

# Latex allergy: what has the epidemic taught us?

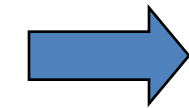
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Respiratory Medicine  
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# History of Latex Allergy

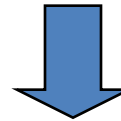
- First described in housewife with contact urticaria (Nutter, 1979)
- Intra-operative anaphylaxis (Turjanmaa, 1984)
- Spina bifida children (Slater, 1989)
- 15 deaths from latex barium enema balloons (Ownby, 1991)

# Latex Glove Manufacture



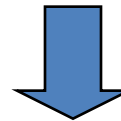
Rubber tapping

Non ammoniated latex (NAL)

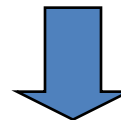


Ammonia 0.7%

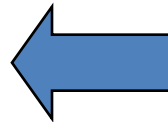
Low ammoniated latex (LAL)



Stabilisation ( Thiurams, antioxidants)  
these chemicals cause Type IV hypersensitivity



Heating, Dipping  
(glove moulds  
dipped into liquid latex)



Washing/Leaching,  
+/-  $\gamma$  irradiation  
+/- powdering





# Epidemiology

- A disorder dependent on exposure to latex proteins
- Wider community - < 1% (Liss, 1999)
- Health care workers **sensitisation** historically between 4 and 22% (now much less)
- Overt **allergy** less than half of sensitised group

# The Alfred Hospital nurses latex allergy study

- 140 nurses from ICU and theatre
- 22% of group positive skin test to one of 5 latex glove eluates (**sensitisation**)
- Symptoms of local hand irritation were equally frequent in skin-prick negative group

Douglas et al, Prevalence of IgE-mediated allergy to latex in hospital nursing staff, Aust NZ J Med 1997; 27: 165-169.

TABLE 3  
Symptoms Associated with Latex Glove Usage

	Latex skin prick test positive	Latex skin prick test negative
Skin dryness	26 (84%)	80 (73%)
Itch	18 (58%)	52 (48%)
Skin erythema	17 (55%)	48 (44%)
Urticaria	4 (13%)*	4 (4%)*
Eczema	10 (32%)	29 (27%)
Eye symptoms	4 (13%)	6 (6%)
Nasal symptoms	5 (16%)	10 (9%)
Dyspnoea	1 (3%)	0 (0%)
Asthma	0 (0%)	0 (0%)
Rapid onset†	23	65
Delayed onset†	7	5

The nurses were asked to estimate the time delay between putting on gloves and onset of each symptom. If the first symptom began less than one hour after putting on the gloves, the subject has been classified as a rapid responder.

\* $p < 0.05$  (comparing latex skin prick test positive and negative nurses for this symptom).

†The delay of onset of symptoms was answered by 30 of the nurses who were latex skin prick test positive and 70 nurses who were skin prick test negative.

From Douglas et al, Aust NZ J Med 1997; 27: 165-169.

# Diagnosis of Latex allergy

- Symptoms of immediate type allergy (hand itching alone **not** predictive) on latex exposure
- Associations – risk group (spina bifida, HCW), fruit allergy, existing hand dermatitis, atopy
- Demonstration of latex specific IgE by skin prick test, RAST test or challenge testing

Latex allergens have a high capacity  
to cross-link effector-cell bound IgE

## Prevalence of self reported symptoms on latex exposure among latex allergic subjects

Symptom	Latex allergic (n=32)	Latex non-allergic (n=19)	p
Local skin itching and erythema	90.6	20.3	<0.01
Local skin hives	87.5	0	<0.01
Generalised erythema	43.8	5.3	<0.01
Sneezing	62.5	0	<0.01
Facial swelling	68.8	0	<0.01
Throat swelling	43.8	0	0.01
Asthma, shortness of breath or wheezing	71.9	5.3	<0.01
Anaphylaxis	40.6	0	0.01

Sutherland, 2003

# Skin testing in latex allergy may be associated with anaphylaxis

- Commonest skin test reagent causing anaphylaxis (228/ 100, 000) (Valvasevi et al, Ann Allergy Asthma Immunol 1999: 132-6.)
- 9/118 consecutive patients tested with glove extract had systemic reactions (4 anaphylaxis) (Kelly et al, J Allergy Clin Immunol 1994: 813-6.)
- NAL skin test reagent Greer 16.1% mild systemic reactions. Not licensed yet. (Hamilton et al, J Allergy Clin Immunol 1998:482-90.)

