

Basic Immunology: IgE Memory

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The discovery of IgE

S. G. O. Johansson, MD, PhD Stockholm, Sweden

In 1919, the search began for the factor, later called reagin, that could mediate an allergy, such as allergic asthma, in sera of allergic subjects. In 1967, the fifth class of immunoglobulins, IgE, was discovered and found to be able to carry reagin activity. This discovery has had immense importance for the understanding, diagnosis, and treatment of allergic diseases. (J Allergy Clin Immunol 2016;137:1671-3.)

Key words: Allergy, inflammation, mast cell, immunoglobulin, sensitization, reagin

In 1919, one of Dr Ramirez's patients with aplastic anemia who had just received a blood transfusion had an asthma attack while taking a horse ride in Central Park in New York. Ramirez was observant and noticed that the blood came from a donor with allergy to horses. He published a case report stating that blood could transfer allergy to horses and cause asthma. Two years later, in 1921, Prausnitz and Küstner passively sensitized the skin of healthy subjects and transferred positive

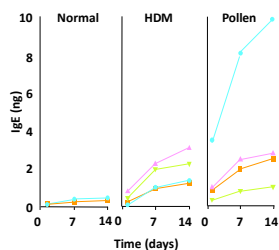
Importance of Bone Marrow Plasma Cells in IgE Memory

- Holt *et al.* Long-lived IgE and IgG-secreting cells in rodents manifesting persistent antibody responses. *Cellular Immunol* 89, 281-8, 1984
- Eckle-Dorna *et al.*, The majority of allergen-specific IgE in the blood of allergic patients does not originate from blood-derived B cells or plasma cells. *Clin Exp Allergy* 42, 137-55, 2012
- Luger *et al.* Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. *J Allergy Clin Immunol*, 124, 819-26, 2009
- Luger *et al.* Allergy for a lifetime. *Allergol Int* 2010, 59, 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

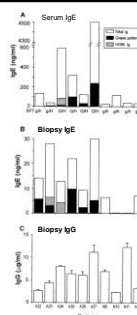
Local IgE synthesis



Smurthwaite *et al.*, *Eur. J. Immunol.* 31, 3422-31, 2001

A large fraction of local IgE is allergen-specific

Smurthwaite *et al.*, *Eur. J. Immunol.* 31, 3422-31, 2001



Relevance of local IgE synthesis

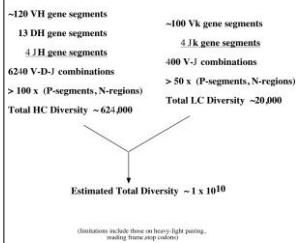
- Rate of IgE synthesis *ex vivo* = 3.6×10^9 molecules/day/mm³
- Rate of loss of IgE from mast cells = 10^7 molecules/day/mm³
Assumptions:
1. number of mast cells/mm³
2. number of IgE receptors/mast cell
3. rate of dissociation of IgE from mast cells in tissues
- IgE synthesis = $3.6 \times 10^9 \gg$ IgE loss = 10^7 molecules/day/mm³
- Conclusion: Local IgE Production is 100X more than required to saturate the mast cells in the tissue and maintain immediate hypersensitivity.

Gould et al., Annu Rev Immunol 2003, 21: 579-628

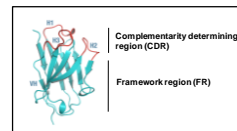
The ontogeny of memory IgE plasma cells

- 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

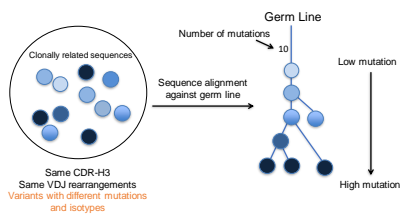


Using the junctional sequences to identify clonal families

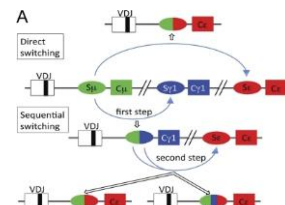


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Family trees reveal clonal lineage and relationships

