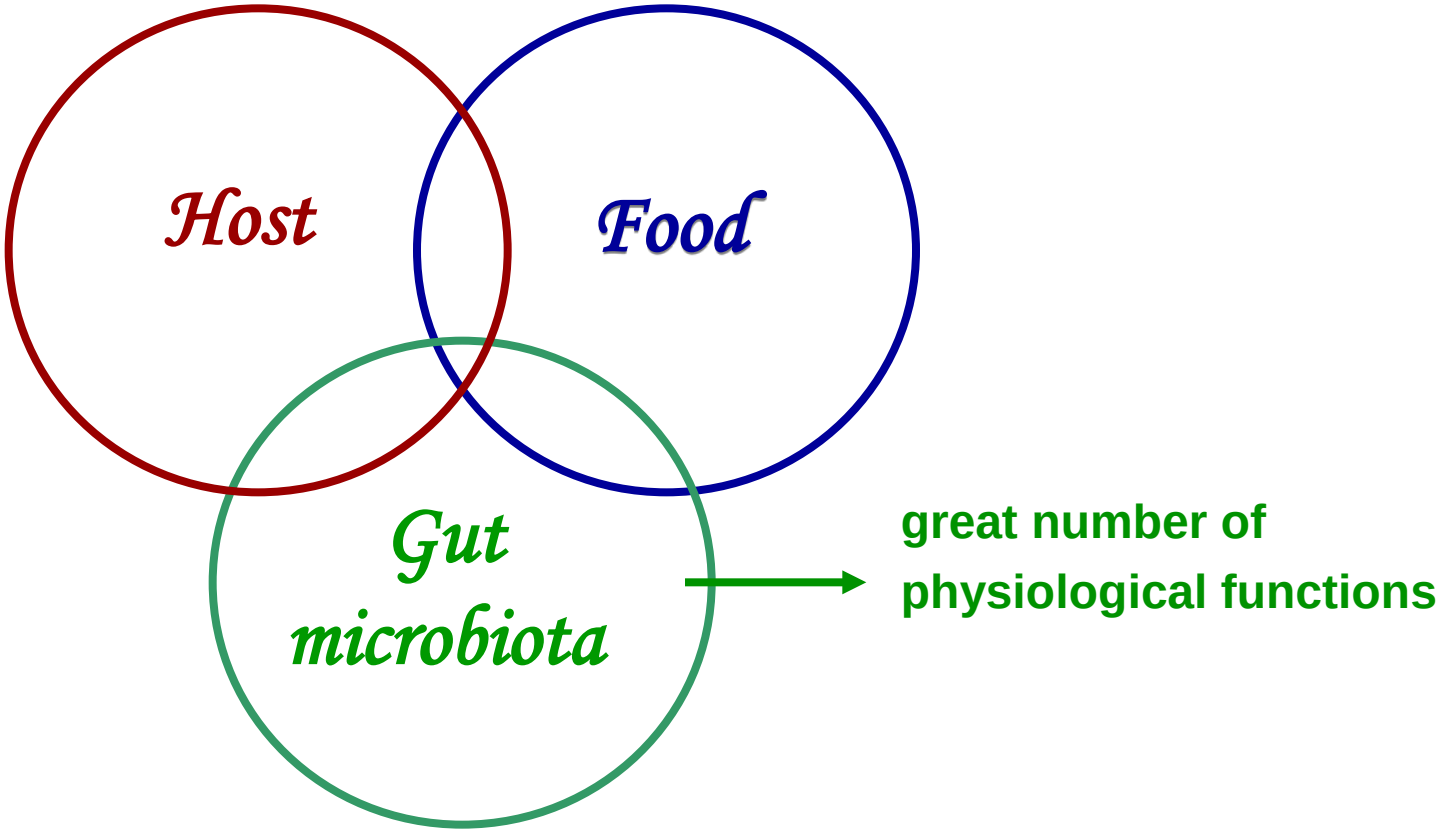


- **Administration of recombinant LAB producing BLG prevents sensitization by induction of a moderate Th1- type response:**
  - Decrease anti-BLG IgG1 antibody response
  - Decrease IL5 and increase IFN γ production by reactivated spleen cells
- **Administration of non recombinant LAB in presence or absence of BLG did not demonstrate significant preventive effect**

