Therapeutic Repurposing of Human IgE Monoclonal Antibodies (mAbs)

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IgE: last to be discovered, not due to importance, due to concentration

- Discovered 1967, over 100 years after IgG
- IgE is $< 300,000^{th}$ of the concentration of IgG
- Highest known human receptor-ligand affinity
- Responsible for allergy and worm immunity
IgE is at the heart of type 2 immunity

Parasitic worm infections: ancient purpose
Allergic disease: modern problem

Histamine
Leukotriene
Prostaglandin
Proteases
Antimicrobial peptides
Chemotactic factors

No one has studied natural human IgE as a mAb

Nature Medicine 11, 381-382
Parasitic worm infections have shaped our immunity

> 2 billion infections worldwide
What proteins correctly induce type 2 immunity & IgE?
IgE-mediated allergic disease is important

- U.S. prevalence of allergic disease: 1 in 5 and climbing
- 5th leading chronic disease in all ages, 3rd in children
- 15 million people in U.S. have allergies to food
- Food allergy, particularly those to nuts, are characterized by severe reactions = anaphylaxis
- Two peanut proteins are most to blame: Ara h 2 & 6
- Current treatment for food allergy = avoidance
How do we make natural human IgE mAbs

1. PBMC isolation
2. B cell expansion in culture
3. Identify IgE B cells
4. Fusion with myeloma partner
5. Grow and select hybridoma
6. Purify human IgE mAb
We have natural human IgE mAbs

- Peanut (Ara h 2 & 6)
- Cashew nut
- Walnut
- Pecan nut
- Milk
- Wheat
- Alpha-gal
- Egg
- Dust mite (Der p 1 & 2)
- Dog (Can f 1)
- Cat (Fel d 1)
- Grasses and weeds
- Aspergillus & Alternaria
- Helminths
Novel strategy for allergy therapeutics & immunotherapies
Approach

① Express Ara h 2- and Ara h 6-specific human IgE mAbs as recombinant therapeutic IgG

② Assess the blocking potential of these therapeutic IgGs against peanut-allergic patient sera *in vitro*

③ Assess the ultimate therapeutic effect of IgG mAbs in passive anaphylaxis *in vivo*
Peanut allergen-specific human IgE mAbs

Table 1. Select human peanut-specific IgE mAbs.

<table>
<thead>
<tr>
<th>Human IgE mAbs</th>
<th>IgE mAb reactivity</th>
<th>Fine specificity</th>
<th>Allergen size (kDa)</th>
<th>Allergen family</th>
</tr>
</thead>
<tbody>
<tr>
<td>5C5</td>
<td>Peanut</td>
<td>Ara h2</td>
<td>17</td>
<td>2S albumin</td>
</tr>
<tr>
<td>13D9</td>
<td>Peanut</td>
<td>Ara h2</td>
<td>17</td>
<td>2S albumin</td>
</tr>
<tr>
<td>15A4</td>
<td>Peanut</td>
<td>Ara h2</td>
<td>17</td>
<td>2S albumin</td>
</tr>
<tr>
<td>1H9</td>
<td>Peanut</td>
<td>Ara h6</td>
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<td>2S albumin</td>
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</tr>
<tr>
<td>1C10</td>
<td>Peanut</td>
<td>Ara h9</td>
<td>9</td>
<td>nsLTP</td>
</tr>
</tbody>
</table>

All IgE mAbs were obtained from the peripheral blood cells of subjects known to have severe peanut allergy. MAb reactivity was determined using Phadia diagnostics and/or by Western blot. nsLTP = non-specific lipid transfer protein.
Produce authentic natural & recombinant peanut allergens

5C5 binding to nAra h2

EC_{50} = 4.1 ng/mL

5C5 binding to rAra h2

EC_{50} = 4.3 ng/ml
Testing therapeutic peanut-specific IgG mAbs is underway

Serum blocking studies will identify/map functional antigenic sites

Transgenic human mouse model of anaphylactic shock

**Human IgE/Mouse Controls**

**First Peanut-specific IgE mAbs**

Temperature (°C)

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% Survival

Minutes after challenge

- IgE/anti-kappa
- IgE/saline
- PBS/anti-kappa
- PBS/saline

- 1H9 & 8F3
- 5C5 & 13D9
- 13D9 & 15A4
- 5C5 & 15A4
Summary

• We can now make/study for the first time natural human allergen- and helminth-specific IgE mAbs

• They can be used as diagnostic tools and to standardize allergenic extracts for immunotherapy (IT)

• We are testing the therapeutic effect of peanut-specific mAbs as a new therapeutic strategy for IT
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