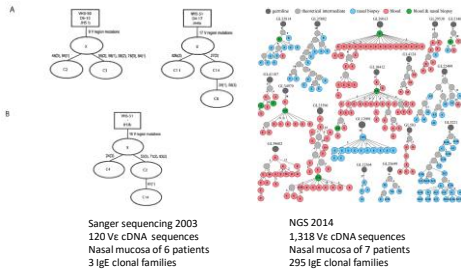


Direct & sequential switching to IgE



Xiong et al., J. Exp. Med. 2012; 209: 353-642

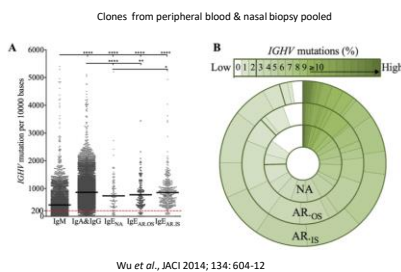
Characteristics of clonal trees



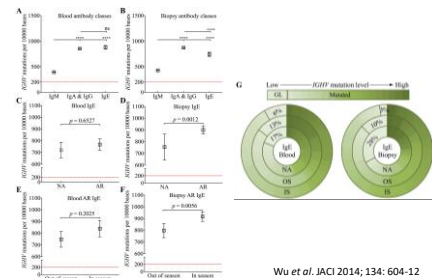
Mining data from 4 different studies

- 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
- Antibodies and superantibodies in patients with chronic rhinosinusitis with nasal polyps. Chen *et al.*, *J Allergy Clin Immunol* 138, 1195-204, 2016
- 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

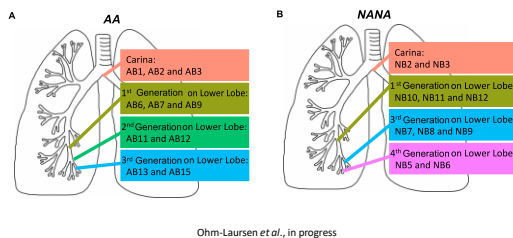
Levels of somatic hypermutation



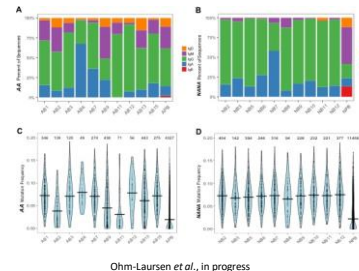
Relative frequency of somatic hypermutation



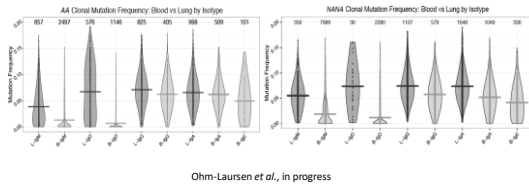
"Connectivity"



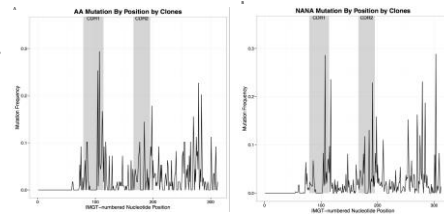
B cell repertoire in asthma



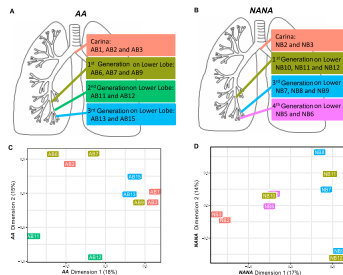
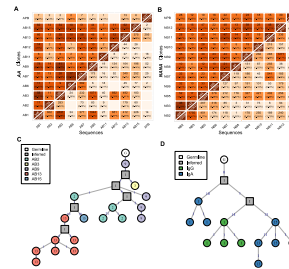
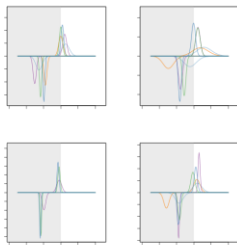
Levels of somatic hypermutation in lung vs. blood in different isotypes



