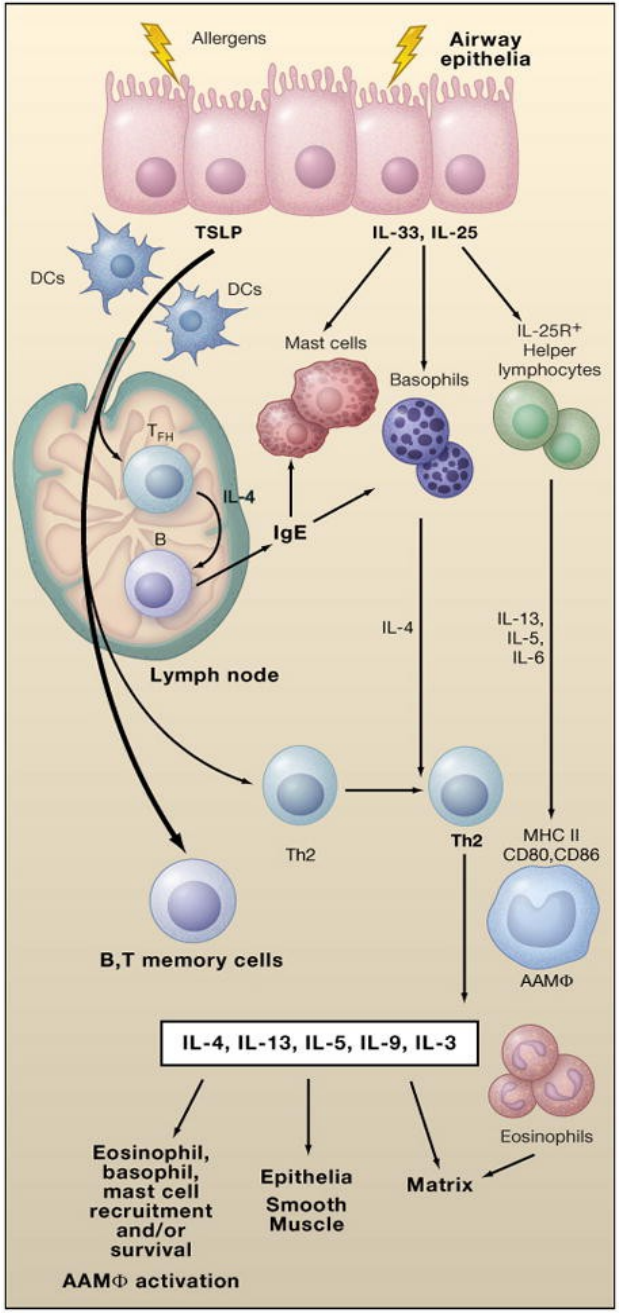


Mechanisms in immune responses in asthma (reproduced from Locksley, Cell, 2010, 140:777-783)

Why study T cell epitopes in allergic disease?

- T cells contribute to pathology directly and indirectly through modulation of immune responses
- Involvement in SIT
- Epitopes as a tool to monitor disease and intervention
- Epitopes have also potential as immunotherapeutic vaccine compositions

Today's talk

- **How to identify allergen-derived epitopes?**
- Are T cell epitopes derived from the same allergen proteins that bind IgE?
- Use of epitopes to assess immunological changes associated with SIT maintenance phase
- Correlates of SIT efficacy

The process of epitope identification and characterization

HLA binding/ motif predictions

Test in allergic patient PBMCs for recall of IL5 production

Problem: HLA molecules are extremely polymorphic

Solution: Address most common HLA allelic variants

Overall hypothesis: A large fraction of the response can be accounted for by promiscuous epitopes

Validation of large scale allergen screening strategy in the timothy system

- We considered 10 proteins (Phl p 1, 2, 3, 4, 5, 6, 7, 11, 12, 13)
  - These allergens have been shown to elicit IgE reactivity
- 687 overlapping peptides spanning these allergens were synthesized
- Peptide pools were tested for reactivity with T cell lines obtained by *in vitro* restimulation with timothy extract

Phl p region					Total response	
Region no.	Antigen	Position	Sequence	Len	Donors	SFC
1	Phl p 1	46	STWYGKPTGAGPKDN	15	2	860
2	Phl p 1	71	KPPFSGMTGCGNTPI	15	2	540
3	Phl p 1	96	FEIKCTKPEACSGEPVVVHI	20	2	124
4	Phl p 1	111	VVVHITDDNEEPIAP	15	1	153
5	Phl p 1	121	EPIAPYHFDLSGHAF	15	1	647
6	Phl p 1	131	SGIAFGSMAKKGDEQ	15	2	1823
7	Phl p 1	151	GELELQFRRVKCKYP	15	2	1410
8	Phl p 1	161	KCKYPEGTKVTFHVE	15	1	37
9	Phl p 1	171	TFHVEKGSNPNYLALLVKYVNGDGD	25	2	803
10	Phl p 1	196	VVAVDIKEKGKDKWI	15	1	277
11	Phl p 1	211	ALKESWGAIWRIDTP	15	1	997
12	Phl p 1	241	GTKTEAEDVIPEGWK	15	1	690
13	Phl p 1	249	VIPEGWKADTAYESK	15	1	447
14	Phl p 2	61	EHGSDEWVAMTKGEGGVWTF	20	2	1874
15	Phl p 2	76	GVWTFDSEEPLQGPF	15	1	457
16	Phl p 2	86	LQGPFNFRFLTEKGMKNVFDDVVPEKYTIG	30	4	280
17	Phl p 3	1	AVQVTFTVQKGSDPKKLVLNIKYTRPGDSL	30	5	3037
18	Phl p 3	41	EEWEPLTKKGNVWEV	15	3	706
19	Phl p 3	51	NVWEVKSSKPLVGPF	15	5	1950
20	Phl p 3	61	LVGPFNFRFMSKGGMRNVFDEVIPT	25	4	913
21	Phl p 4	191	MLLRKYGIAAENVID	15	1	140
22	Phl p 4	221	GIVVAWKVRLLPVPP	15	1	53
23	Phl p 4	321	FVHLGHRDNIEDDLL	15	1	73
24	Phl p 4	336	NRNNTFKPFAEYKSDYVYQPFPK	23	2	2610
25	Phl p 4	356	FPKEVWEQIFSTWLL	15	1	97
26	Phl p 5a/5b	70	INAGFKAALAAAAGVPPADKY	21	4	1747
27	Phl p 5b	76	PAADKFKTFEAAFTS	15	1	160
28	Phl p 5af	76	PKGGAESSSKAALTS	15	1	97
29	Phl p 5b	121	TPEAKFDSFVASLTE	15	1	220
30	Phl p 5af	111	KYDAYVATLSEALRI	15	1	2047
31	Phl p 5a	126	TSKLDAAYKLAYKTA	15	1	1103
32	Phl p 5b	136	ALRVIAGALEVHAVK	15	1	543
33	Phl p 5a	181	VIPAGELQVIEKVDAAFKVA	20	2	200
34	Phl p 5a	196	AFKVAATAANAAPAN	15	7	10631
35	Phl p 5a/5b	208	PANDKFTVFEAAFNDAIKE	19	5	1170
36	Phl p 5a/5b	231	AYESYKFIPALEAAVKQAYAATVAAA	26	2	2246
37	Phl p 5a/5b	251	ATVATAPEVKYTVFETALKKAITAMS	26	4	3011
38	Phl p 5b	251	ITAMSEVQKVSQPAT	15	1	33
39	Phl p 11	111	RYANPIAFFRKEPLK	15	1	927
40	Phl p 12	26	LGHDGTVWAQSADFP	15	1	30
41	Phl p 13	36	KTDCTKEVEEAWASA	15	1	110
42	Phl p 13	96	LAKYKANWIEIMRIK	15	2	260
43	Phl p 13	126	AVWGKNSCAKNYNCK	15	1	330
TOTAL					86	45863

Phl p 5a = Phl p 5.0103; Phl p 5b = Phl p 5.0203; Phl p 5 af = Phl p 5.01 core fragment.