# Stallergenes latex extract

- 93% sensitivity (43/46 latex allergics diagnosed), 100% specificity at 1:200 dilution (100 IR) 22ug/ml . Low ammoniated latex preparation (RRIM clone 600)
  - Turjanmaa et al, Latex allergy diagnosis: in vitro and in vivo standardization of a natural rubber latex, *Allergy* 1997 50: 41-50
- Alfred Hospital Experience – over 200 skin tests performed. Five systemic reactions. None in RAST negative subjects.
  - Sr Sue McLellan personal communication – SAS records.

Different latex allergens  
are clinically important in  
different risk groups

# Reported Prevalences of IgE reactivity to individual latex allergens

Allergen	Spina bifida	HCW	Reference
Hev b 1	81 54	52.3 13	(Chen <i>et al.</i> , 1997) (Kurup <i>et al.</i> , 2000)
Hev b 2	38	48	(Kurup <i>et al.</i> , 2000)
Hev b 3	100 85	0 19	(Yeang <i>et al.</i> , 1996) (Kurup <i>et al.</i> , 2000)
Hev b 4	46	61	(Kurup <i>et al.</i> , 2000)
Hev b 5	56	92	(Slater <i>et al.</i> , 1996)
Hev b 6.01	38	45 69	(Kurup <i>et al.</i> , 2000) (Alenius <i>et al.</i> , 1996)
Hev b 6.02		48	(Alenius <i>et al.</i> , 1996)
Hev b 6.03		21	(Alenius <i>et al.</i> , 1996)
Hev b 7	23 3 39.5	23 49	(Kurup <i>et al.</i> , 2000) (Seppala <i>et al.</i> , 2000) (Wagner <i>et al.</i> , 2001)
Hev b 8		35	(Fuchs <i>et al.</i> , 1997)
Hev b 9		14.5	(Wagner <i>et al.</i> , 2000)
Hev b 10		33	(Wagner <i>et al.</i> , 2001)
Hev b 11w		23	(O'Riordain <i>et al.</i> , 2001)

# Hev b 5, 6 and 7 are important among HCW

- Combination of rHev b 5,6,7 has 93% sensitivity, 100% specificity for diagnosis of latex allergy by SPT Yip et al. Skin prick test reactivity to recombinant latex allergens. Int Arch Allergy Immunol 2000;121(4): 292-9
- There is significant monosensitisation to each Hev b 5 (17%), Hev b 6 (10%) and Hev b 7 (10%).

Some latex  
allergens share  
significant  
homology with  
food allergens

# Food allergy in latex allergy

- 50% of subjects with latex allergy have food allergy
- Commonest fruits are banana, avocado, kiwi fruit
- Major allergens of banana and avocado are chitinases that share significant homology with hevein (Hev b 6.02)

Current *in vitro* IgE  
assays are non  
ammoniated latex  
(NAL)

# RAST testing for latex allergy

- No approved skin testing reagent in Australia or US – therefore **RAST testing critical**
- Pharmacia Cap system is most widely used and validated
- Fully automated system where allergen bound to an activated cellulose matrix
- Serum added undiluted, washed and detected with monoclonal anti-IgE (fluorescent substrate)



# Performance of CAP assay

Study	Sensitivity	Specificity	N=allergic	N=non allergic	Gold Standard
Hamilton*	<b>76.3%</b>	<b>96.7%</b>	117	195	History and positive Skin test
Ownby#	<b>79.5%</b>	<b>90.2%</b>	83	60	History and positive Skin test