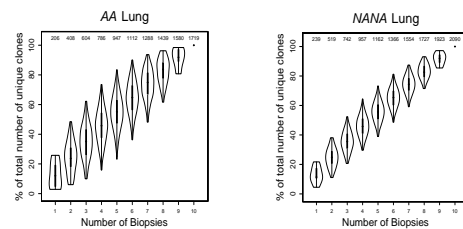
Expected hot spots of mutation



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.

Ontogeny of IgE B cells?



Acknowledgements

My lab (past)

Dr. Lyn Smurthwaite
Dr. Pooja Takhar
Dr. Heather Coker
Dr. Louisa James
Dr. Jun-Bo (Leo) Chen
et al.

Clinical Colleagues

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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.

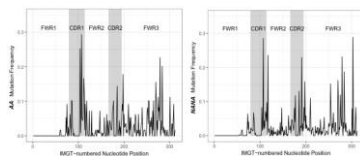
My lab (current)

Dr. Yu-Chang (Bryan) Wu
Dr. Line Ohm-Laursen
Dr. Faruk Ramadani
Dr. Holly Bowen
et al.

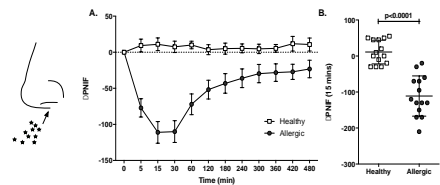
Biopsy donors



Expected hot spots of mutation

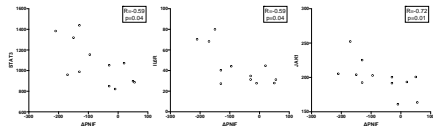


Relation between clinical response to allergen and local inflammation



James *et al.*, in progress

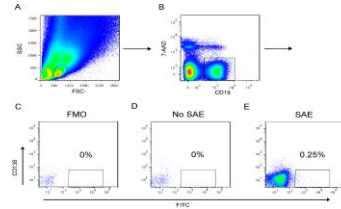
Gene expression vs. clinical response to allergen



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.

Spearman correlations

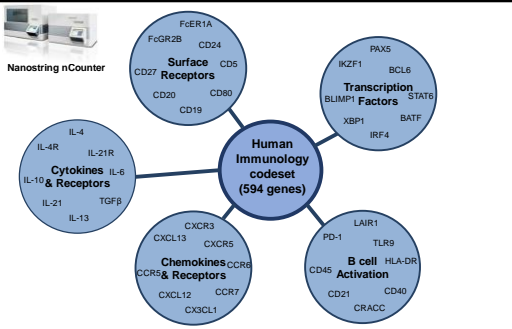
Isolation of single B cells expressing allergen specific antibodies



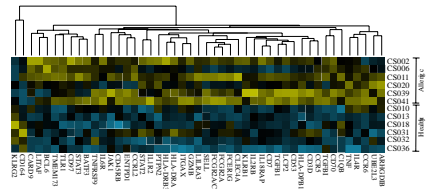
Chen et al., 2016



Nanostring nCounter



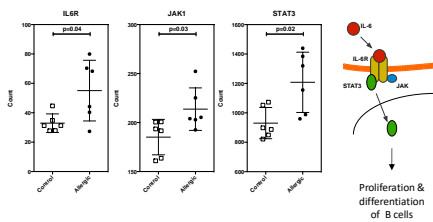
Signature of allergic inflammation



47 genes were differentially expressed between allergic and non-allergic controls

