Importance of Bone Marrow Plasma Cells in IgE Memory

- Ettle-Dorma et al. The majority of allergen-specific IgE in the blood of allergic patients do not originate from blood-derived B cells or plasma cells. Clin Exp Allergy 42, 137-55, 2012
- Luger et al. Allergy for a lifetime. Allergy 65, 1-8

Our “Projectory”

- Look back at some of our earlier work relating to IgE plasma cells in the respiratory tract
- Describe ongoing studies by next generation sequencing (NGS) of the expressed immunoglobulin genes in rhinitis, asthma
- End with possible implications of results for future directions

Local IgE synthesis

A large fraction of local IgE is allergen-specific

- Normal
- HDM
- Pollen

Time (days)

Relevance of local IgE synthesis

* Rate of IgE synthesis *ex vivo* = 3.6x10^9 molecules/day/mm^3
* Rate of loss of IgE from mast cells = 10^7 molecules/day/mm^3

Assumptions:
1. number of mast cells/mm^3
2. number of IgE receptors/mast cell
3. rate of dissociation of IgE from mast cells in tissues

* IgE synthesis = 3.6x10^9 >> IgE loss = 10^7 molecules/day/mm^3

* Conclusion: Local IgE Production is 100X more than required to saturate the mast cells in the tissue and maintain immediate hypersensitivity.


The ontogeny of memory IgE plasma cells

* What drives these events and what are the underlying mechanisms and functional outcomes?
* What can we learn from next generation sequencing (NGS) of the B cell repertoires?

Generation of Diversity: By the Numbers

- 40 V H gene segments
- 35 000 gene segments
- 11 H gene segments
- 40 V J combinations
- > 100 V-J combinations

Total H C diversity = 1.2x10^9

Estimated Total Diversity = 1 x 10^10

Using the junctional sequences to identify clonal families

Family trees reveal clonal lineage and relationships

Germ Line

Number of mutations

Sequence alignment against germ line

Low mutation

High mutation

Same CDR-H3

Same VDJ rearrangements

Variants with different mutations and isotypes

Direct & sequential switching to IgE

Xiong et al., J. Exp. Med. 2012; 209: 353-642
Characteristics of clonal trees

Sanger sequencing 2003
120 Vε cDNA sequences
Nasal mucosa of 6 patients
3 IgE clonal families

NGS 2014
1,318 Vε cDNA sequences
Nasal mucosa of 7 patients
295 IgE clonal families

Levels of somatic hypermutation

Clones from peripheral blood & nasal biopsy pooled

Wu et al., JACI 2014; 134: 604-12

Relative frequency of somatic hypermutation

Wu et al., JACI 2014; 134: 604-12

“Connectivity”

Ohm-Laursen et al., in progress

B cell repertoire in asthma

Ohm-Laursen et al., in progress

Mining data from 4 different studies

- Influence of seasonal exposure to grass pollen on local tissue and peripheral blood IgE repertoires in patients with allergic rhinitis. Wu et al., J Allergy Clin Immunol 134, 604-12, 2014
- Relation between the B cell repertoire, local inflammation and the clinical response to allergen in allergic rhinitis. James et al., in progress
- Both local and distant connectivity between immunoglobulin clones in the human lung mucosa revealed by new generation sequencing. Ohm-Laursen et al., in progress
Levels of somatic hypermutation in lung vs. blood in different isotypes

Expected hot spots of mutation

Is the response Ag-driven?

Sampling and saturation of the repertoire
Summary with Questions:

The respiratory tract mucosa is a site for the development of IgE plasma cells.

• B cells migrate into the mucosa and undergo (antigen-dependent?) somatic mutation and immunoglobulin class switching to multiple isotypes, including IgE.

• Clonal expansion (selection?) and cell differentiation into plasma cells occurs in the mucosa.

• Cells migrate out of the mucosa and may re-enter at other sites.

• We don’t know the fates of all the various cells, but in immunized mice, nasal allergen challenge generates short-lived plasma cells some of which migrate to the bone marrow to become IgE memory plasma cells (Luger et al., 2009).

• We can’t rule out a contribution of local lymphoid tissue and mucosal tissue to some of these processes, e.g., class switching and somatic hypermutation followed by homing (chemotaxis) of selected B cell populations into the mucosa.

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Dr. Louisa James
Dr. Jiun-Bo (Leo) Chen et al.

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Prof. Christopher Corrigan (King’s College London)
Prof. Sebastian Johnston (Imperial College London)
Dr. Harsha Kariyawasam (University College London) et al.

My lab (current)
Dr. Yu-Chang (Bryan) Wu
Dr. Line Ohm-Laursen
Dr. Farah Flamandini
Dr. Holly Bowan et al.

Biopsy donors

Expected hot spots of mutation

Relation between clinical response to allergen and local inflammation

James et al., in progress
The elevated expression levels of the majority of the 47 pro-inflammatory genes in the AR patients correlated with the clinical response to allergen challenge, as exemplified here by STAT3, IL6R and JAK1.

The ground breaking discovery was that 47 genes were differentially expressed between allergic and non-allergic controls. Heat map generated in nSolver v2.0 based on p<0.05.

