



Characterisation of Allergenic Proteins in Lupin Seeds : Relationship between Lupin and Peanut Allergens

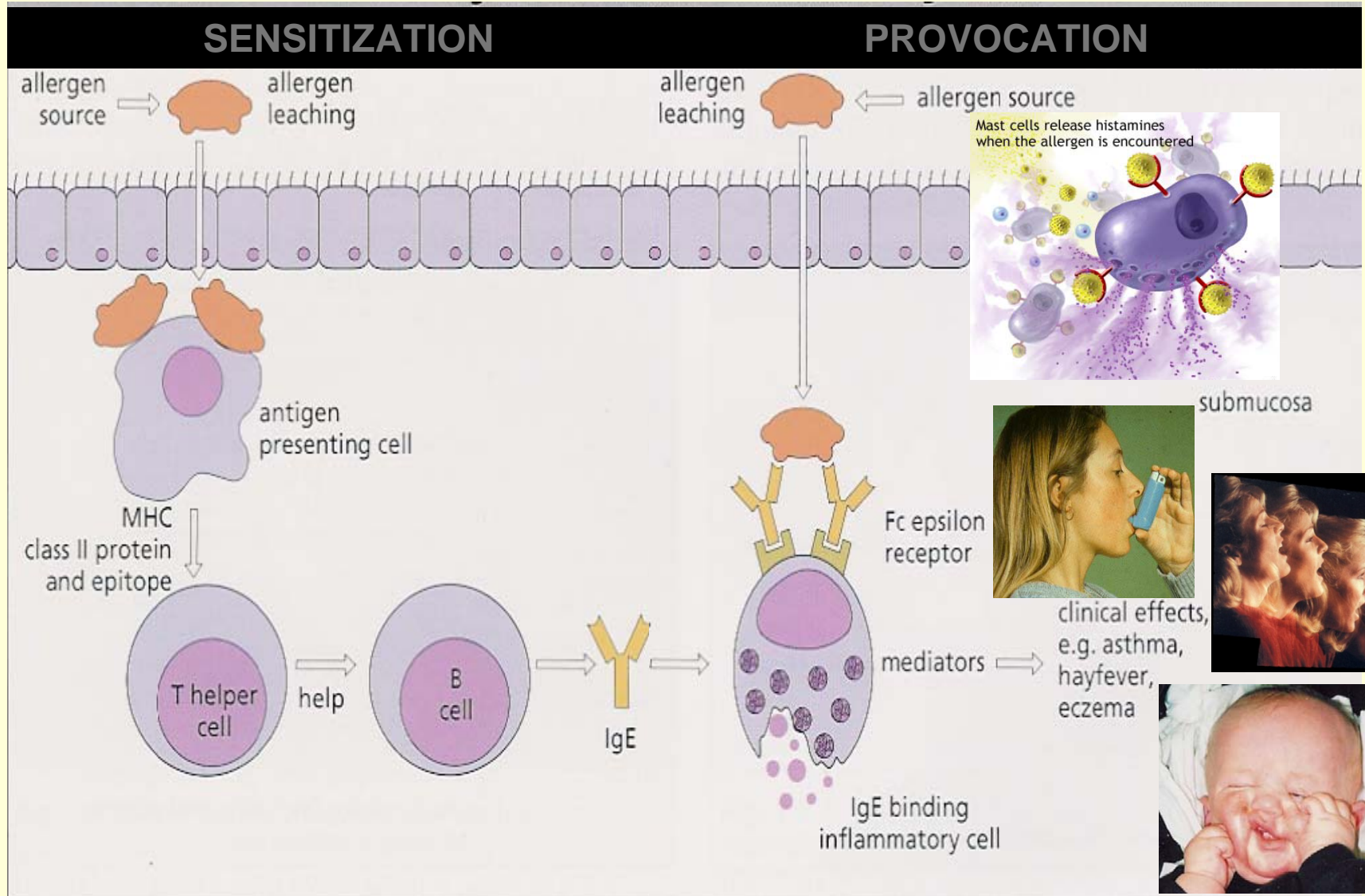
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Emilie Cameron, Martin Stuckey, William Smith, Susan
Prescott, Valencia Soutter, Robert Loblay

Lupin seeds can be allergenic



- Cases of lupin allergy in literature
 - anaphylaxis reported
- Prevalence in general population not clear
 - others allergic to only lupin or lupin and other legumes
 - linked with allergy to peanut
- National lupin allergy project
 - Prevalence in peanut allergic people
 - What are the allergens?
 - Relationship between lupin and peanut allergens?

