Novel biological functions of IgE antibodies
Definition of „Allergy“
In: Münchner Med. Wochenschrift 1906
Triggering allergy effector cells causes symptoms

- Sensitization phase: Production of IgE
- Effector phase: Symptoms

- Rhinocconjunctivitis
- Asthma
- Food allergy
- Urticaria
- Anaphylaxis
Oral, intestinal

Food allergy

Asthma, Atopic Triad

Respiratory
IgE incidence tripled during last decades. Predicted 40% in New Zealand.

What makes an allergen an allergen?
How is the specificity of antibodies guaranteed?

From the following article:
Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data
Stephan C. Bischoff. *Nature Reviews Immunology* 7, 93-104 (February 2007)
**B-cell crosslinking by allergens**

- Flexible, multivalent antigens?
- Multivalent, multiepitope antigens?
- Monovalent antigen/allergen?
- No secreted IgE
**B-cell crosslinking by allergens**

*non-productive*  

*productive*

- Multimeric antigens/allergens
- Monovalent antigen/allergen
- Flexible, multivalent antigens
- B-cell crosslinking by allergens
- Non-productive
- Productive

- Secreted IgE
- Multimeric antigens/allergens

- IgE cells

- Secreted IgE
Table 1: Examples of allergens, which are able to form dimers or multimers (sorted by year of publication).

<table>
<thead>
<tr>
<th>Allergen (source)</th>
<th>Year of publication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gad c 1 (Baltic codfish)</td>
<td>1967</td>
<td>(Aas and Jebsen, 1967)</td>
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<tr>
<td>Ole e 1 (olive pollen)</td>
<td>2001</td>
<td>(Huecas et al., 2001)</td>
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<tr>
<td>Api m 4 (bee venom)</td>
<td>1982</td>
<td>(Terwilliger et al., 1982)</td>
</tr>
<tr>
<td>ABA 1 (Ascaris, nematodes)</td>
<td>1990</td>
<td>(McGibbon et al., 1990)</td>
</tr>
<tr>
<td>A2U (rat urinary allergen)</td>
<td>1992</td>
<td>(Bocskei et al., 1992)</td>
</tr>
<tr>
<td>Mus m 1 (MUP; mouse urinary protein)</td>
<td>1992</td>
<td>(Bocskei et al., 1992)</td>
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<tr>
<td>Gly m Bd 30K (soy bean)</td>
<td>1993</td>
<td>(Ogawa et al., 1993)</td>
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<tr>
<td>Sol i II (fire ant)</td>
<td>1993</td>
<td>(Hoffman, 1993)</td>
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<tr>
<td>Tropomyosin (e.g. fish)</td>
<td>1995</td>
<td>(Gimona et al., 1995)</td>
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<tr>
<td>Alt a 1 (Alternaria, fungi)</td>
<td>1996</td>
<td>(De Vouge et al., 1996)</td>
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<tr>
<td>Bet v 1 (birch pollen)</td>
<td>1996</td>
<td>(Wellhausen et al., 1996)</td>
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<tr>
<td>Bos d 2 (BDA20) and Bos d 5 (bovine dander allergens)</td>
<td>1996</td>
<td>(Rautiainen et al., 1996)</td>
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<tr>
<td>Ara h 1 (peanut)</td>
<td>1998</td>
<td>(Shin et al., 1998)</td>
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<tr>
<td>Phil p 1 (grass pollen)</td>
<td>1998</td>
<td>(Petersen et al., 1998)</td>
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<tr>
<td>Aca s 13 (Acarus siro, dust mite)</td>
<td>1999</td>
<td>(Eriksson et al., 1999)</td>
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<tr>
<td>Equ c 1 (horse dander)</td>
<td>1999</td>
<td>(Gregoire et al., 1999)</td>
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<td>Tropomyosin (seafood)</td>
<td>1999</td>
<td>(Reese et al., 1999)</td>
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<tr>
<td>ABA-1 (Ascaris, nematodes)</td>
<td>2000</td>
<td>(Xia et al., 2000)</td>
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<tr>
<td>Ara h 1 (peanut)</td>
<td>2000</td>
<td>(Maleki et al., 2000)</td>
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<tr>
<td>Equ c 1 (horse dander)</td>
<td>2000</td>
<td>(Lascombe et al., 2000)</td>
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<tr>
<td>Ves v 5 (wasp venom)</td>
<td>2000</td>
<td>(Suck et al., 2000)</td>
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<td>Ves v 5 (wasp venom)</td>
<td>2000</td>
<td>(Suck et al., 2000)</td>
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<td>Beta-lactoglobulin (bovine milk)</td>
<td>2001</td>
<td>(Sakurai et al., 2001)</td>
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<tr>
<td>Ara h 2 (peanut)</td>
<td>2002</td>
<td>(Sen et al., 2002)</td>
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<tr>
<td>Gad m 1 (Atlantic codfish)</td>
<td>2002</td>
<td>(Das Dores et al., 2002)</td>
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<tr>
<td>Phl p 5b (grass pollen)</td>
<td>2002</td>
<td>(Rajashankar et al., 2002)</td>
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<tr>
<td>Phl p 7 (grass pollen)</td>
<td>2002</td>
<td>(Verdino et al., 2002)</td>
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<tr>
<td>Profilin (panallergen)</td>
<td>2002</td>
<td>(Wopfner et al., 2002)</td>
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<tr>
<td>Cav p 1 (guinea pig dander)</td>
<td>2003</td>
<td>(Fahlbusch et al., 2003)</td>
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<tr>
<td>Fel d 1 (cat dander)</td>
<td>2003</td>
<td>(Gronlund et al., 2003)</td>
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<tr>
<td>Bet v 1 (birch pollen)</td>
<td>2005</td>
<td>(Schöll et al., 2005)</td>
</tr>
<tr>
<td>Dac g 5 (grass pollen)</td>
<td>2005</td>
<td>(van Oort et al., 2005)</td>
</tr>
<tr>
<td>Ara h 1 (peanut)</td>
<td>2006</td>
<td>(van Boxtel et al., 2006)</td>
</tr>
<tr>
<td>Der p 1 (house dust mite)</td>
<td>2006</td>
<td>(de Halleux et al., 2006)</td>
</tr>
<tr>
<td>Can f 1 and 2 (dog dander)</td>
<td>2007</td>
<td>(Kamata et al., 2007)</td>
</tr>
<tr>
<td>Bla g 2 (cockroach)</td>
<td>2008</td>
<td>(Li et al., 2008)</td>
</tr>
<tr>
<td>Der p 21 (house dust mite)</td>
<td>2008</td>
<td>(Weghofer et al., 2008)</td>
</tr>
<tr>
<td>Per a 3 (cockroach)</td>
<td>2008</td>
<td>(Bellinghausen et al., 2008)</td>
</tr>
<tr>
<td>Per a 4 (cockroach)</td>
<td>2009</td>
<td>(Tan et al., 2009)</td>
</tr>
</tbody>
</table>
What makes a protein an effector allergen, i.e. triggering symptoms?