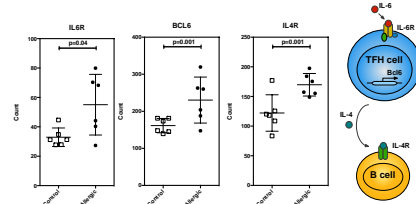
Heat map generated in nSolver v2.0 based on $p < 0.05$

Activation of the IL-6R signaling pathway

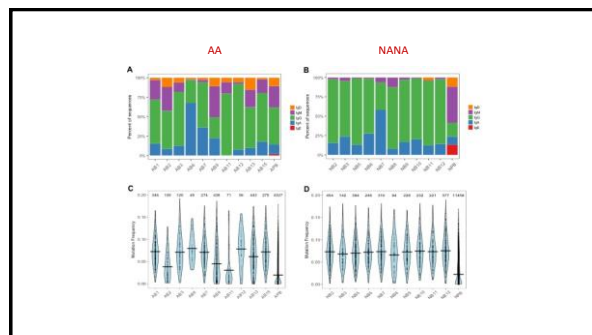
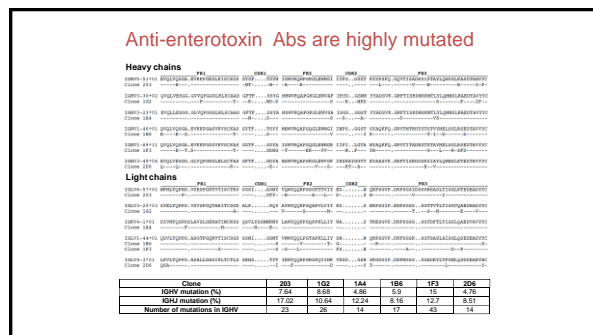
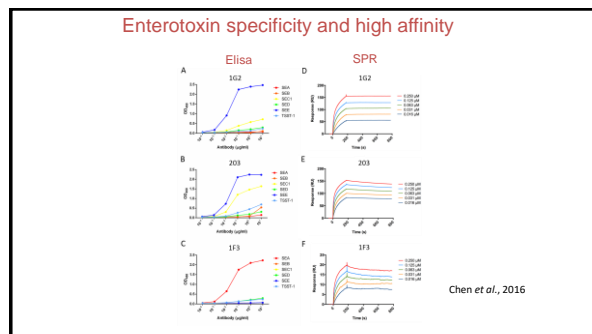
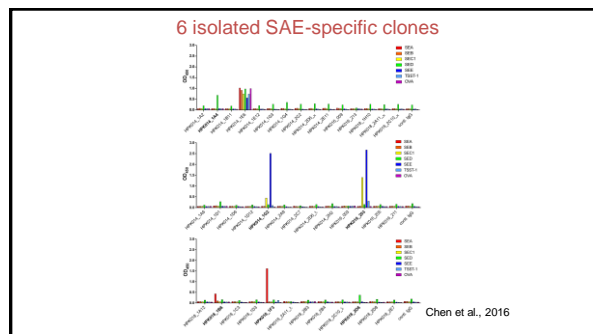


P-values were calculated with Mann-Whitney tests

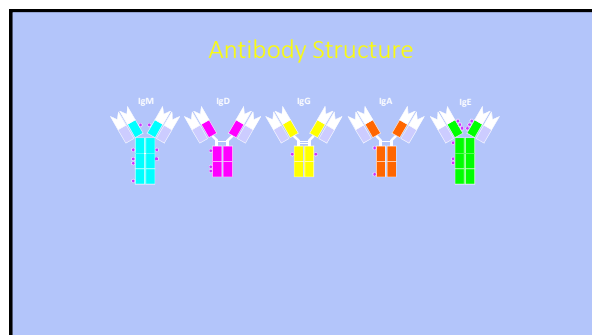
Up-regulation of Bcl-6 and IL-4R expression