Allergic reactions



Prevalence of lupin allergy in
peanut allergic people

Lupin oral challenge

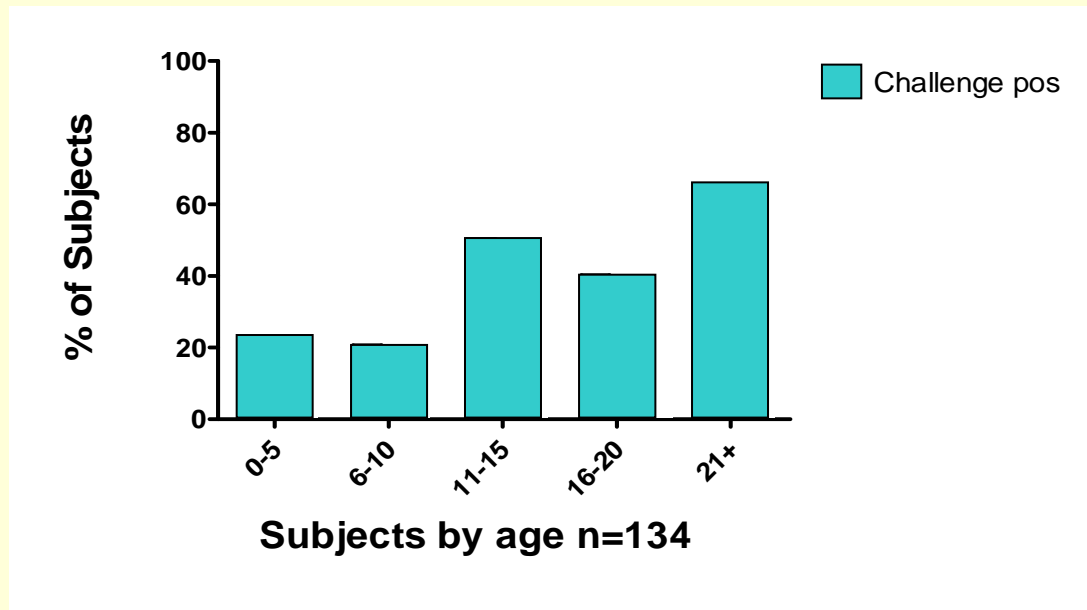
- Lupin skin tests were done on people who were sensitised to peanut
- 27.7% were sensitised
 - 19% in Perth group; n=350; 31% in Sydney, n=901)
- Oral challenge for those with positive skin test to both (134 volunteers)
 - Fed increasing amounts of lupin containing foods ("twisties", bread made with lupin flour) to see if they had an allergic reaction to lupin
 - Serum collected and IgE levels determined

Lupin oral challenge results

| Participants | Challenge +ve | Symptoms |
|----------------|---------------|--|
| Children (112) | 28 (25%) | Anaphylaxis (4), respiratory, oropharyngeal, cutaneous, gastrointestinal, cardiovascular disturbance |
| Adults (22) | 9 (41%) | |
| Total (134) | 37 (27.6%) | |

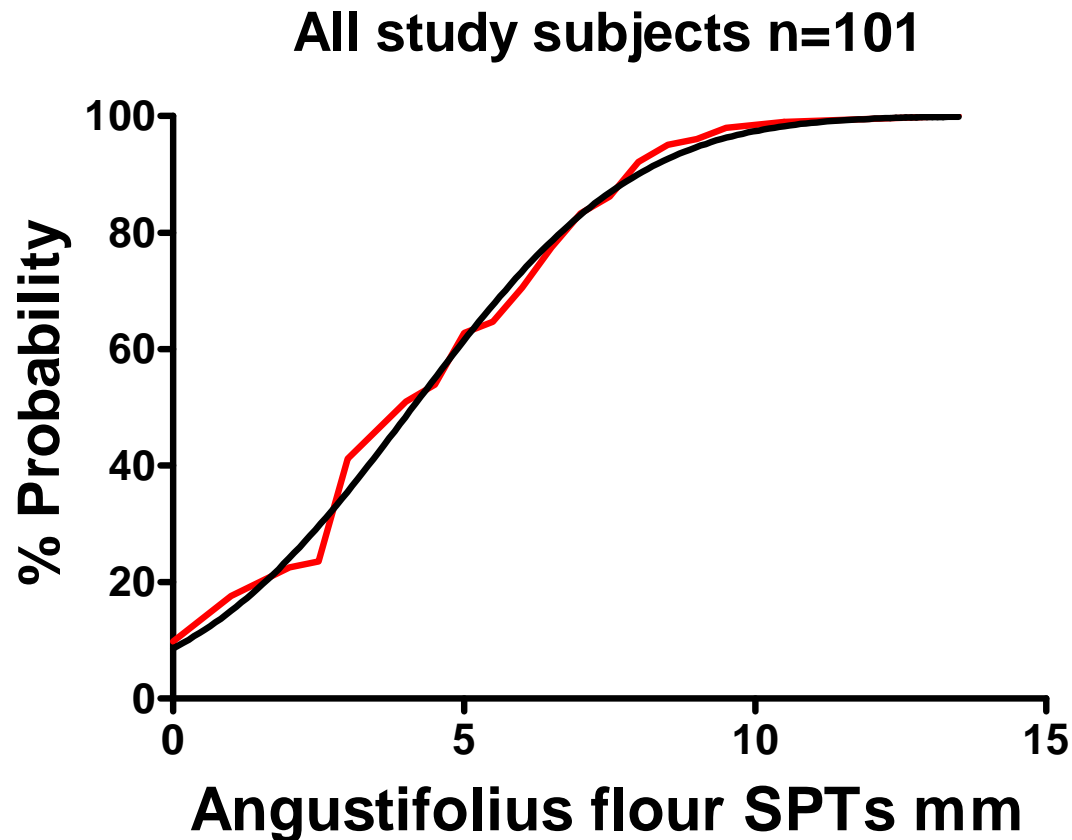
- 7.6% peanut sensitised individuals react to lupin