43 antigenic regions were identified

Nine antigenic regions account for about half of the responses

No. regions	No. positive donors	Total spots (region)	Fraction of total spots	Cumulative fraction of spots
1	**7**	**10631**	**0.232**	**0.232**
3	**5**	**6157**	**0.134**	**0.366**
4	**4**	**5951**	**0.130**	**0.496**
1	**3**	**706**	**0.015**	**0.511**
11	2	12750	0.278	0.789
23	1	9667	0.211	1.000
275	-	45863	1.00	

Total

Dominant epitopes can be predicted on the basis of their sequence characteristics, and binding to multiple HLAs

Allergen Epitope Identification in the Bla g (German cockroach) system

- No T cell epitopes identified in humans thus far
- Identify dominant epitopes to characterize responses
- Synthetized 195 peptides from 6 known allergens (Bla g 1,2,4,5,6,7)
- Enrolled 34 allergic donors from Johns Hopkins and LIAI
- Tested extract stimulated PBMC cultures with 13 pools containing 12-18 peptides for IL5 and IFNg production
- Positive pools deconvoluted identified 25 different epitopes/antigenic regions

Immunodominance of Bla g epitopes

Antigen	Position	Total SFC	% response	Sum
Bla g 5	181	4953	0.20	0.20
Bla g 5	66	2443	0.10	0.30
Bla g 5	16	2342	0.10	0.40
Bla g 5	96	2103	0.09	0.49
Bla g 5	156	1730	0.07	0.56
Bla g 6	66	1433	0.06	0.62
Bla g 6	6	1245	0.05	0.67
Bla g 5	46	1237	0.05	0.73
Bla g 1	331	1200	0.05	0.78
Bla g 5	166	1140	0.05	0.83
Bla g 5	131	987	0.04	0.87
Bla g 2	11	855	0.03	0.90
Bla g 6	11	757	0.03	0.93

Large scale screen for other common allergens

- Epitope identification studies from an additional 28 allergen sources
- Various trees, grass, fungi, animal allergens
  - Commonly used at the two clinical sites (extracts)
  - Allergen sequences available from IUIS
- The predictive strategy validated in the Timothy grass model system was utilized
- 257 antigenic regions identified
- First-ever human T cell epitopes from 16 common allergen sources

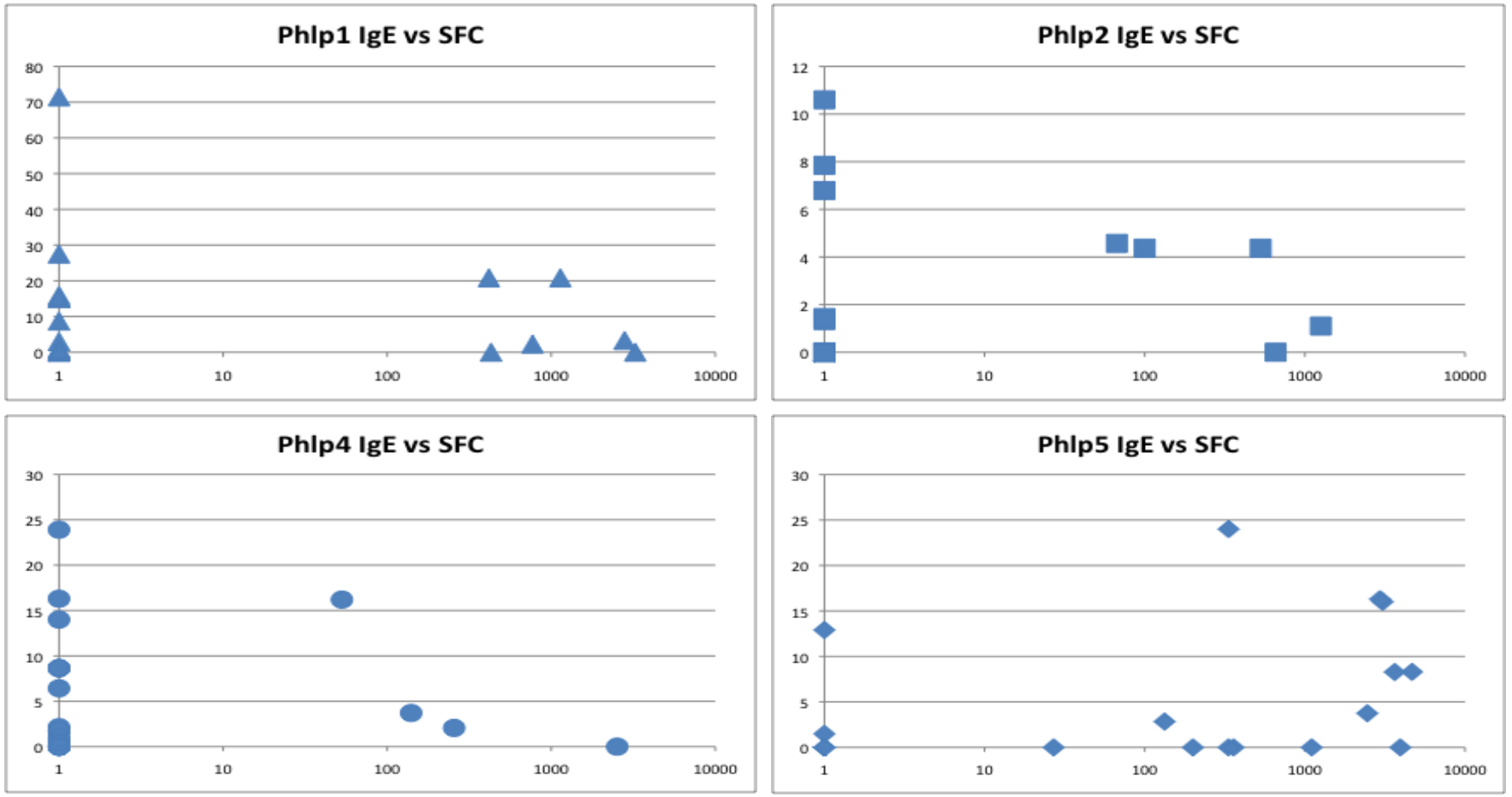
Epitope identification Studies: Conclusions

- A majority of the response is directed against few immunodominant epitopes
- These epitopes can be identified/predicted on the basis of MHC binding characteristics
- A specific peptide prediction and selection strategy was implemented for the large scale allergen screen study

Today's talk

- How to identify allergen-derived epitopes?
- **Are T cell epitopes derived from the same allergen proteins that bind IgE?**
- Use of epitopes to assess immunological changes associated with SIT maintenance phase
- Correlates of SIT efficacy