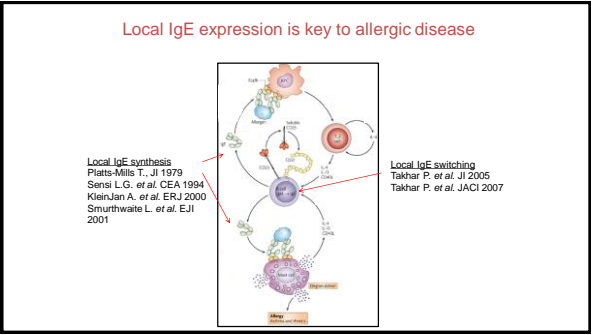
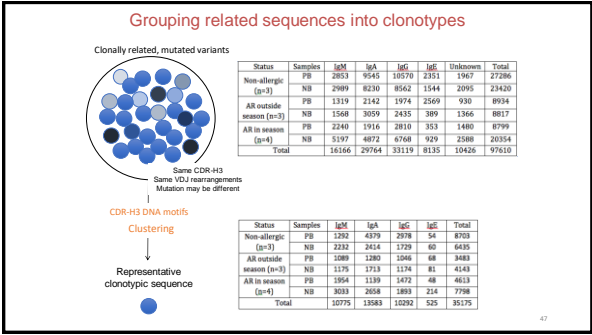
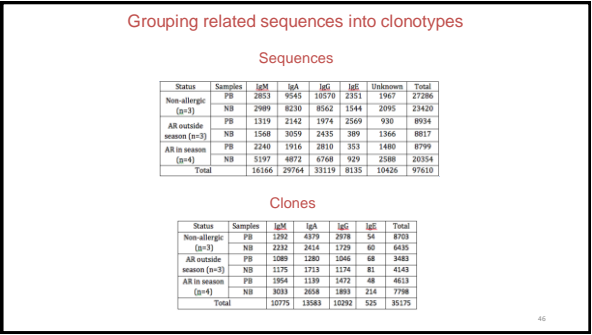
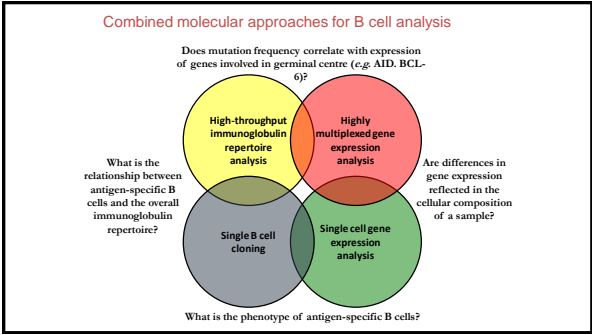
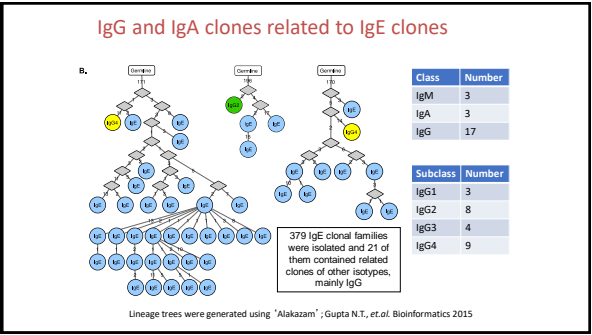
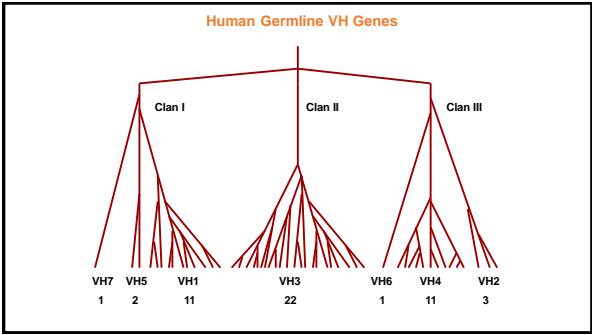