Challenge Results by Age



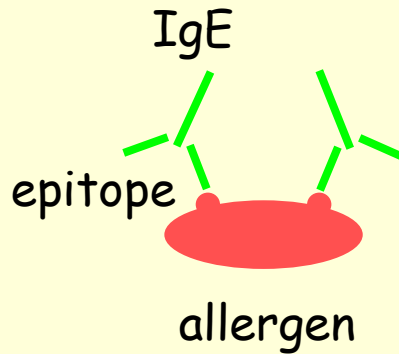
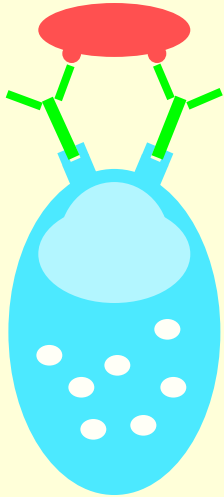
- More adults reacted to lupin
 - May reflect that many children stop reacting as they age
 - Adults are those that did not grow out of allergy

Predictive value of skin tests for reaction to lupin?

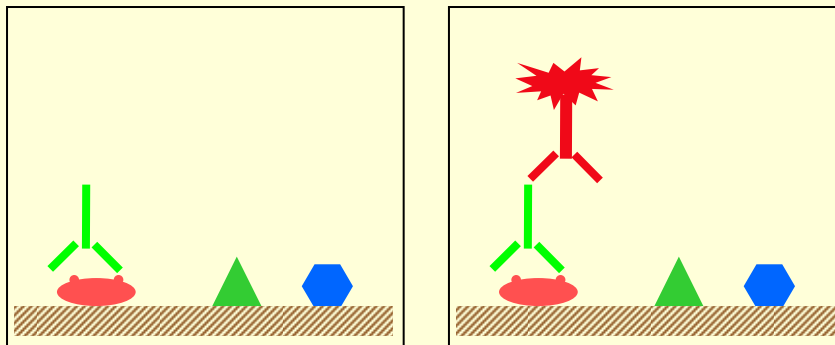


Identifying Lupin Allergens

IgE : a key to studying allergenic molecules



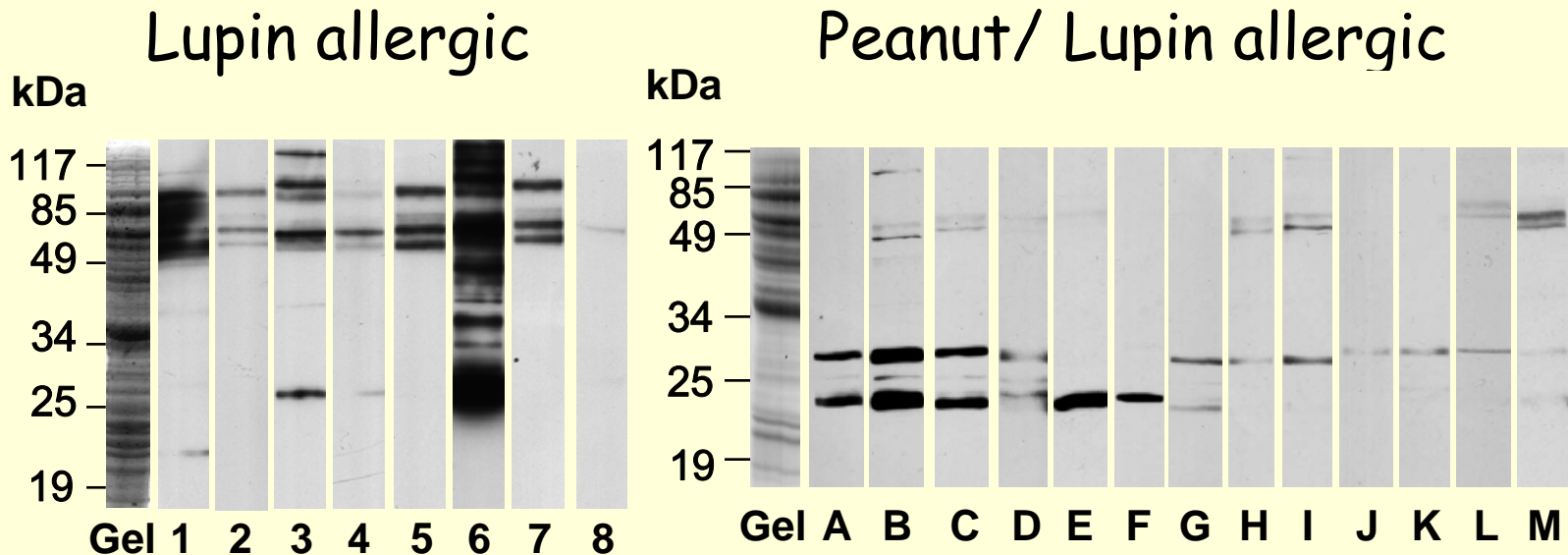
- IgE is present in serum of people who have allergies
- IgE binds to an epitope on the allergen