47 genes were differentially expressed between allergic and non-allergic controls. Heat map generated in nSolver v2.0 based on p<0.05.
Anti-enterotoxin Abs are highly mutated

<table>
<thead>
<tr>
<th>Clone</th>
<th>203</th>
<th>1G2</th>
<th>1A4</th>
<th>1B6</th>
<th>1F3</th>
<th>2D6</th>
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<tbody>
<tr>
<td>IGHV mut (%)</td>
<td>7.64</td>
<td>8.68</td>
<td>4.86</td>
<td>5.90</td>
<td>15.02</td>
<td>4.76</td>
</tr>
<tr>
<td>IGHJ mut (%)</td>
<td>17.02</td>
<td>10.64</td>
<td>12.24</td>
<td>8.16</td>
<td>12.70</td>
<td>8.51</td>
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<tr>
<td>Number of mutations in IGHV</td>
<td>23</td>
<td>26</td>
<td>14</td>
<td>17</td>
<td>43</td>
<td>14</td>
</tr>
</tbody>
</table>

Table IV: Distribution of GDT and CT in anti-enterotoxin GP allergic patients following GP allergen challenge
Combined molecular approaches for B cell analysis

Does mutation frequency correlate with expression of genes involved in germinal centres (e.g. AID, BCL-6)?

What is the relationship between antigen-specific B cells and the overall immunoglobulin repertoire?

What is the phenotype of antigen-specific B cells?

Grouping related sequences into clonotypes

Sequences

<table>
<thead>
<tr>
<th>Class</th>
<th>Number</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>46</td>
<td>39</td>
<td>37</td>
<td>3</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Number</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
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</thead>
<tbody>
<tr>
<td>Total</td>
<td>46</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

Grouping related sequences into clonotypes

Cells derived from the same progenitor

CDR-H3 fingerprints

Clustering

Same CDR-H3

Mutation may be different

CDR-H3 DNA motifs

Clonally related, mutated variants

Representative clonotypic sequence

Local IgE expression is key to allergic disease

Local IgE expression

Platts-Mills T., JI 1979

Sensi L.G., et al. CEA 1994

Kleijmeer M., et al. ERJ 2000

Smurthwaite L., et al. EJI 2001

Local IgE switching

Takhar P., et al. JACI 2005

Takhar P., et al. JACI 2007
Epitope specificity of anti-SEEs

Class switch recombination (IgM→IgA1)

B cell phenotyping

Gene expression analysis of the nasal mucosa

Nanostring uses molecular "barcodes" and single molecule imaging to detect and count transcripts allowing highly multiplexed measurement of gene expression.
Gene expression analysis of the nasal mucosa

Hybridised target RNA is immobilized, orientated and counted

Experimental workflow

Day 1
overnight hybridisation of RNA with Reporter and Capture ProbeSets (at 65°C)

Day 2
Immobilisation of hybridised target to nanostring cartridge (automated on nCounter Prep station)

Samples analysed on nCounter Digital Analyser

* GEE nCounter facility, UCL

IgE+ PC differentiation is promoted by sequential CSR

• Both direct (Iε → Cμ; IgM → IgE) and sequential (Iε → Cγ; IgM → IgG → IgE) switching detected in our IgE lo and IgE hi cells

However, only sequential switching detected in IgE+ CD138+ cells

Analysis of antibody genes reveals the diversity of the immunoglobulin repertoire

Theoretical size of the human immunoglobulin repertoire: >10^12

Approximate number of B cells in a human: 10^9

Analysis of nasal immunoglobulin repertoires by high throughput sequencing

1. 0.5 μg of RNA converted to cDNA
2. Separate nested PCR reaction were performed for each antibody class in 8 replicates
3. Following purification of PCR products by gel extraction samples were pooled in equal quantities
4. PCR products were sequenced
IgE is more mutated in allergic versus healthy subjects

Advances in sequencing technology provide a greater insight into antibody repertoires

Increased expression of genes associated with cellular migration

Increase in antigen presentation within the nasal mucosa

Experimental Pipeline

Fluidigm Biomark platform: analysis of 96 genes in 96 single cells

McKenna J, Zeng Y, Chien YH, Quake SR. Correlation of Gene Expression and Genome Mutation in Single B Cells (2011)
VDJ recombination in the H-chain locus

Ig Germline Gene Repertoire

<table>
<thead>
<tr>
<th>Number of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH2</td>
</tr>
<tr>
<td>VH3</td>
</tr>
<tr>
<td>VH4</td>
</tr>
<tr>
<td>VH5</td>
</tr>
<tr>
<td>VH6</td>
</tr>
<tr>
<td>VH7</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

(VHD:23 / IGHJ:6 / IGHC:9) + 10^7 V-D-J-C Regions

Somatic hypermutation

- Encodes Ab with altered Ag binding site
- may result in higher or lower affinity for Ag

VH domain gene and protein

Blood antibody classes

<table>
<thead>
<tr>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgE</th>
</tr>
</thead>
</table>

Biopsy antibody classes

<table>
<thead>
<tr>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgE</th>
</tr>
</thead>
</table>

Pellec (Gevaert et al., Allergy 2005)

Nasal biopsy (Coker et al., J. Immunol., 2003)
Table 1. IgE related to other classes in lineage trees.

<table>
<thead>
<tr>
<th>IgE related to</th>
<th>Non-allergic</th>
<th>All (inc &amp; out of season)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>1 (0.05%)</td>
<td>0</td>
</tr>
<tr>
<td>IgG &amp; IgE</td>
<td>2 (0.19%)</td>
<td>1 (0.01%)</td>
</tr>
<tr>
<td>IgG</td>
<td>0</td>
<td>1 (0.15%)</td>
</tr>
<tr>
<td>IgE &amp; IgG</td>
<td>0</td>
<td>1 (0.15%)</td>
</tr>
</tbody>
</table>

*Absolut number observed. **Frequency is calculated based on the clone counts in Table E3, comparison between NA and JR.

Gould et al. 2006, Trends Immunol. 27: 446-452