Induction de Tolérance: Est-ce Toujours d’Actualité?

Gideon Lack
Avoidance

Exposure
Childhood exposure to pets including cats was associated with lower sensitization to cats in adulthood, particularly among those with a positive family history of atopy (OR 0.68 95%CI 0.51 to 0.93.


Dog ownership in early childhood protects against the development of inhalant sensitisation and this effect cannot be attributed to the simultaneous exposure to endotoxin.

Chen CM. Eur Respir J 2008; 31: 963–973

Exposure to 2 or more dogs or cats in the first year of life may reduce subsequent risk of allergic sensitization to multiple allergens during childhood.

Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study

Methods

- Population cross-sectional study of 226 children (aged 12 – 14 years).
- 47 of whom had asthma.
- Dust samples were obtained from four different areas within the children's homes and assayed for cat allergen and dust mite.
- Antibodies to cat and mite allergens measured by isotype (IgG and IgG4).
- Sensitization/Specific IgE/SPT.
Prevalence of sensitisation to cat allergens and of IgG antibody to Fel d 1 ≥125 units/mL for six equal-exposure groups for cat allergen

Clinical and immunologic survey in beekeepers (n = 200) in relation to their sensitization

Relationships between allergic reactions, HBV skin tests, and estimated number of stings received annually by beekeepers (BKs)

Non allergic BKs
Local reactions;
Systemic reactions.

Conclusion:
High numbers of sting protect against sensitization and systemic reactions

Randomised controlled avoidance studies
High-risk children (n = 251) were prenatally randomized to stringent environmental control [active (n = 133)] or no intervention [control (n = 118)].

Woodcock A. Am J Respir Crit Care Med; 170: 433–439.
Manchester Asthma and Allergy Study: Low-allergen environment can be achieved and maintained during pregnancy and in early life

House dust mite allergen levels (GM and 95% CI) in nursery room at birth, 6 months, and 12 months

Crib mattress, total allergen recovered

Crib mattress, allergen concentration

Higher Prevalence of Mite Sensitisation with Stringent Environmental Control

Woodcock A. Am J Respir Crit Care Med; 170: 433–439.
The development and prediction of atopy in high-risk children: **Follow-up at age seven years** in a prospective randomized study of combined maternal and infant food allergen avoidance.

Why have avoidance studies failed?

1. Development of allergy or tolerance is unrelated to allergen.

2. Avoidance measures have been insufficient.

3. The concept of avoidance is wrong
Should we expose infants to food allergens?
Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy

Methods

- 5171 Jewish school children in UK and 5615 Jewish school children in Israel were compared for food allergies and apoty.
- Questionnaire based assessment of peanut allergy validated by challenges.
- Infant weaning for peanut and other foods was determined in infants using a validated FFQ.

Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy

How early is early?
Cohort analysis for cow’s milk allergy

Cohort= 13,234

Successful contact was not established
203 = 1.5% of the cohort

Sample
13,019 = 98.4% of cohort

Lost after initial contact
12 = 0.1% of the cohort

Adverse event to CMP suspected
381 = 2.9% of sample

Rule of CMP in complaint was ruled out
244 = 1.9% of sample

Non IgE mediated adverse event
71 = 0.5% of sample

IgE-CMA
66 = 0.5% of sample

Early cow’s milk introduction associated with reduced incidence of cow’s milk protein allergy.

Cohort analysis for egg allergy

3552 eligible infants approached
2589 (73%) participated

2141 (83%) negative SPT
Not egg allergic
100 declined challenge
Excluded
1 refused to eat all doses
Excluded
6 reacted later on same day
Egg allergic

448 (17%) positive SPT
(wheal size ≥ 1mm)
340 underwent oral food challenge (OFC)
128 tolerated one whole egg white during OFC
6 reacted after dose of egg at home
Egg allergic
108 did not report any late reactions
Not egg allergic
8 possible late reactions (not clear)
Excluded

8 had previous reactions
Egg allergic
211 reacted during OFC
Egg allergic
Introduction of cooked egg at 4-6 months is associated with reduction in egg allergy

Randomised controlled exposure studies

Food allergens
**LEAP Study – Immune Tolerance Network**

Recruitment of 4-11 month old children with eczema and/or egg allergy for randomisation and stratification.

**Randomisation/Stratification**

- Intervention group: Peanut consumed 3 times per week (n≈320)
- Control Group (n≈320): Peanut avoidance

**Age**
- 4-11 months
- 1 yr* (2.5 yr*)
- 5 yr* (5 yr*)
Primary Endpoint

- The proportion of participants with peanut allergy at 60 months of age. Peanut allergy is defined by the Double Blind Placebo Controlled Food Challenge.
Secondary Endpoints

- The proportion of participants with allergic sensitisation to food allergens (30 and 60 months)

- The proportion of participants with allergic rhinoconjunctivitis and asthma (30 and 60 months)

- The proportion of participants with food allergy at 60 months

- Incidence of adverse events, laboratory anomalies, and nutritional evaluations

- Results of cellular and humoral immune response to peanut and other specific allergens
Is Oral Tolerance Induction a Primary or Secondary Prevention Strategy?