Western blot of proteins separated by electrophoresis

- Can be used to identify allergenic proteins separated by gel electrophoresis

IgE binding to lupin seed proteins

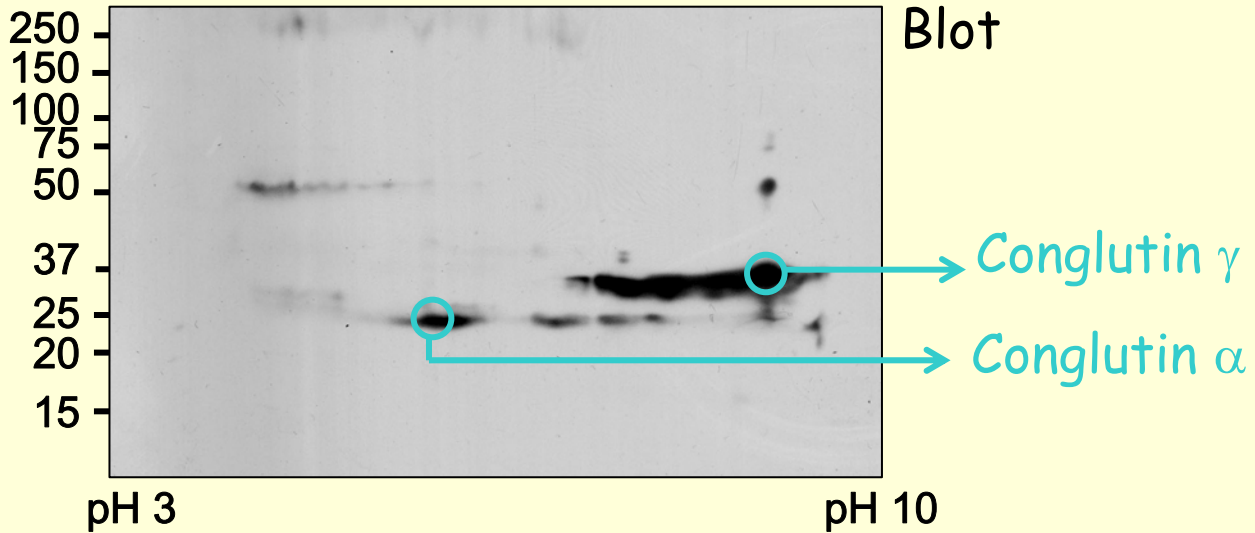
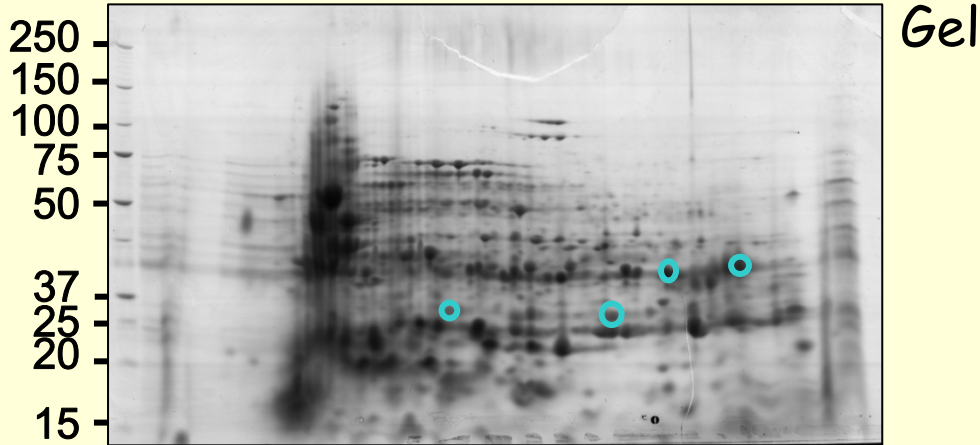


Western Blots of proteins separated by SDS-PAGE

- For most "lupin only" individuals the major allergen is conglutin β
 - Identified by mass spec analysis (Goggin et al 2008 J. Ag. Food Chem.)
- Peanut/lupin individuals react to different protein