Relationship between RAST IgE titers and antigenicity for T cell responses



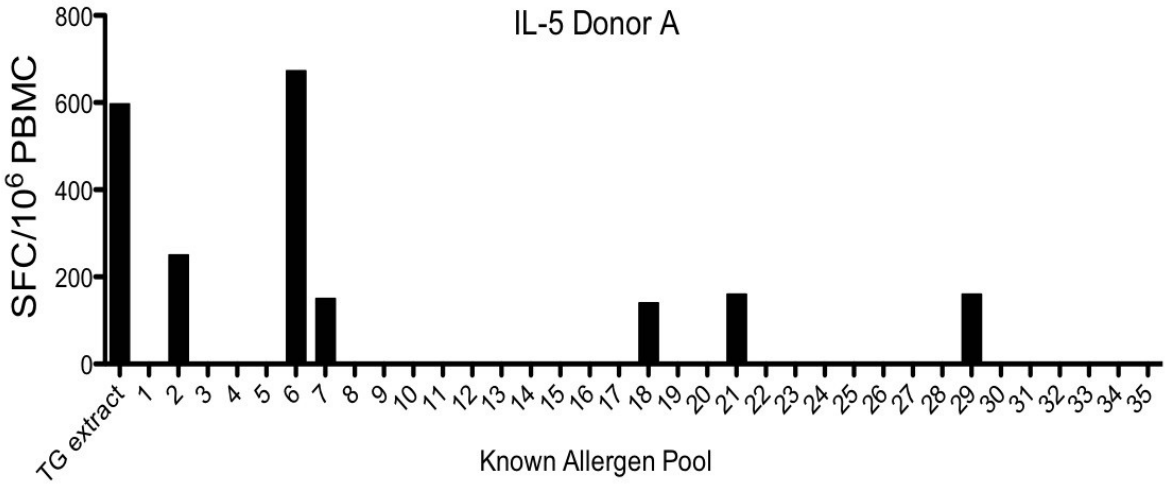
- Each dot represents a donor. Data from 19 donors shown.
- No T cell response measured in Phlp 6, 7, and 12.
- Only one donor showed positive response to Phlp 11

Lack of correlation between IgE prevalence of and T cell responses to individual Bla g proteins

Allergen	Donors with responses to: Both	T cell only	IgE only	Neither	p value
Bla g 1	1	3	2	28	0.33
Bla g 2	1	1	7	25	0.42
Bla g 4	0	0	4	30	1.0
Bla g 5	4	5	8	17	0.69
Bla g 7	0	4	4	26	1.0

Observation: Known TG allergens do not account for all T cell responses

A



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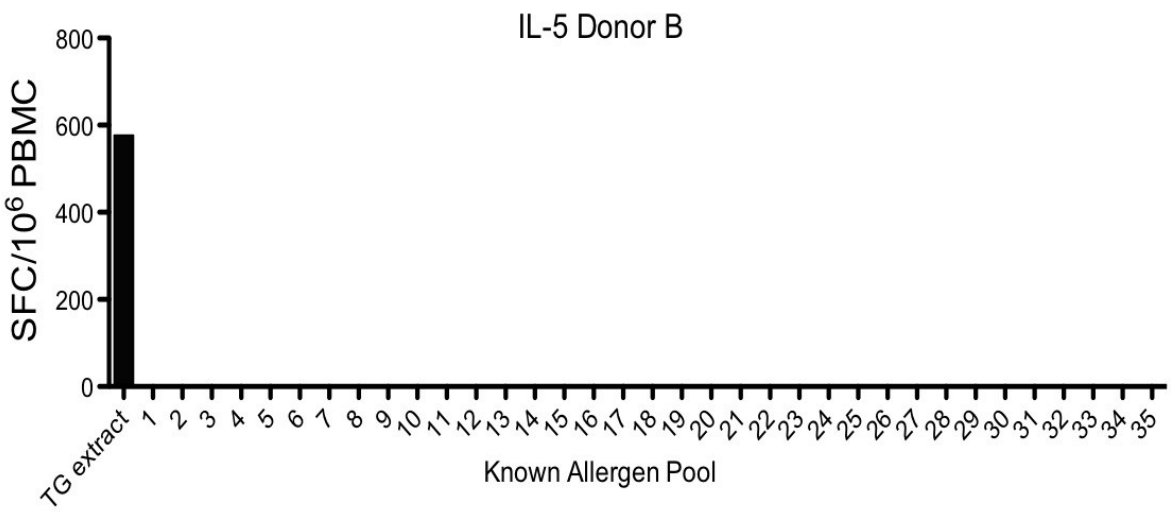


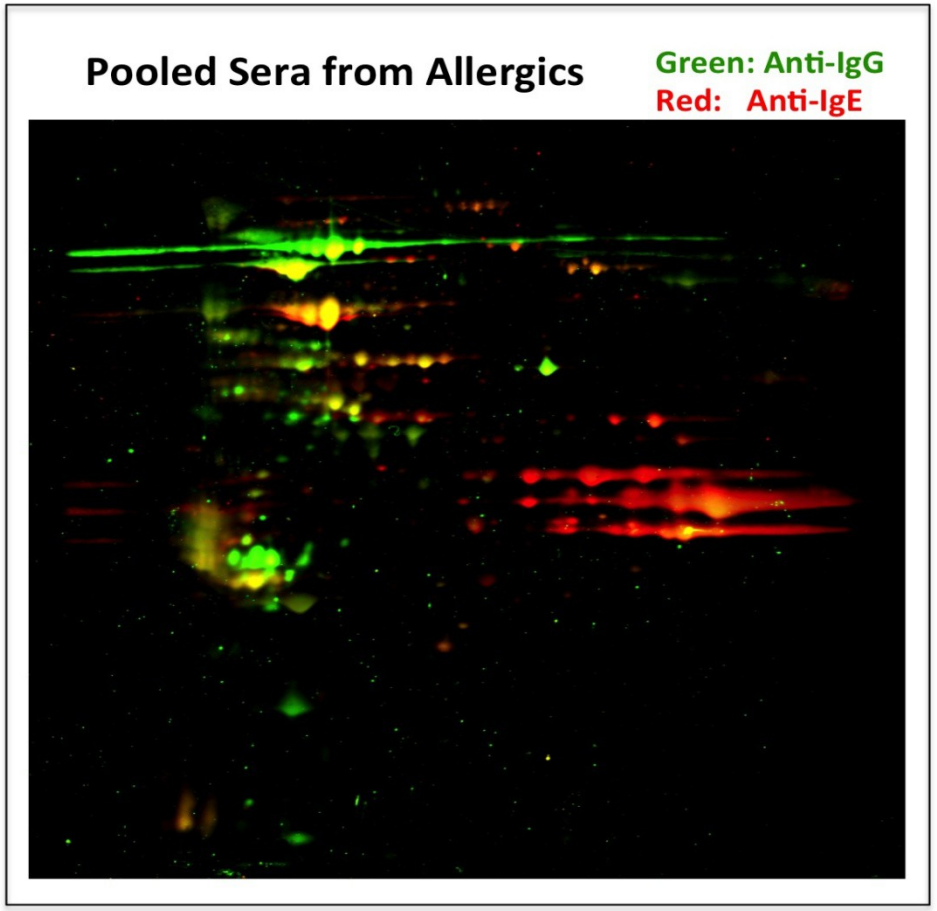
- 30% of donors are category B
- What do they recognize in TG extract? Other proteins?
- Problem: No other proteins from TG are known

Approaches to T cell antigen discovery

- **Transcriptomics**: Obtain mRNA sequences from TG pollen
- **Proteomics**: Identify pollen proteins by mass spec of extract using transcriptome as reference
- **Immunomics**: Predict peptides binding promiscuously to HLA class II molecules and test them for T cell recognition