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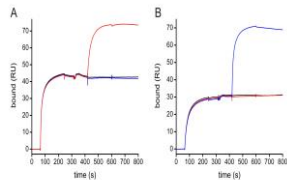


	Culture Medium				Allergen Challenge			
	AT	CM	KG	SS	AT	CM	KG	SS
<i>x</i> GLT					+	+		
μ GLT		+					+	+
γ 1 GLT		+		+	+	+	+	+
γ 3 GLT	+	+			+	+	+	+
γ 4 GLT					+	+		
1e-C μ CT		+				+	+	+
1e-C γ CT				+	+	+		+
1e-C γ 1 CT					+		+	+
1e-C γ 3 CT						+		+
1e-C γ 4 CT			+					+

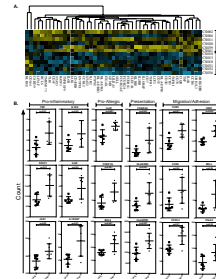




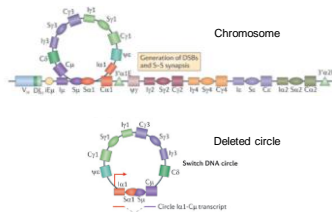
Epitope specificity of anti-SEEs



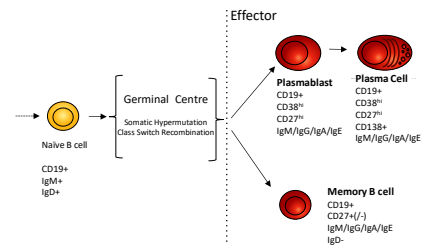
Transcriptome analysis



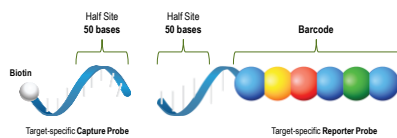
Class switch recombination (IgM → IgA1)



B cell phenotyping

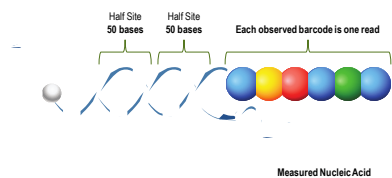


Gene expression analysis of the nasal mucosa



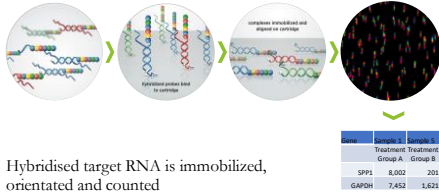
Nanostring uses molecular "barcodes" and single molecule imaging to detect and count transcripts allowing highly multiplexed measurement of gene expression

Gene expression analysis of the nasal mucosa



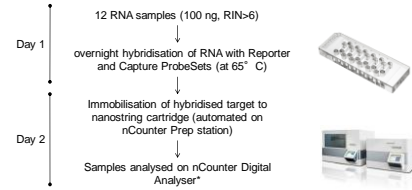
Nanostring uses molecular "barcodes" and single molecule imaging to detect and count transcripts allowing highly multiplexed measurement of gene expression

Gene expression analysis of the nasal mucosa



Hybridised target RNA is immobilized, orientated and counted

Experimental workflow



* GEE nCounter facility, UCL

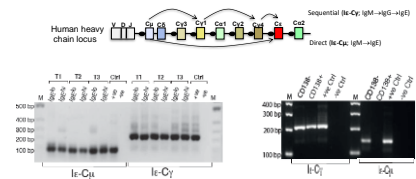
TABLE I. Lineage tree frequency with clonal relatedness between IgE and other classes

IgE related to:	NA group	ARJS and AR.OS groups
IgM	3* (0.29%)†	2 (0.31%)
IgA and IgM	1 (0.10%)	0
IgA	2 (0.19%)	2 (0.31%)
IgG	0	1 (0.15%)
IgG and IgM	0	1 (0.15%)

*Absolute number observed.