Identification of allergenic proteins

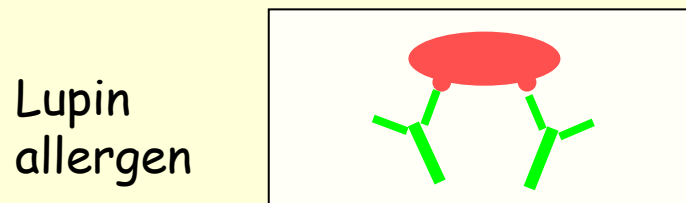
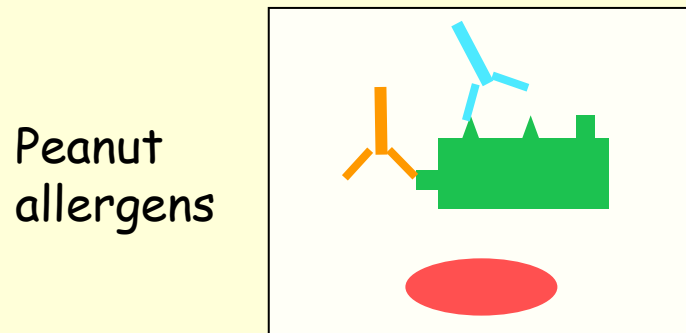
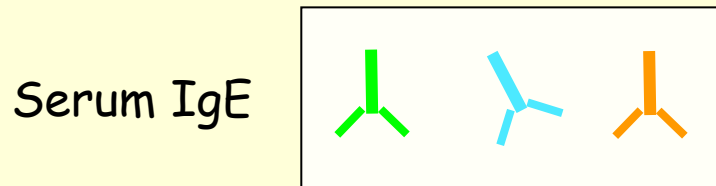
kDa



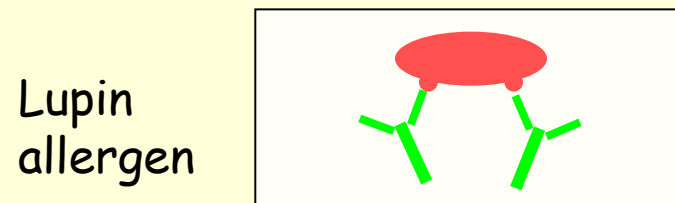
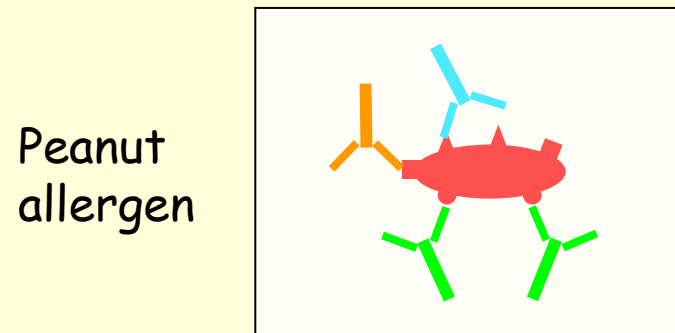
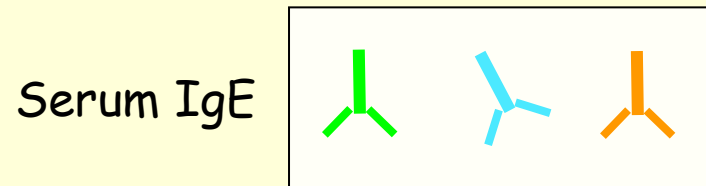
Peanut and lupin

- Some people react only to lupin
- Others react to peanut and lupin
 - May just be a coincidence (separate sensitisation)
 - May be because the allergens are similar (cross-reactive)
- Our aim was to determine whether peanut and lupins share cross-reactive allergens