Novel protein identification by gel separation and MS

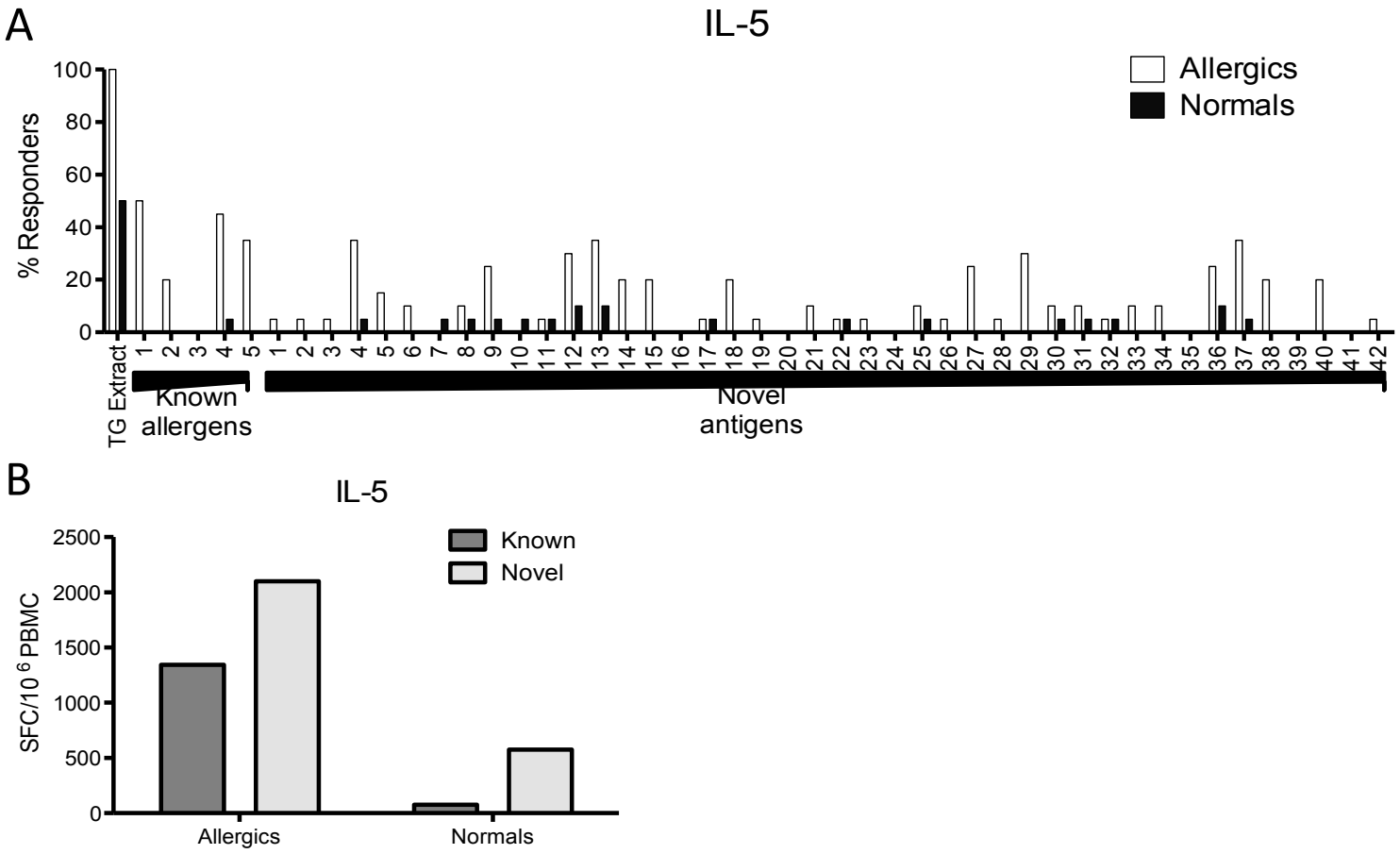
- Pollen extract separated on 2D gel
- Spots picked, based on antibody or protein staining
- Spots cut out from gel, analyzed in mass spectrometer
- 83 new proteins from 2D gel + 10 proteins from whole extract mass spec were chosen for further studies



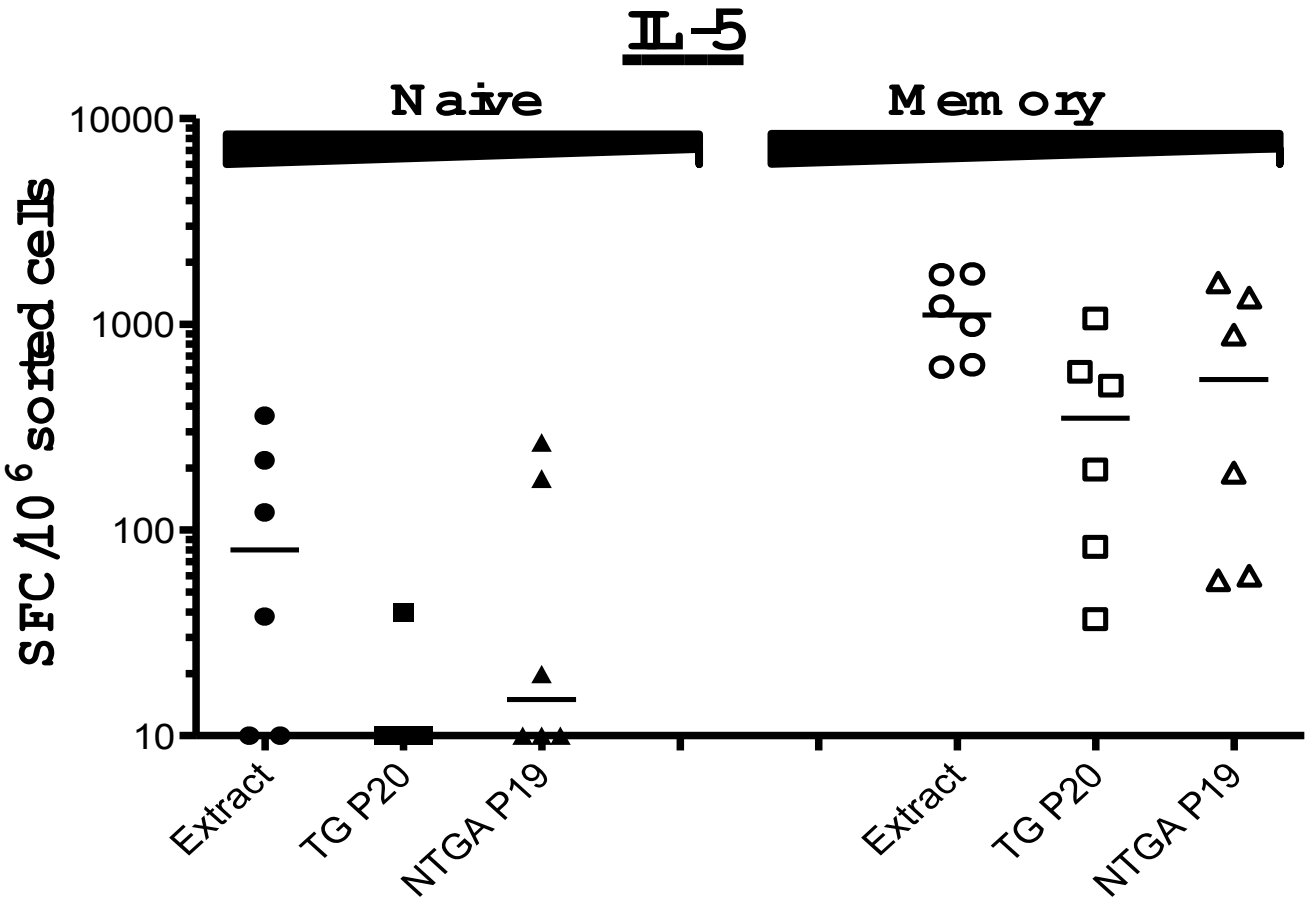
T cell antigen identification based on HLA class II binding predictions