Cortes-Perez et al., CVI 2007; IAAI 2009

Influence of the gut microbiota

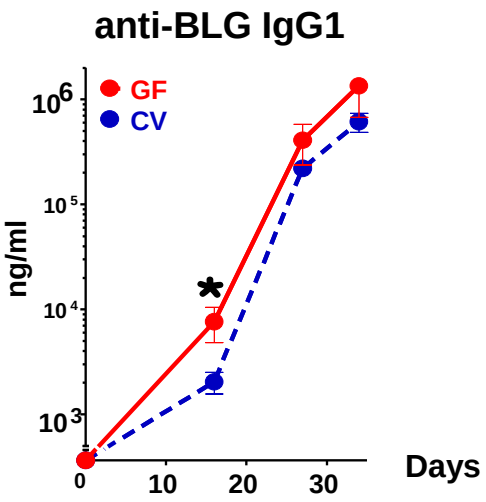
In the gut

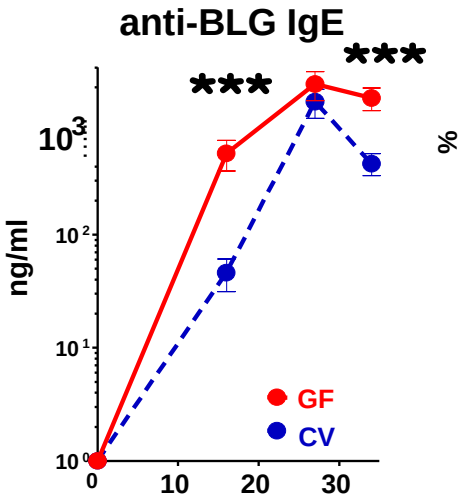


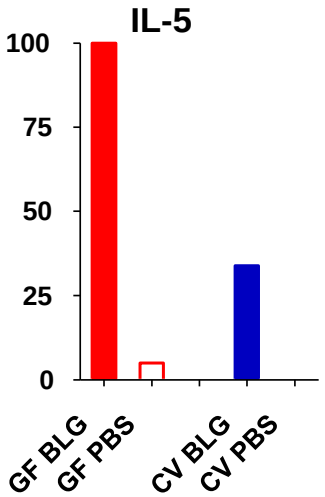
➲ Influence of the gut microbiota on the sensitizing properties of a food protein

Sensitizing properties of BLG in GF *vs* Cv Balb/c mouse

i.p. sensitization with IFA

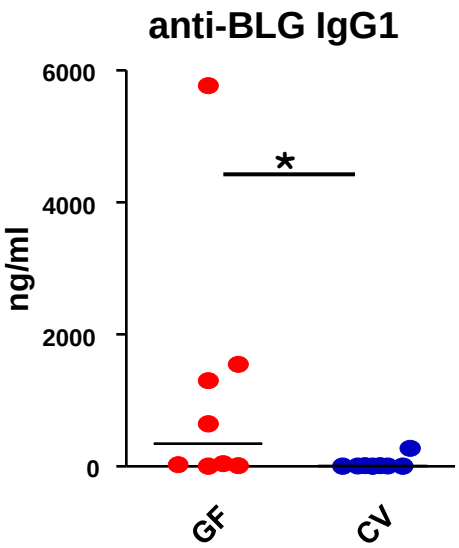


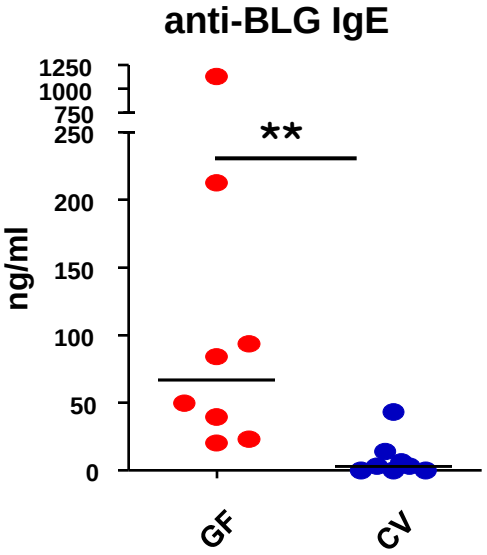


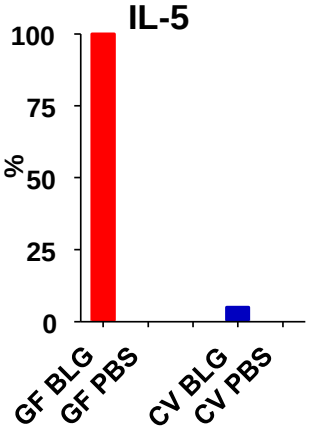


Hazebrouck et al, IAAI 2009

i.p. sensitization without adjuvant







Morin et al, MNFR 2011

The Balb/c mouse model as a tool for studying factors and mechanisms that modulate the sensitizing properties of a protein/food

Summary

- 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

The Balb/c mouse model as a tool for studying factors and mechanisms that modulate the sensitizing properties of a protein/food

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

Acknowledgments

INRA Food Allergy Team