![Graph showing the percentage with IgE to foods over age in months for Peanut, Egg, and Milk. The graph includes data points for 490 participants.](image-url)

- **Peanut**: Brown line
- **Egg**: Red line
- **Milk**: Blue line

**n = 490**
EAT Study - Early Weaning Trial

Pregnant women
20/40 scan

2500 subjects

Early weaning onto allergenic foods

Randomization (3 months)

Current weaning recommendations

3 year assessment
Food allergy
Eczema
Atopic wheeze
Cumulative allergy

EAT
Enquiring About Tolerance
Endoscopic and biopsy findings in patients with and without celiac disease. (A) High-definition endoscopic photo of normal small intestine. The villi are clearly visible with no evidence of atrophy or scalloping of the folds. (B) Biopsy specimen of normal small intestine (hematoxylin-eosin; original magnification, × 100). (C) PillCam image of small intestine in a patient with celiac disease, showing scalloping of the mucosal folds (arrows) characteristic of a malabsorption pattern. There is also evidence of villous atrophy compared with normal. (D) Biopsy specimen of small intestine in a patient with celiac disease (hematoxylin-eosin; original magnification, × 100). Note the loss of villous architecture.

Presutti RJ. Am Fam Physician 2007;76:1795-1802
Randomisation of 1000 high risk infants

**Intervention group** – daily gluten during the period of breastfeeding (Dose is 3% of normal 6 month old consumption).

**Control Group** - Receives a milk sugar powder placebo during the period of breastfeeding from the age of 4 months.

**Endpoint = CD**
Gluten challenge, antibodies, histology

'Bhigh-risk': First degree family member has coeliac disease.

Age 4 months 3 yr*♦

10 European countries
Food antigen exposure is a necessary but not a sufficient condition for oral tolerance

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Oral tolerance induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral food antigen</td>
<td>No</td>
</tr>
<tr>
<td>Bacteria</td>
<td>No</td>
</tr>
<tr>
<td>Breast milk</td>
<td>No</td>
</tr>
</tbody>
</table>

- No tolerance induction

<table>
<thead>
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<tr>
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</tr>
<tr>
<td>Breast milk</td>
<td>+</td>
</tr>
</tbody>
</table>

- Tolerance induction
The failure of oral tolerance induction is functionally coupled to the absence of T Cells in Peyer’s patches under germfree conditions

Specific pathogen free
Germ free
Gnotobiotic:  
- *Bifidobacterium infantis*
- *Escherichia coli*
- *Clostridium perfringens*
- *Staphylococcus aureus*

**OVA**

**Sensitisation**

**Sacrifice**

- Oral tolerance induction

- Ip OVA + alum

**Readouts:**
- OVA IgE
- OVA IgG1
- OVA IgG2a
- Cellular responses
- Adoptive transfer experiments

Maeda Y. Immunobiol 2001; 204: 442–457
Serum Ab titer in the mice that underwent oral tolerance induction

GF: Germ free
SPF: Specific pathogen free
BiG: Bifidobacterium infantis - associated gnotobiotic
EcG: Escherichia coli - associated gnotobiotic
CpG: Clostridium perfringens - associated gnotobiotic
SaG: Staphylococcus aureus - associated gnotobiotic

Maeda Y. Immunobiol 2001; 204: 442–457
Histological analyses of Peyer’s patches
HE staining

Specific pathogen free mice

Germ-free mice

Maeda Y. Immunobiol 2001; 204: 442–457
# Effect of Peyer’s patch cell transfer on tolerance induction

<table>
<thead>
<tr>
<th>Cell</th>
<th>Donor cells</th>
<th>Number</th>
<th>Recipient mice</th>
<th>Ag-specific IgG1 (unit/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>$4719 \pm 136^c$</td>
</tr>
<tr>
<td>+</td>
<td>Whole PP cells</td>
<td>3</td>
<td>-</td>
<td>$148 \pm 112$</td>
</tr>
<tr>
<td>+</td>
<td>T cell-depleted PP cells</td>
<td>6</td>
<td>-</td>
<td>$576 \pm 185$</td>
</tr>
</tbody>
</table>

\[ p < 0.01^d \] \quad NS

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\( ^a \) Donor cells were obtained from PPs of SPF mice, which had been fed 20 mg OVA in days -7 to day -4 and then sacrificed on day 0 to collect PP cells.

\( ^b \) GF mice as recipients were given i.v. 3´106 of donor cells on day 0, and challenged i.p. three times with OVA in alum on day 0, day 14 and day 28.

\( ^c \) Mean ± SD

\( ^d \) Statistically significant based on Student’s *t*-test.