- Predict peptides binding to a panel of 25 HLA class II molecules (DR, DP, DQ)
- Synthesize 822 peptides that bind promiscuously (>12 HLA variants)
- Test peptides as pools for IL-5 production in PBMC from TG allergic donors

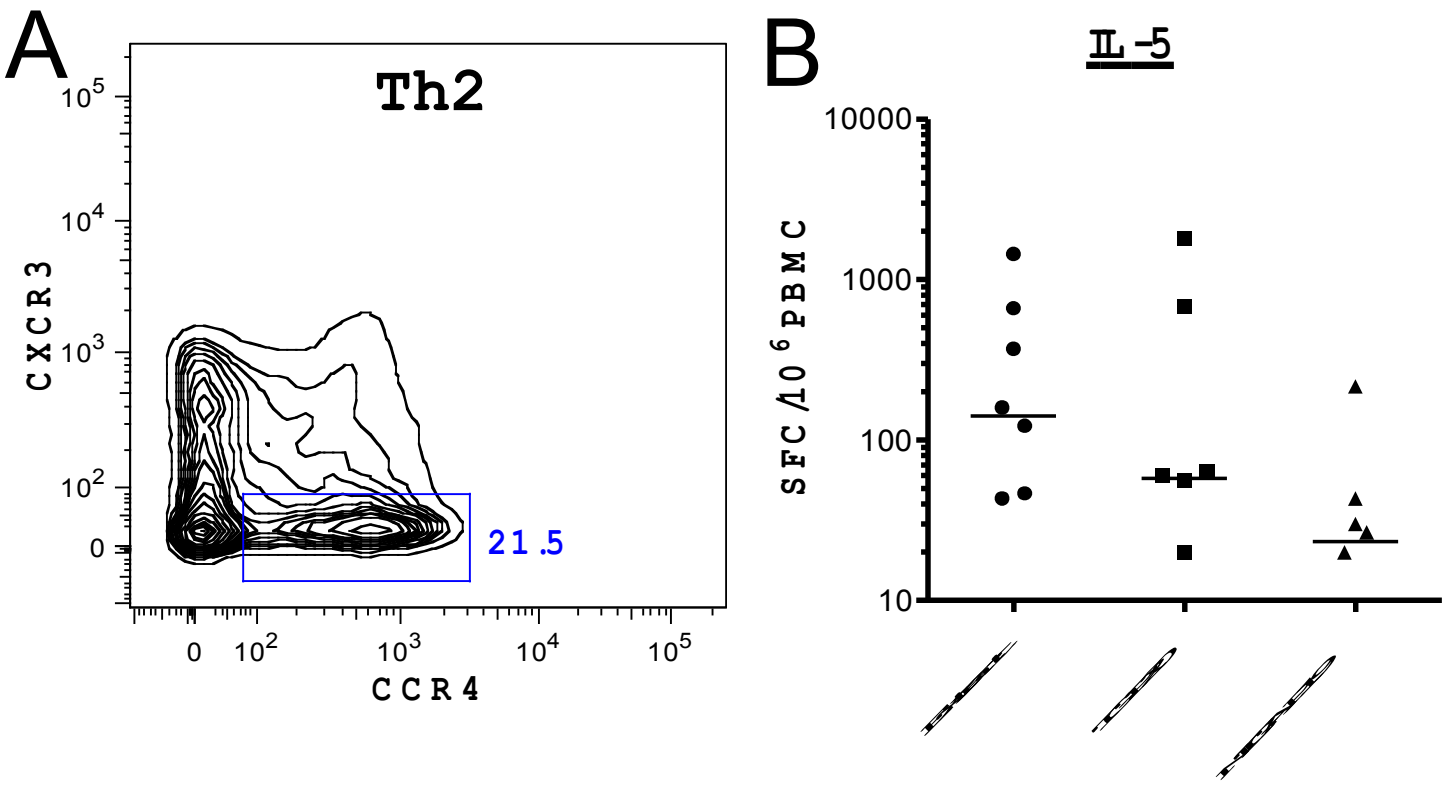
A majority of Th2 cells in allergic donors target novel epitopes



Memory T cells are the source of IL-5 T cell responses after *in vitro* expansion



Responses to conventional and novel TG antigens can be detected directly ex vivo



NTGAs: Conclusions

- Novel TG proteins elicited Th2 responses, explaining the reactivity gap between known allergens and whole extract
- IL-5 production to several antigens not targeted by IgE or IgG, suggesting unlinked T-cell help
- The universe of T cell inducing antigens is much broader than that of IgE binding allergens