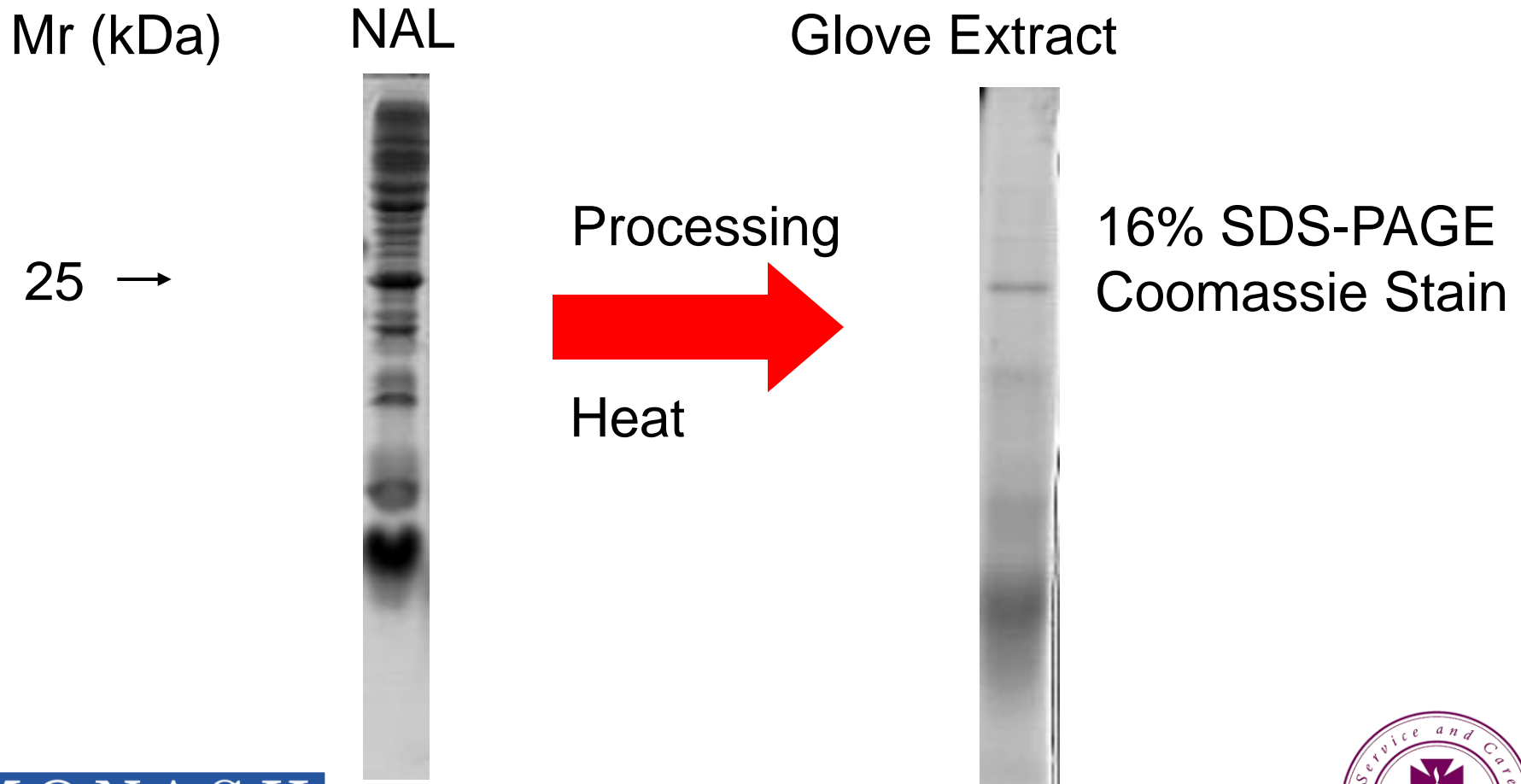
\*Diagnostic Performance of FDA cleared serologic assays for latex allergy, Hamilton et al, JACI 1999; 103: 925-30

#A blinded multicenter comparison of two commercial in vitro tests for latex-specific IgE antibodies, Ownby et al, Ann Allergy Asthma Immunol 2000; 84: 193-196

# Potential limitations of current IgE assays

- CAP system is Non Ammoniated Latex (NAL)
- Source of allergen exposures is latex gloves
- Some proteins may be under-represented or their IgE epitopes altered in current NAL diagnostic assays