Cross-reactivity versus separate sensitisation

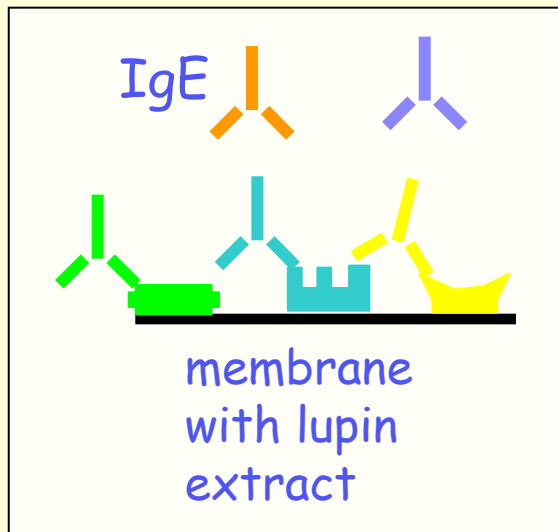
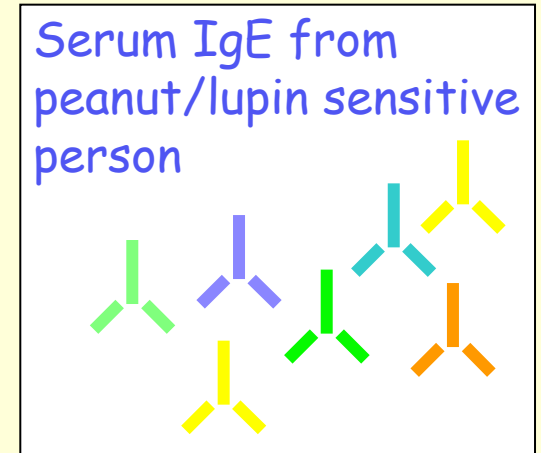
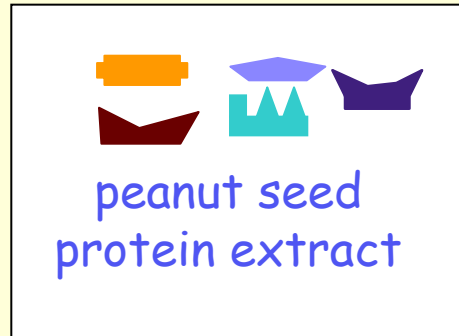