†Frequency is calculated based on clone counts in Table E3.

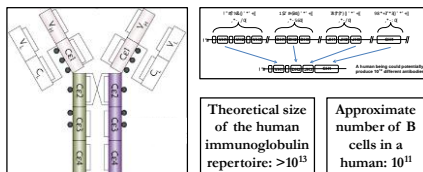
IgE+ PC differentiation is promoted by sequential CSR



* Both direct [Ig-Cμ: IgM→IgE] and sequential [Ig-Cμ: IgM→IgG→IgE] switching detected in our IgE^{hi} and IgE^{int} cells

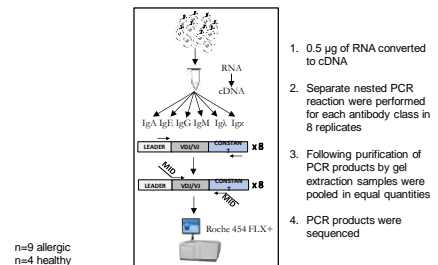
* However, only sequential switching detected in IgE^{CD138} cells

Analysis of antibody genes reveals the diversity of the immunoglobulin repertoire

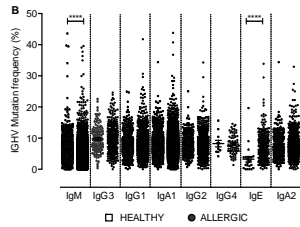


* 10^{13} =10 trillion
 10^{11} = 100 billion

Analysis of nasal immunoglobulin repertoires by high throughput sequencing

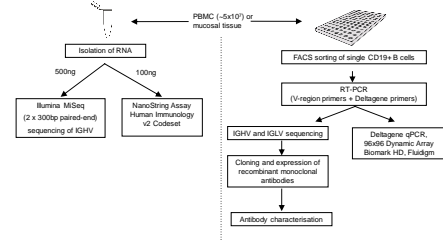


IgE is more mutated in allergic versus healthy subjects

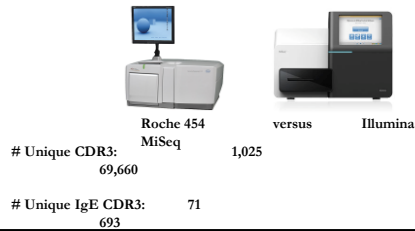


P-values were calculated with Mann-Whitney tests: ****p<0.0001.

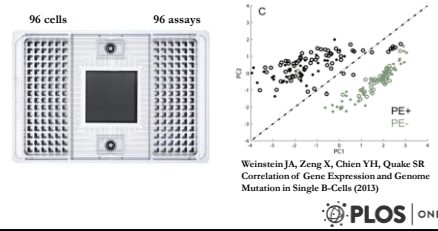
Experimental Pipeline



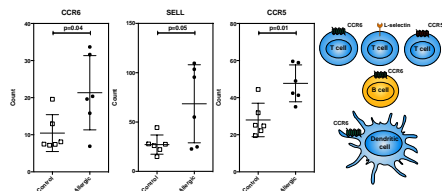
Advances in sequencing technology provide a greater insight into antibody repertoires



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

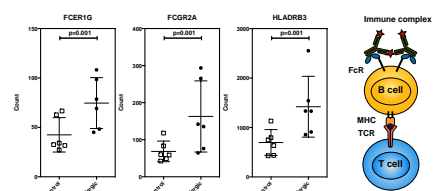


Increased expression of genes associated with cellular migration



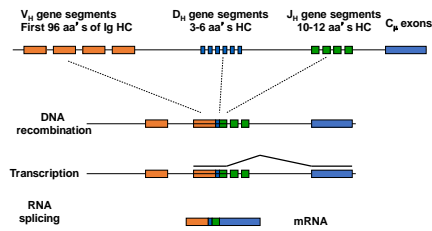
P-values were calculated with Mann-Whitney tests