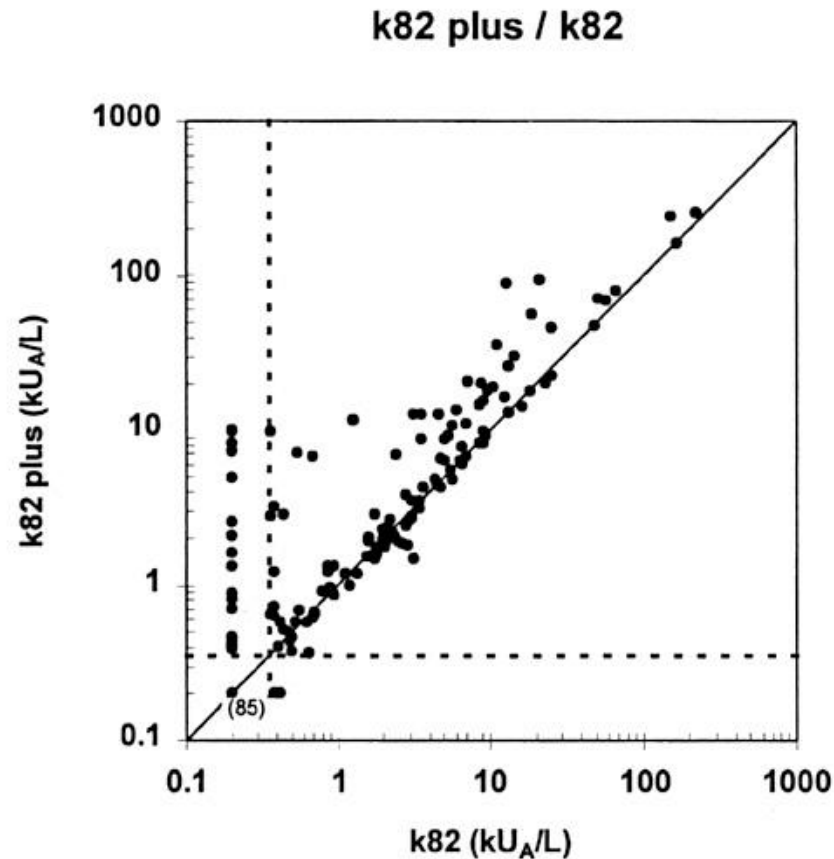
# Difference in protein profile between NAL and latex glove extract



# Hev b 5 in latex allergy

- Hev b 5 is an important allergen recognised on skin testing by 18/29 (62%) of latex allergic health care workers (HCWs). In 5/29 (17%) it was the sole sensitiser. Yip et al, Skin Prick Testing to recombinant latex allergens, (Int Arch Allergy Immunol 2000; 121: 292-299)
- Hev b 5 is in low abundance in commercially available capture assays Chen, Z. et al, The absence of Hev b 5 in capture antigen may cause false negative results in serologic assays for latex specific IgE antibodies(Abstract), J Allergy Clin Immunol 105; S 83.
- “k82 plus” – (NAL CAP spiked with rHev b 5/MBP) results in greater diagnostic sensitivity (an additional 16/222 or 7% of serum samples became positive) Lundberg et al, Recombinant spiked allergen extract, Allergy 2001, 56: 794-795.