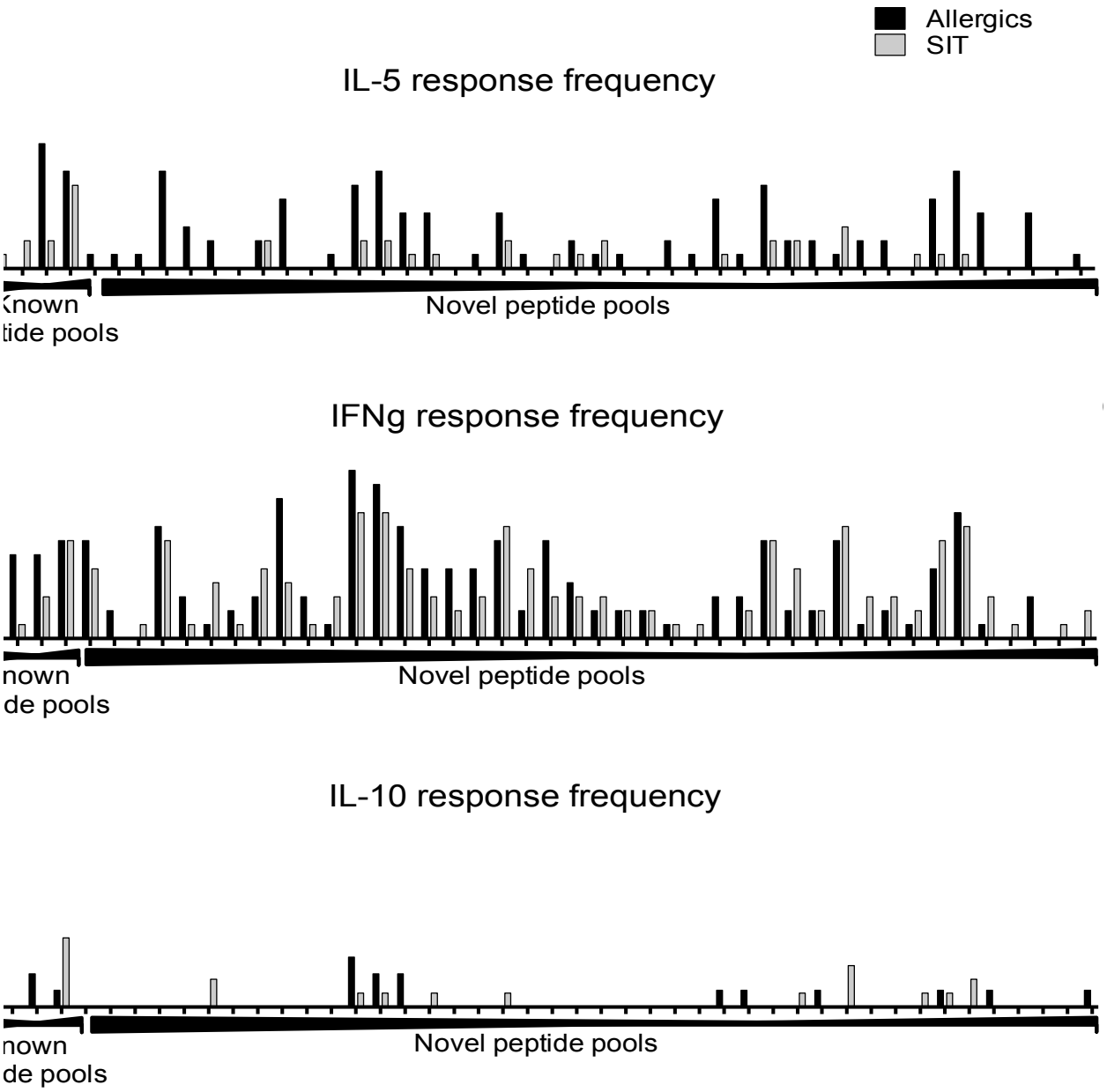
Today's talk

- How to identify allergen-derived epitopes?
- Are T cell epitopes derived from the same allergen proteins that bind IgE?
- **Immunological changes associated with SIT maintenance phase**
- Epitopes as biomarkers of SIT efficacy

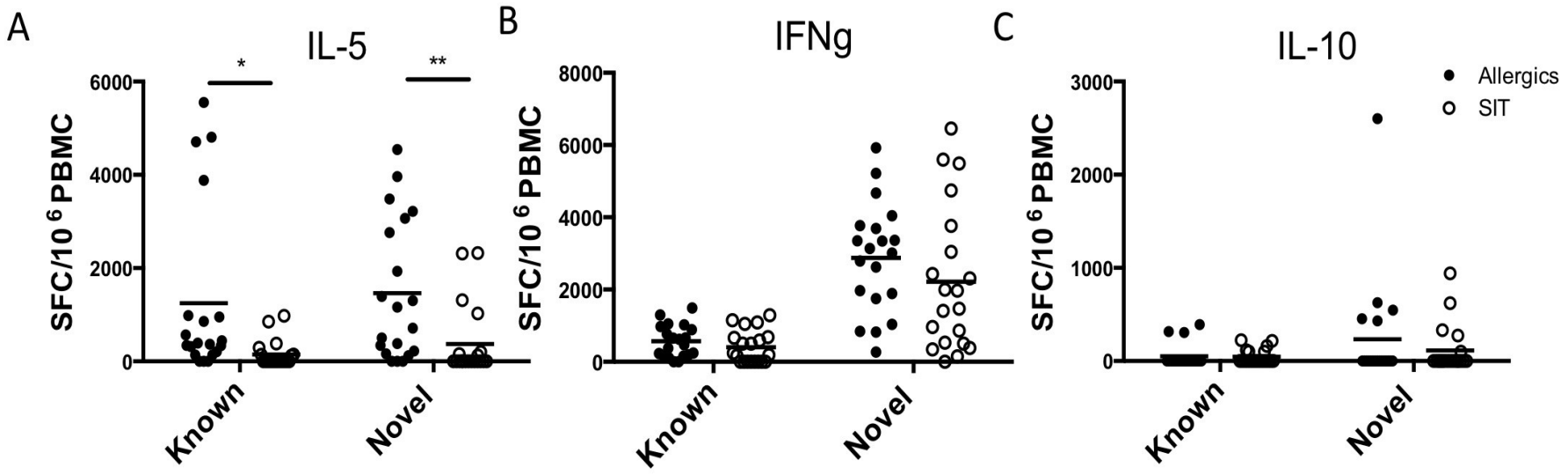
Use of Epitopes to investigate allergic responses

- SIT limitations
  - Safety
  - Incomplete understanding of mechanism of action
  - Lack of immunomarkers of efficacy
- Analyzed NTGAs epitopes in a crosssectional study
  - 13 NTGA triggered IL5 responses in 20% of donors
  - IL5, IL10 IFN-gamma and IL17

T cell recognition of known and novel TG peptide pools in allergic and SIT-treated donors.