Increase in antigen presentation within the nasal mucosa

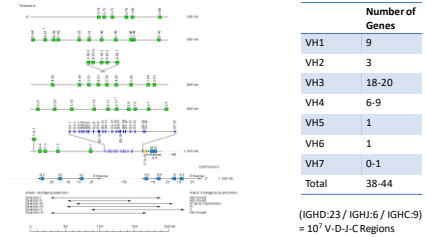


P-values were calculated with Mann-Whitney tests

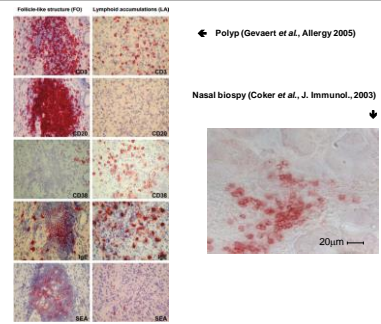
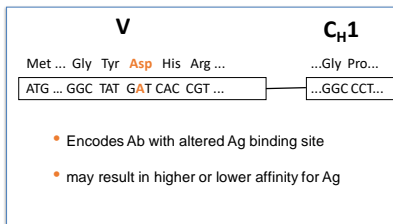
VDJ recombination in the H-chain locus



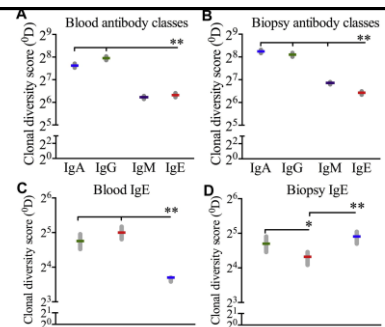
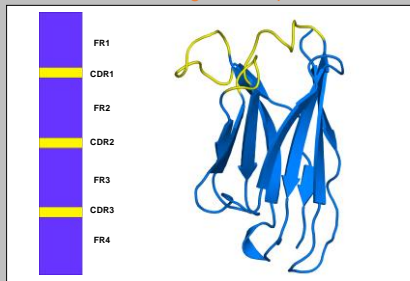
Ig Germline Gene Repertoire



Somatic hypermutation



VH domain gene and protein



Re-do

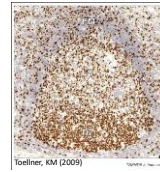
Table 1. IgE related to other classes in lineage trees.

IgE related to	Non-allergic	AR (in & out of season)
IgM	3 ¹ (0.29%) ²	2 (0.31%)
IgA & IgM	1 (0.19%)	0
IgA	2 (0.19%)	2 (0.31%)
IgG	0	1 (0.15%)
IgG & IgM	0	1 (0.15%)

¹Absolute number observed; ²Frequency is calculated based on the clone counts in

Table E3; ³Comparison between NA and AR.

Germinal center reactions focus the Ab repertoire

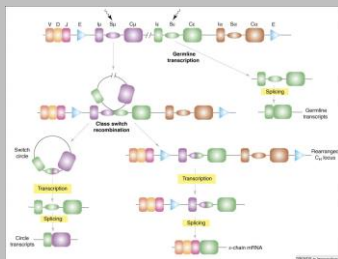


B cells stained in a section from secondary lymphoid tissue showing a germinal centre

- **Somatic hypermutation** introduces point mutations in the CDRs of the Ab V-regions, which may increase the affinity for Ag expressed by B cells in competition for Ag presented by FDCs during *affinity maturation* in the germinal centers of secondary lymphoid tissue.

- **Class switch recombination** changes the constant-regions of the heavy-chain & thereby Ab class (IgG, IgA or IgE) & **effector function**.

Class switching from IgM to IgE



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