# rHev b 5 increases sensitivity of CAP



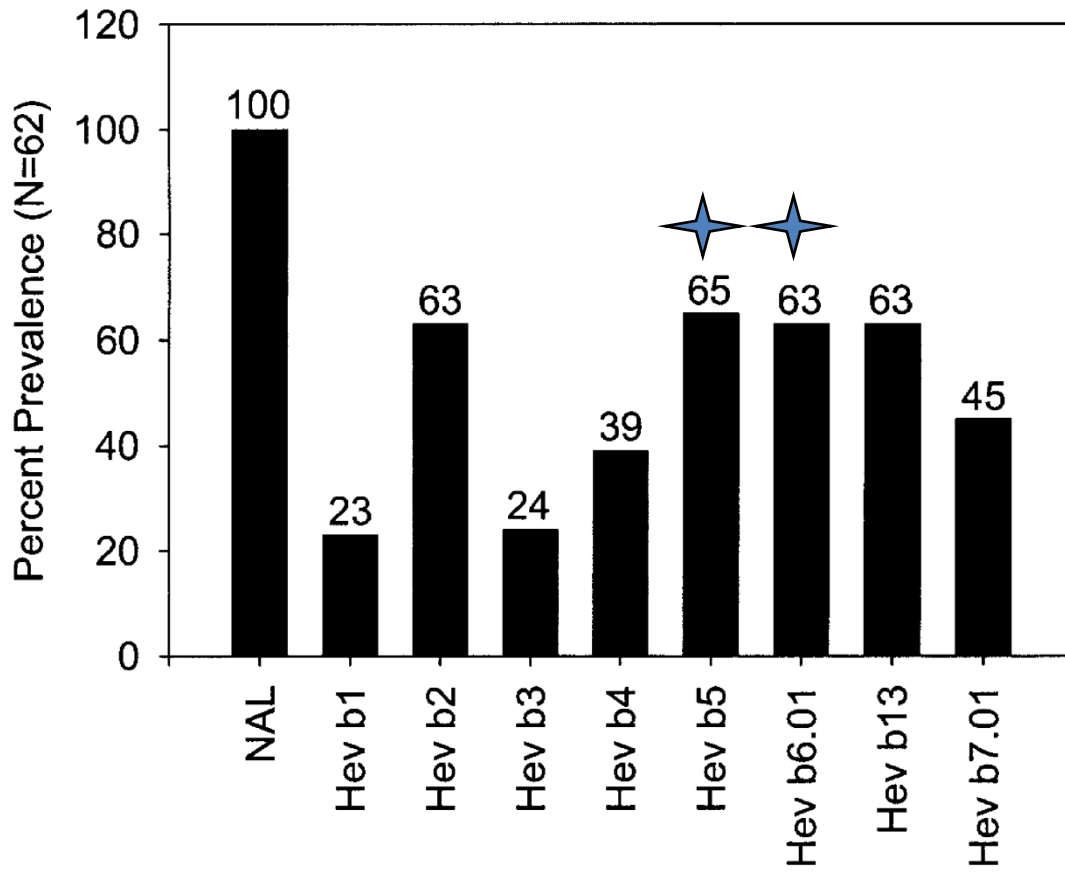
Lundberg, Allergy 2001 56: 794-795

*CRC for Asthma*

# The human immune response to Hev b 5

- Hev b 5 cloned and expressed simultaneously in 1996
  - Slater, J Biol Chem 1996: 25394-9, Akasawa, J Biol Chem 1996: 25389-93.
- Reacted with IgE of 92% of HCW, 56% spina bifida patients
- Highly charged, proline rich
- Uncertainties – no specific mAbs expressed as fusion protein with MBP.