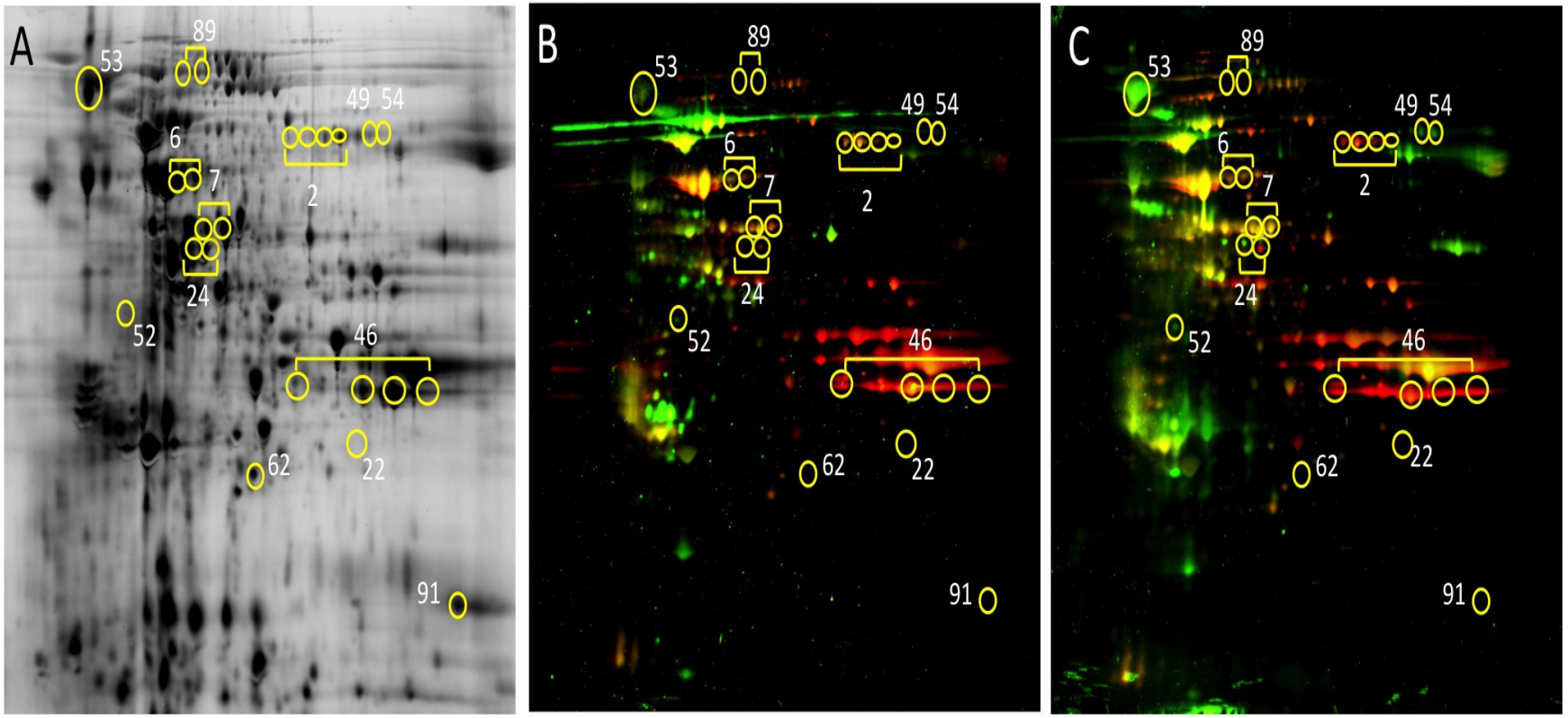


Cytokine production in allergic and SIT donors to known and novel Phl p



Biochemical characteristics of NTGA

2D gel and immunoblot of TG extract with pooled sera from allergic and SIT



Immunological characteristics

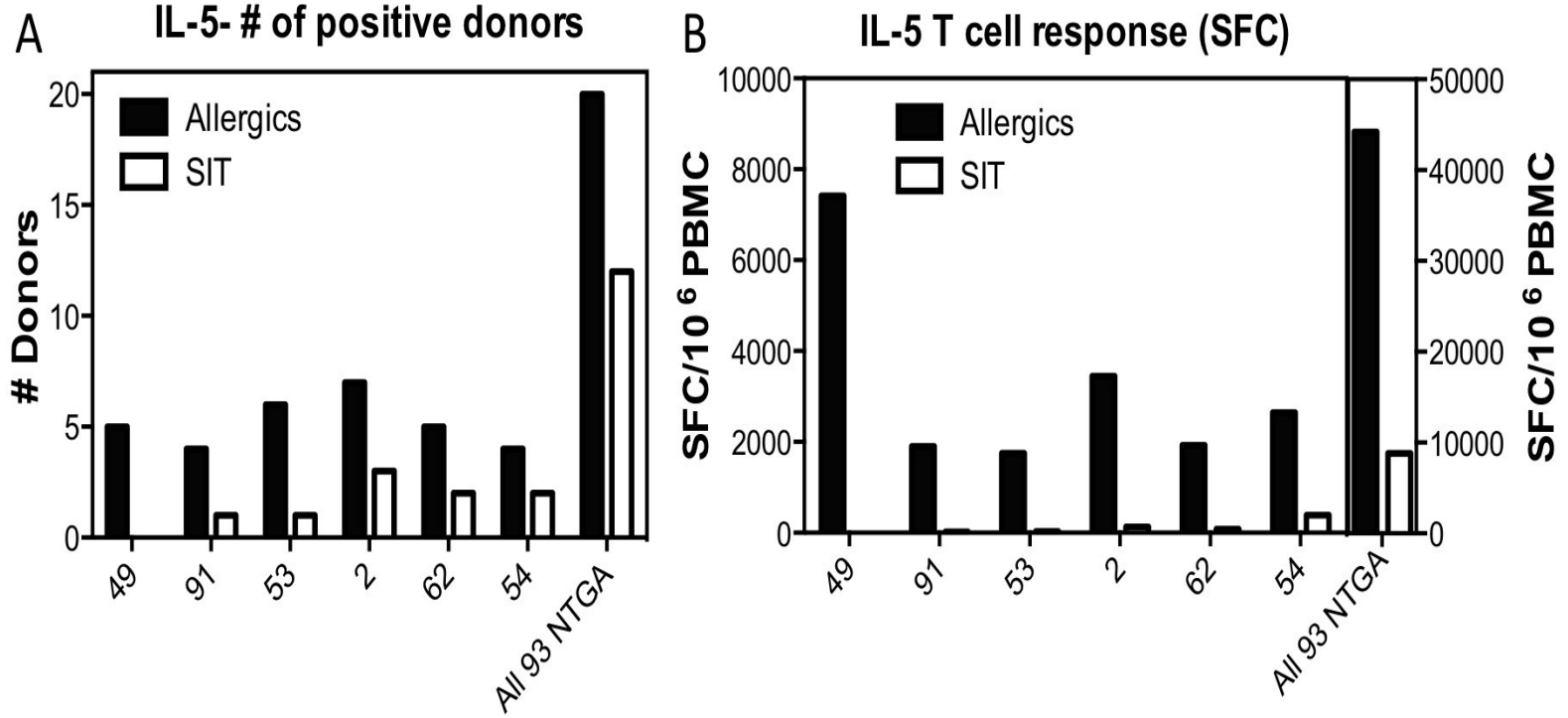
Changes associated with SIT maintenance phase. Conclusions

- For both Known and novel allergens/antigens
  - Decrease in IL5
  - No difference in IFN
  - No difference in IL10
- A subsets of antigens/epitopes particularly downmodulated was selected for additional studies in new donors

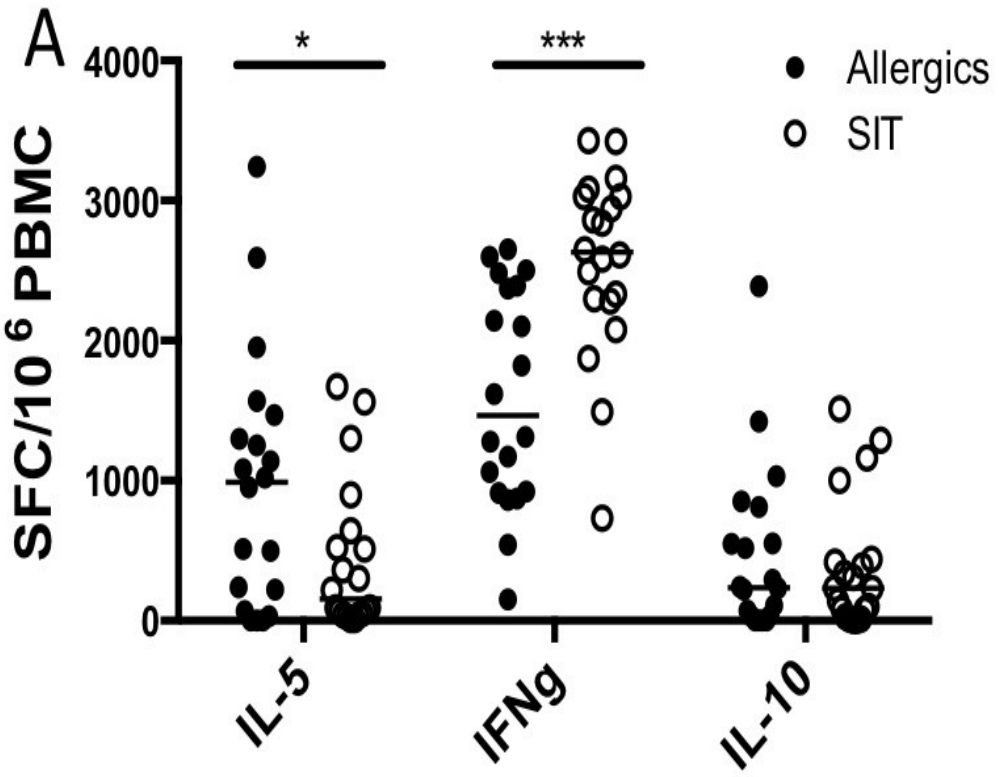
Today's talk

- How to identify allergen-derived epitopes?
- Are T cell epitopes derived from the same allergen proteins that bind IgE?
- Immunological changes associated with SIT maintenance phase
- **Epitopes as biomarkers of SIT efficacy**