**Multivalency?**

*Multivalent antigen crosslinks* bound IgE….
*Immunobiology, by Janeway & Travers 1997.*

...The immediate phase reaction occurs as a result of **cross-linking with multivalent allergen**….
*Current Opinion in Allergy and Clinical Immunology 2002, by R. Pawankar*

Aggregation of FcεRI, typically by the binding of **di- or multivalent allergen** recognized by the IgE, induces mast cell and basophil activation.
**Effector cell crosslinking by allergens**

*non-productive*

*productive*

**INTERPHASES**
- BETWEEN ALLERGEN AND IgE
- BETWEEN IgE and FcεRI, CD23

Flexible, multivalent antigens

enhanced cell survival & FcεRI expression

monovalent antigen/allergen

monomeric IgE

**IgE-effector cells?**

mediators
chemokines

cytokines

multimeric antigens/allergens
Bet v 1 monomer does not trigger HR

A Bet v 1 trimer does not trigger HR

Disagreement with hypothesis?

Figure 3. A birch pollen allergic patient (left) and a nonatopic person (right) were skin prick tested on the forearm with various concentrations of rBet v 1 monomer and trimer (insert). Wheal areas were surrounded.

Epitope spacing is critical for Crosslinking

Does IgE against innocuous antigens make sense?
The function of IgE: Protection against parasites?

- Highly elevated IgE levels in sera
- Eosinophils elevated to 50% of leucocyte counts

“…a causal association between geohelminth infection and atopic disorders has not been established.“

“…evidence from experimental animal models infected with helminth parasites and treated with anti-IgE antibodies do not support a critical role for IgE in mediating protection against helminths.“
*Cooper PJ et al, Allergy. 2008 Apr;63(4):409-17*

*Read more in: Survival of the fittest: allergology or parasitology?*  
IgE: exclusively cooperation with cells

With eosinophils, mast cells, basophils, macrophages:
Effector functions

• **ADCC - Cytotoxicity**: IgE and Fc\(_\varepsilon\)RI
• **ADCP - Phagocytosis**: IgE and CD23

With DCs and other APCs, IgE supports
Antigen transport and immunity

• **Antigen focussing**: IgE and Fc\(_\varepsilon\)RI, CD23

*From: Taking our breath away: dendritic cells in the pathogenesis of asthma*

Immune cells present in tumor tissue (Red and Green)

"Chronic infiltration of innate immune cells belonging to the myeloid cell lineage, including mast cells, macrophages, and neutrophils, correlates with poor disease prognosis."

Source: Nature review in Cancer 2006
Cancer mortality (n= 1,102,247) and allergies.

„…significant inverse associations between asthma and hay fever and cancer mortality ….“


Anti-IgE therapy and Cancer.

„…Xolair patients had a 0.5% malignancy rate compared to 0.2% of control patients.“

(FDA Advisory committee)
Evidence for IgE combating tumors

- IgE is more effective in cytotoxicity than IgG


Dense infiltration by macrophages tumor transplanted SCID mice treated with IgE.