# Frequency of skin prick test reactivity to latex allergens for latex glove sensitised health care workers



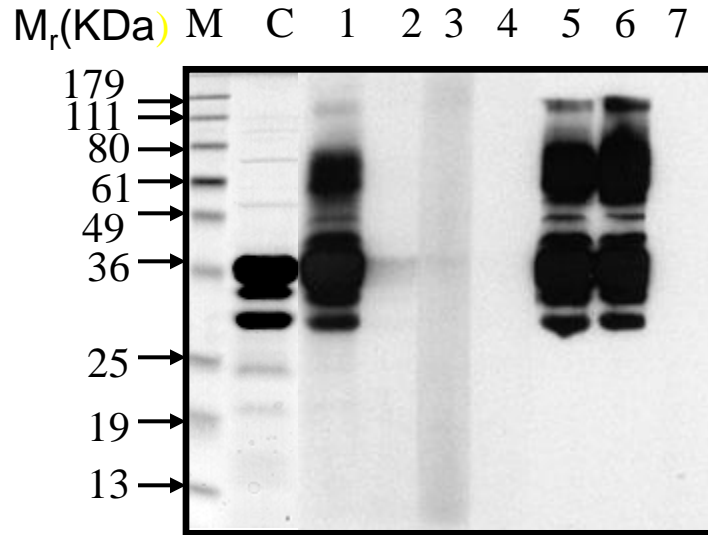
Bernstein et al., JACI 111:610, 2003

*CRC for Asthma*

# Monoclonal Antibody Production

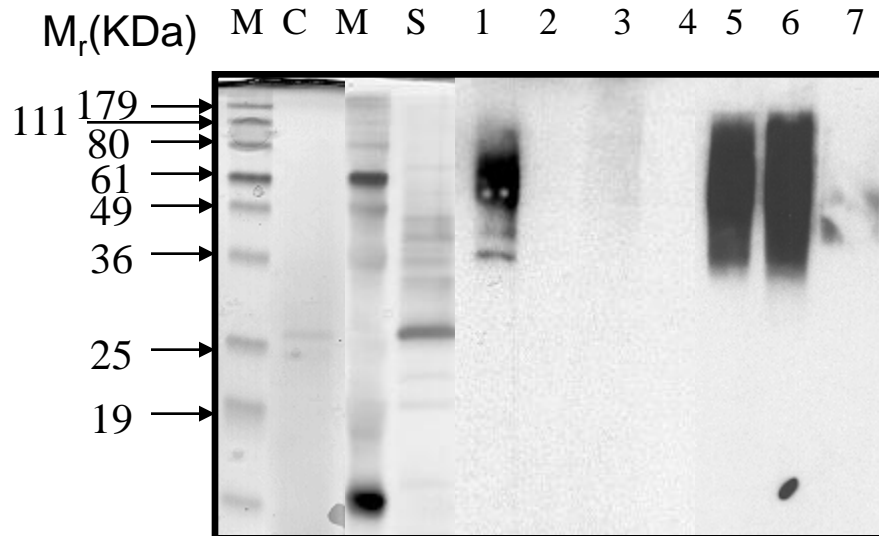
- Useful to act as probes to determine relative abundance of Hev b 5 in latex extracts.
- Use in aero-allergen assays

# Western of rHev b 5



C – Coomassie stain  
 S – Silver stain  
 1 – latex allergic HCW IgE  
 2 – non latex allergic HCW IgE  
 3 – 1 inhibited with Hev b 5  
 4 – no serum control  
 5 – mAb 6F6  
 6 – mAb 6A10  
 7 – isotype control  
 B Inhibition Immunoblot of Glove extract