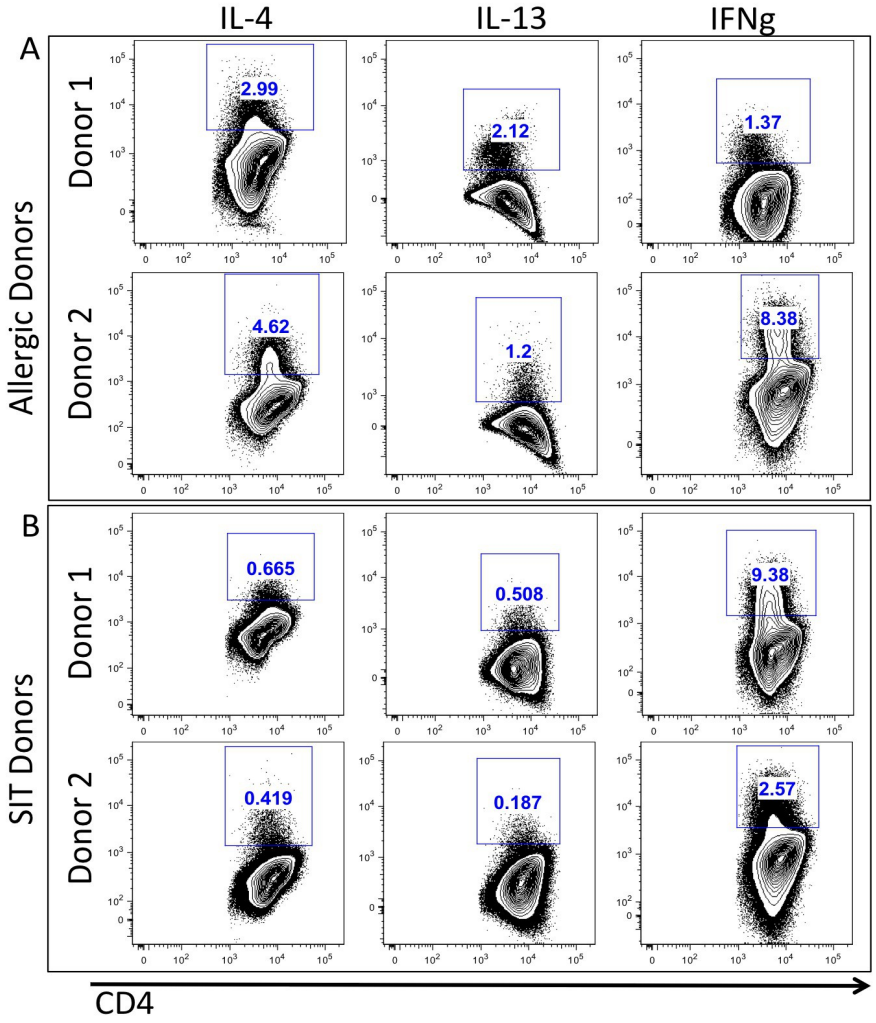
A subset of NTGAs for which IL-5 responses are most down-regulated in SIT



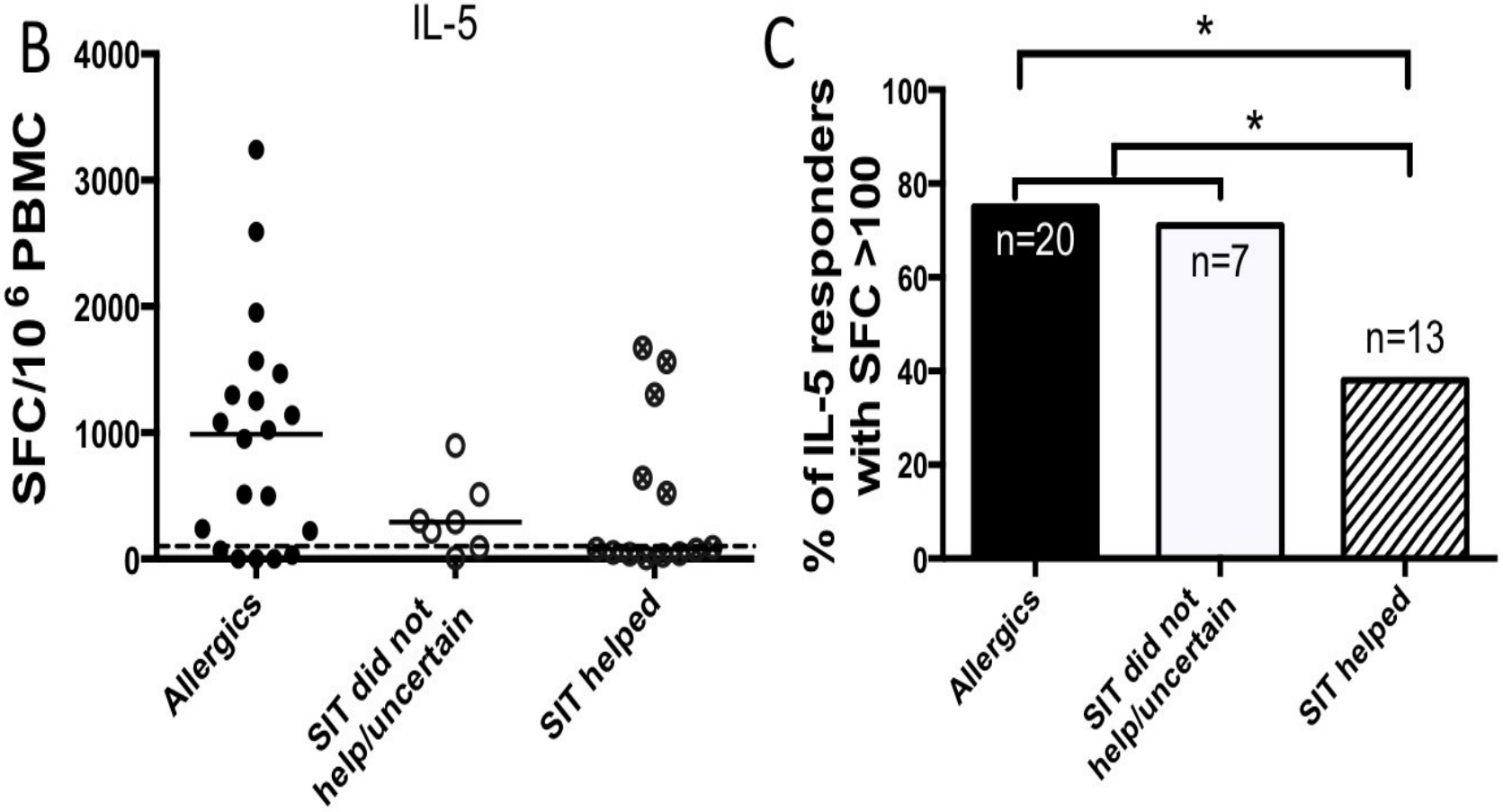
Modulation of cytokine responses to the MNA pool after SIT



The reduction of Th2 responses in SIT donors is not limited to IL-5



Modulation of cytokine responses to the MNA pool correlates with SIT



Theoretical population coverage across different ethnicities

Epitopes as biomarkers of SIT efficacy. Conclusions

- Epitope pools are a marker of SIT efficacy
- Potential use in large scale clinical trials
- Further studies longitudinal studies
  - Onset time and correlation with duration of efficacy
  - Correlation with IgG4 responses

Epitopes and SIT. Summary

- Data does not support a prominent role of induction of IL10 or TH1 responses against known or novel allergens
- Further studies in the Bla g and Phl p systems
  - Characterize responses *ex vivo*
  - Determine changes in a longitudinal fashion
  - Determine signatures of efficacy
- Potential use in immunotherapy

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