Maeda Y. Immunobiol 2001; 204: 442–457
Colostrum
Cytokines

Antigen

Salivary factors
Bacteria

ORAL TOLERANCE
## Cytokine concentrations in colostrum, mature breast milk and saliva

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Saliva</th>
<th>Colostrum</th>
<th>Mature milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>64.5 ± 89.6 pg / mL</td>
<td>17 ± 4 pg / mL</td>
<td>10 ± 2 pg / mL</td>
</tr>
<tr>
<td>IL-2</td>
<td>7.3 ± 3.0 pg / mL</td>
<td>90.1 (50.0–132.0) pg / mL</td>
<td>50.0 (50.0–50.0) pg / mL</td>
</tr>
<tr>
<td>IL-4</td>
<td>19.60 ± 1.21 pg / mL</td>
<td>172 (53–261) pg / mL</td>
<td>83 (13–180) pg / mL</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>35.10 ± 17.94 pg / mL</td>
<td>708 (8–2228) pg/ml</td>
<td>175 (3–792) pg/ml</td>
</tr>
<tr>
<td>IL-6</td>
<td>27.6 ± 26.3 pg / mL</td>
<td>978.80 ± 86.80 pg/ml</td>
<td>86.92 ± 2.47 pg/ml</td>
</tr>
<tr>
<td>IL-8</td>
<td>755.3 ± 700.4 pg / mL</td>
<td>585.70 ± 30.75 pg/ml</td>
<td>200.30 ± 25.01 pg/ml</td>
</tr>
<tr>
<td>IL-10</td>
<td>8.97 ± 1.91 pg / mL</td>
<td>43.95 ± 5.26 pg/ml</td>
<td>35.82 ± 2.98 pg/ml</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>427.93 ± 117.23 pg / mL</td>
<td>708 (8–2228) pg/ml</td>
<td>175 (3–792) pg/ml</td>
</tr>
<tr>
<td>TNF-α</td>
<td>11.2 ± 8.5 pg / mL</td>
<td>402.80 ± 29.65 pg/ml</td>
<td>178.30 ± 14.41 pg/ml</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>24.96 ± 2.38 pg / mL</td>
<td>140 (67 - 186) pg/ml</td>
<td>83 (17 - 114) pg/ml</td>
</tr>
<tr>
<td>IgA1</td>
<td>71.7 ± 21.9 mg/mL</td>
<td>8.55 ± 9.04 mg/mL</td>
<td>10.48 ± 12.94 mg/mL</td>
</tr>
<tr>
<td>IgA2</td>
<td>103.2 ± 42.9 mg/mL</td>
<td>0.36 ± 0.23 mg/mL</td>
<td>0.27 ± 0.19 mg/mL</td>
</tr>
<tr>
<td>IgG1</td>
<td>38.2 ± 46.1 µg/mL</td>
<td>195.0 ± 83.2 µg/mL</td>
<td>35.72 ± 4.40 µg/mL</td>
</tr>
<tr>
<td>IgG2</td>
<td>122.7 ± 183.7 µg/mL</td>
<td>12.3 ± 0.4 µg/mL</td>
<td>4.18 ± 0.69 µg/mL</td>
</tr>
<tr>
<td>IgG3</td>
<td>14.6 ± 21.4 µg/mL</td>
<td>14.7 ±2.5 µg/mL</td>
<td>1.31 ± 0.15 µg/mL</td>
</tr>
<tr>
<td>IgG4</td>
<td>28.9 ± 58.9 µg/mL</td>
<td>2.4 ±0.4 µg/mL</td>
<td>0.516 ± 0.109 µg/mL</td>
</tr>
<tr>
<td>IgM</td>
<td>2.1 ± 1.7 µg/mL</td>
<td>122.30 ± 100.19 mg/dl</td>
<td>25.71 ±20.39 mg/dL</td>
</tr>
<tr>
<td>sCD14</td>
<td>190 x 10³ pg/mL</td>
<td>15 (12–20) pg/mL</td>
<td>8 (6–10) pg/mL</td>
</tr>
</tbody>
</table>
Current practice for feeding infants

Breastfeeding

Hypoallergenic formula
Baby rice, lamb, chicken

Eggs, peanut
Nuts, fish

0 – 6 months

6 – 12 months

12 months – 3 years
Conclusions

1. Avoidance studies to prevent allergy have failed.

2. Complete allergen avoidance is rarely possible.

3. Oral tolerance induction needs to be investigated in RCT’s. Studies are currently in progress.

4. The choice of participants and clinical endpoints is critical.

5. Safety is paramount and studies should have independent data safety monitoring boards.
Conclusions

6. Oral tolerance induction through early antigen exposure is currently being tested in the EAT and the LEAP studies.

7. Oral consumption of allergen is a necessary condition but a sufficient condition for the development of oral tolerance induction.

8. Other facilitating factors may be necessary for OTI to occur – saliva, breast milk, bacteria, cytokines.
Equipoise

Avoidance

Exposure
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- Immune Tolerance Network
- Food Standards Agency
- Medical Research Council
- FAAN
- Food Allergy Initiative