Separate sensitisation

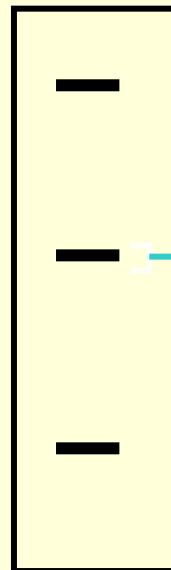


Cross-reactivity

Inhibition assay

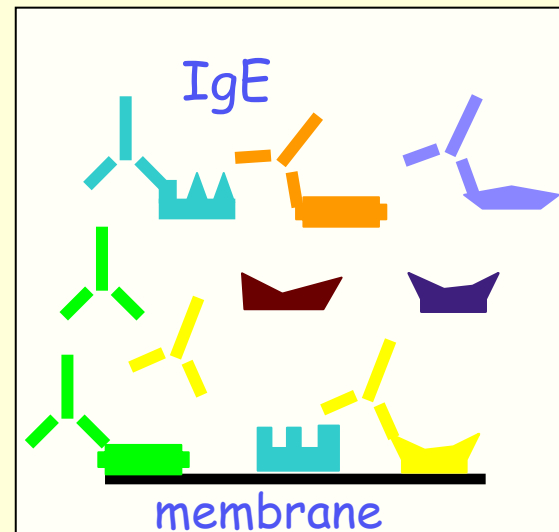


Without peanut extract

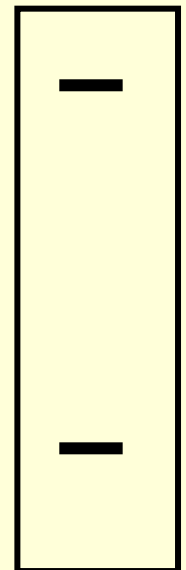


film

Protein with
cross-
reactive
epitope

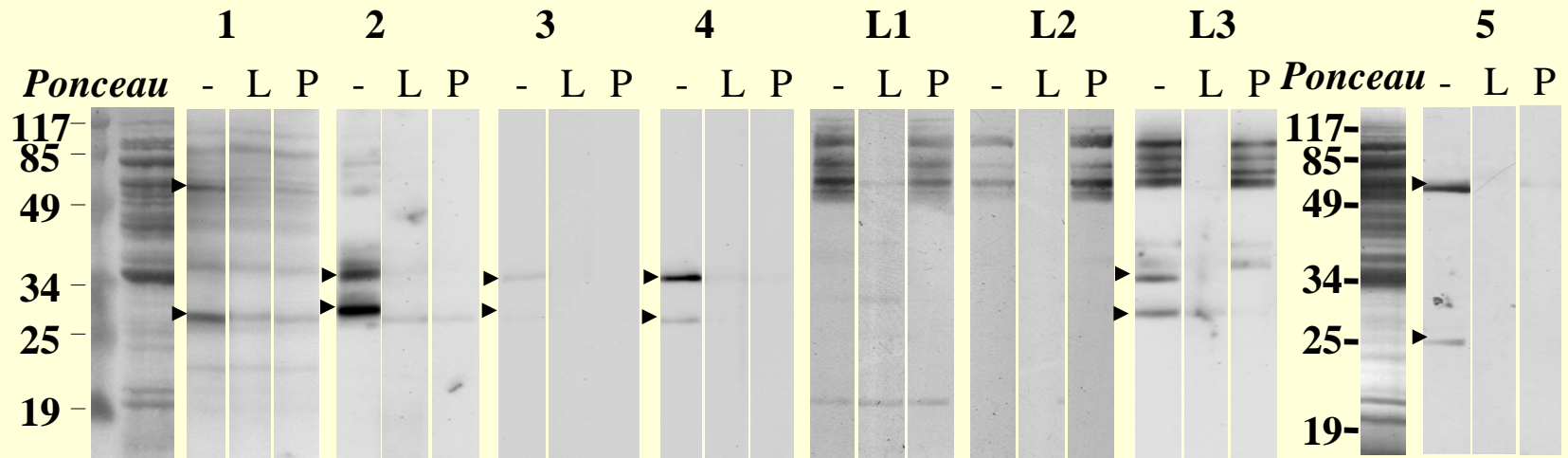


Preincubate with peanut
extract



film

Inhibition assays



- Three lupin proteins appear to show inhibition after preincubation with peanut proteins
 - Indicates presence of an epitope/s that is shared with a peanut protein
- Smaller two proteins correspond to spots identified as conglutin α & γ

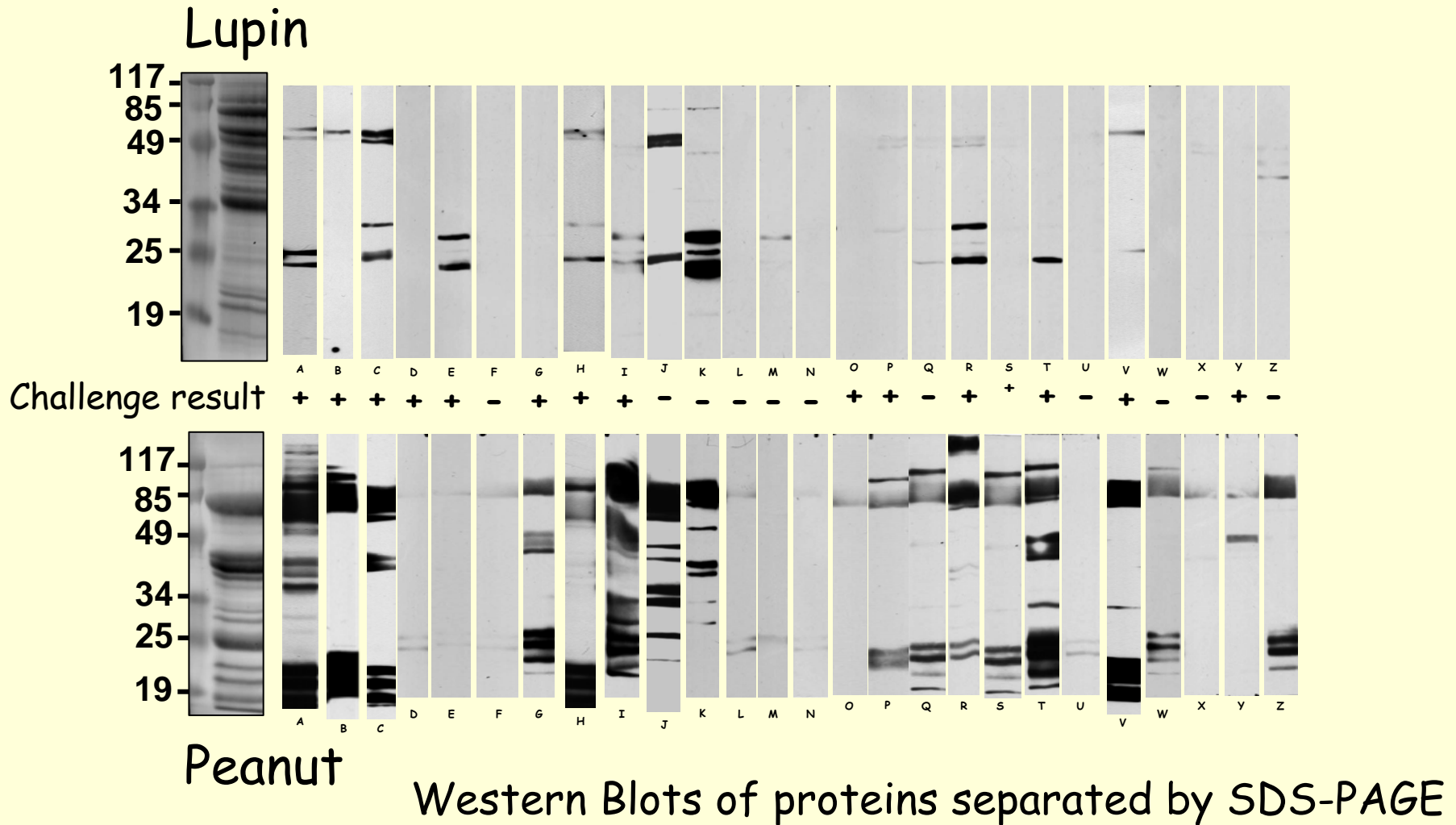
Conclusions

- We have shown that a proportion (27.6%) of peanut allergic people who are sensitized to lupin react clinically to lupin
 - Proportion of peanut sensitized people that react to lupin is ~7.6%
 - A greater proportion of adults react to lupin
- It is possible that reactivity is due to a common (cross-reactive) epitope on a lupin & peanut allergen
 - Perhaps peanut lupin allergic people have IgE to a different epitope than other peanut allergic people
- Cross-reactive allergen may be conglutin α or γ
- People allergic to "lupin only" mainly react to conglutin β
 - does not appear to cross-react with peanut (may depend on person)