IgE inhibits tumor growth in SCID.

From:
## Table I. IgE in allergy and oncology

<table>
<thead>
<tr>
<th>Authors</th>
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<tr>
<td><strong>Definition of Allergy &amp; Era of Reagins</strong></td>
<td></td>
<td>1906</td>
</tr>
<tr>
<td><strong>Identification of IgE</strong></td>
<td></td>
<td>1967</td>
</tr>
<tr>
<td><strong>Definition of AllergoOncology</strong></td>
<td></td>
<td>2006</td>
</tr>
</tbody>
</table>
• Occurrence of natural IgE in head and neck cancer

*Neuchrist et al, Int. Archs Allergy Clin Immun. 1994*

• Occurrence of natural IgE in pancreatic cancer: tumoricidic

*From: Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells.*

Natural IgE in melanoma?

- **IgE**
  - Green: anti human IgE/IgG-FITC
  - Blue: DAPI to stain nuclei

- **IgG**

Melanoma sample
1774/08/1
Natural IgE in melanoma by TissueFAXs

IgE

120 / 83.674 positive

IgG

2.233 / 84.386 positive
Passive immunotherapy with IgE
Passive Antibody-immunotherapy

- ADC, CDC
- Radioimmunoconjugates
- Immune cytokines
- Immunotoxins
- Direct Apoptosis
- Internalisation and downregulation

Colon cancer
Breast cancer

Growth arrest
Herceptin-IgE construction

- **cDNA** encoding heavy and light chains of Herceptin® (source: www.pdb.org; 1n8z)
- **Cloning** into vectors with epsilon heavy chain, and human kappa light chain
- **Transfection** into HEK293 cells
- **Antibodies** purified by affinity chromatography

*Karagiannis and Singer et al, Cancer Immunol. Immunother, 2008*
Herceptin-IgE: affinity to HER-2 and FcεRI

![Graph showing time vs. response for Trastuzumab IgE and NIP IgE](image)

<table>
<thead>
<tr>
<th>ECD\textsuperscript{HER2} surface</th>
<th>sFcεRIα surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>Trastuzumab IgE</td>
</tr>
<tr>
<td>$k_a$ (M\textsuperscript{-1}s\textsuperscript{-1})</td>
<td>$(3.52 \pm 1.36) \times 10^5$</td>
</tr>
<tr>
<td>$k_d$ (s\textsuperscript{-1})</td>
<td>$(2.39 \pm 0.31) \times 10^{-5}$</td>
</tr>
<tr>
<td>$K_a$ (M\textsuperscript{-1})</td>
<td>$1.48 \times 10^{10}$</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Calculated kinetic values of trastuzumab IgE.
Precondition for effector function:
Herceptin-IgE binds to IgE receptors FcεRI and CD23

**FcεRI expressing RBL-SX38**

**CD23 expressing A8866 cells**
Visualization of effector functions

Phagocytosis in 3-color assay

M. Bracher et al., Journal of Imm. Meths 2007
Quantification of effector functions

- αCD89 PE stained cells --> Monocytes U937
- CFSE-stained cells --> Tumor cells
- Double stained cells --> phagocyted tumor cells
- Propidium Iodide positive cells --> Cytotoxicity

M. Bracher et al., Journal of Imm. Meths 2007
Herceptin-IgG or –IgE in 3-color assay

Cetuximab (Erbitux®) targets EGFR

Construction of a Cetuximab-IgE

MTT cell viability assay with EGFR+ A431 cells
Co-culture EGFR positive A-431 cells and RBL-SX38 effector cells with cetuximab IgG or IgE

After 16 hours two separate populations

Mixed populations
Co-culture A-431/RBL-SX38 and IgE (3 h)
Active immunotherapy with IgE
subcutaneous mimotope-vaccine induces Herceptin®-like IgG antibodies

- receptor internalisation
- ADCC, CDC
- *in vivo* tumor arrest in transgenic model

How to induce IgE: Lessons from Food Allergy

Formation of IgE-antibodies against non-digested proteins

Protein and acid inhibitor

Untersmayr & Jensen-Jarolim. JACI 2008
oral mimotope vaccine induces Herceptin®-like IgE antibodies

Riemer AB et al., Cancer Research 2007
Commons of allergens and tumor antigens

**Allergen Prototype**
- Homomultimers
- Aggregation and clustering of epitopes

**AAMPs:**
- Allergen-Associated Molecular Patterns

**Tumor Prototype**
- Homodimers
- Clustering of overexpressed TAAs

**TAMPs:**
- Tumor-Associated Molecular Patterns
Effector cell crosslinking by tumor antigens: ADCC, ADCP

- Natural anti-tumor IgE
- Recombinant IgE antibodies
- IgE-based oral vaccine

overexpressing tumor cell

E-effector cells

mediators chemokines cytokines

multimeric antigens/allergens
Novel biological functions of IgE antibodies

Erika Jensen-Jarolim

Medizinisches Propädeutikum,
18. November 2009