# Western of glove extract



Sutherland et al, Clin Exp Allergy, 2002

## Latex extract

## Hev b 5 content % total protein \*

### Powdered non-sterile latex gloves

- laboratory utility glove 3.0
- household glove 1.6

### Sterile surgical gloves

- powdered nd
- non-powdered nd

Latex sap 1.8

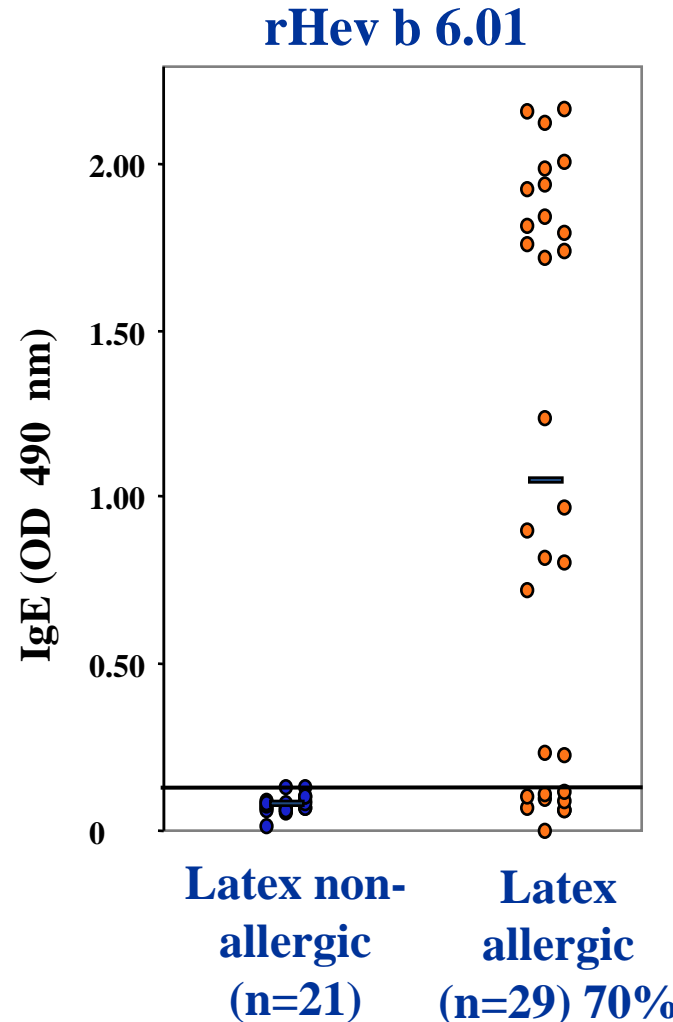
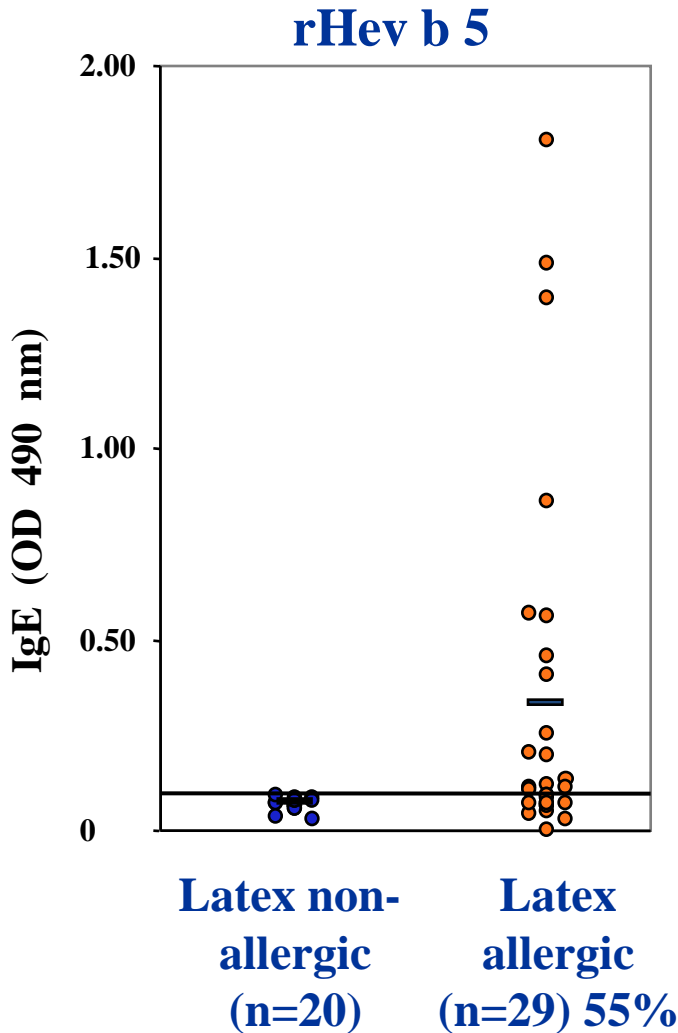
\* detected by mAb inhibition ELISA