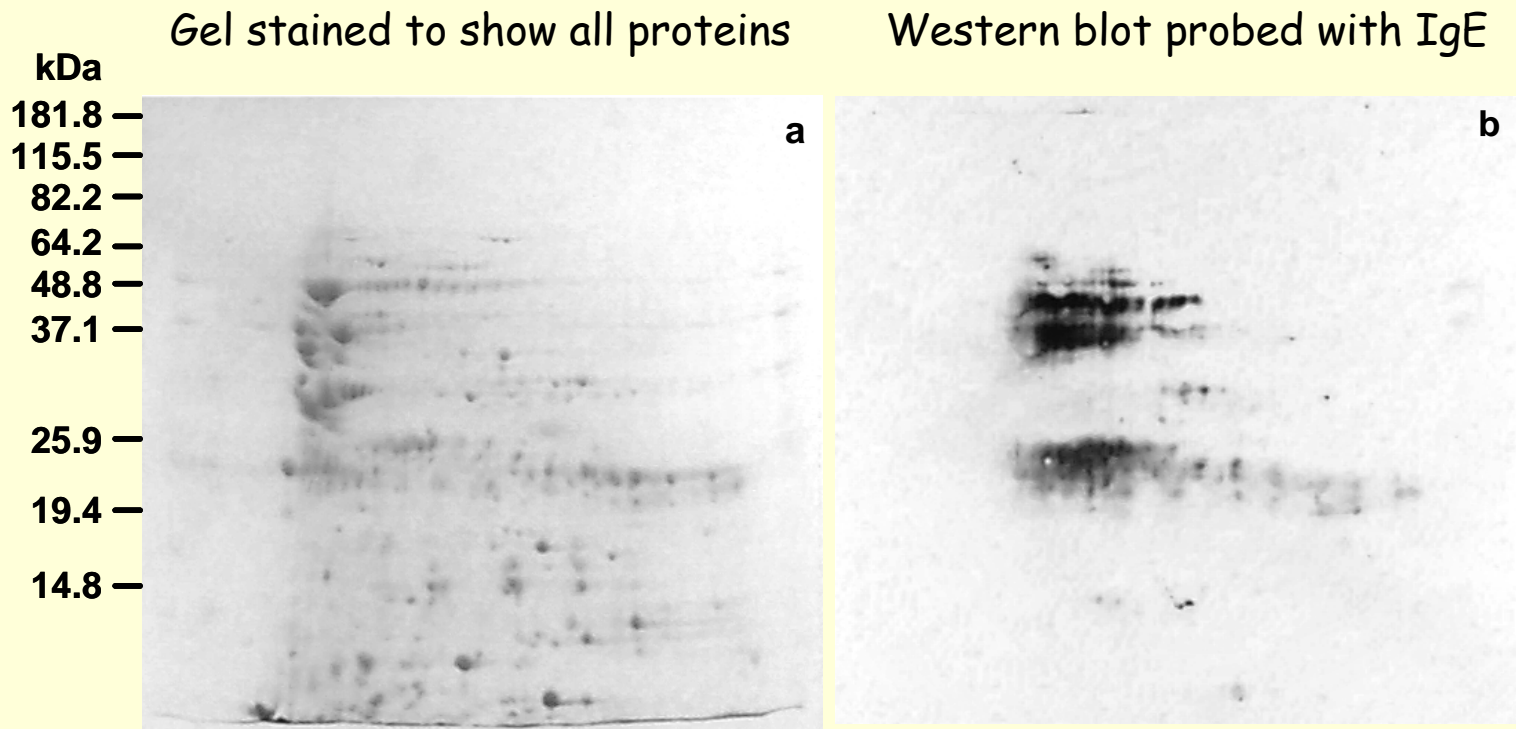
National Lupin Allergy Project

- School of Biological Sciences, University of Sydney
 - Gisela Mir, Penny Smith, (Danica Goggin, UWA)
- Allergy Unit, Royal Prince Alfred Hospital, NSW
 - Helen Colinet, Rob Loblay, Velencia Soutter
- Princess Margaret Hospital for Children, WA
 - Susan Prescott
- Royal Adelaide Hospital, South Australia
 - William Smith
- Project Coordination: George Weston Foods
 - Sherry Duckworth,
- DAFWA Sofia Sipsas
- Funding from Grain Foods CRC, GRDC and Centre for Genomic Medicine

Binding of IgE to lupin and peanut proteins



Allergen ID-“lupin-only”



Allergen ID- spots identified