nd = not detectable

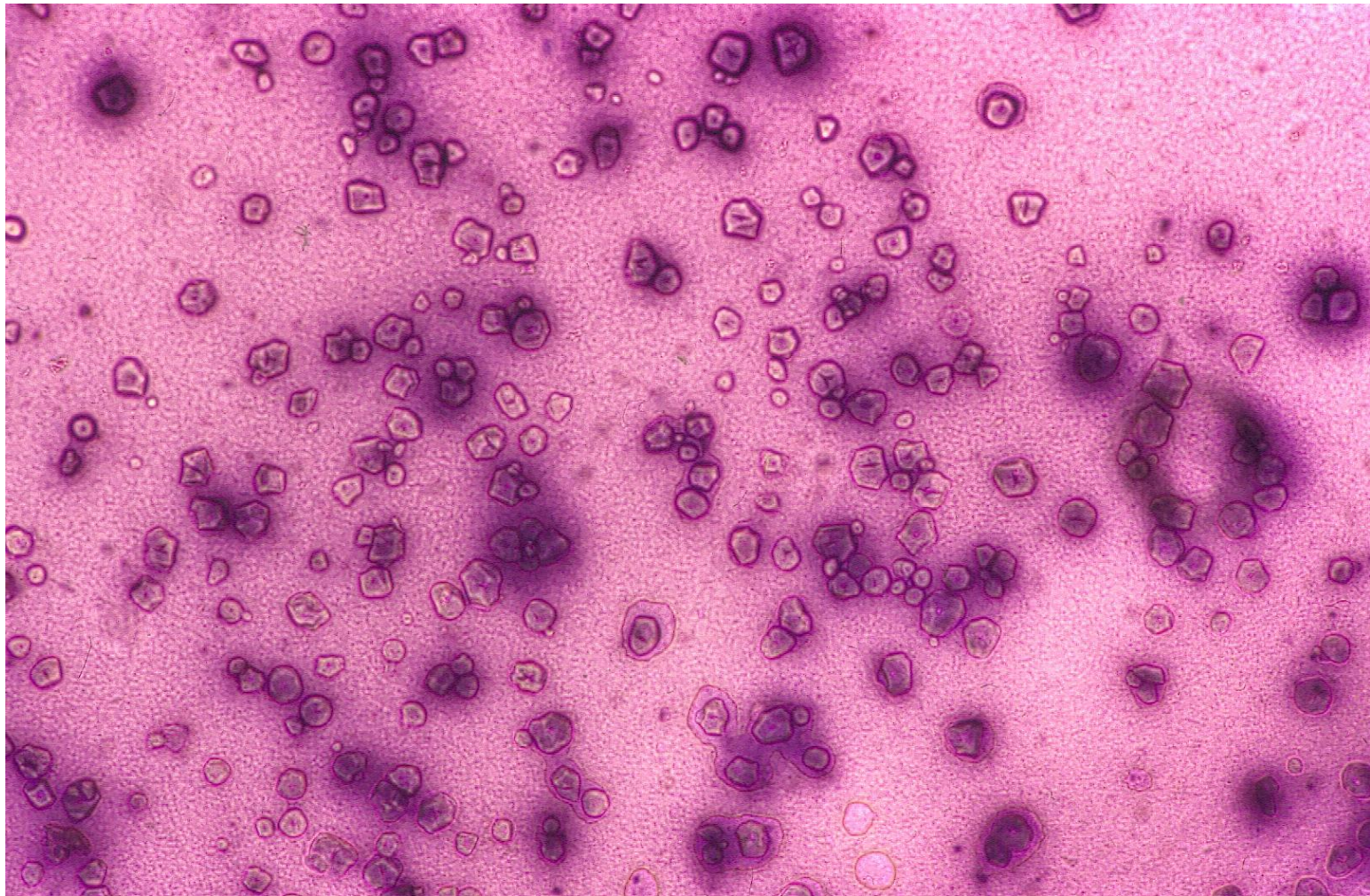
Sutherland et al, Clin Exp Allergy 2002

*CRC for Asthma*

# Major latex allergens in latex glove sensitised patients



94% IgE reactive to Hev b 5 and/or Hev b 6.01



Livingstone powdered latex glove (Hev b 5 staining)

Halogen® assay (courtesy of Dr Teresa Mitakakis)

# Hev b 5 : summary

- Hev b 5 is a major latex allergen and important aero-allergen
- Most high quality surgical gloves and powder free examination gloves have low or undetectable allergen levels
- May be under-represented in commercial capture assays

Latex is an excellent model for  
novel immunotherapeutic  
strategies

# Specific immunotherapy for latex allergy

- trials using current unfractionated extracts

- 17 patients, randomised DBPC, rush sc IT then maintenance – clinical benefit, but high adverse events

(Leynadier et al., JACI 2000)

- 24 patients, DBPC cluster sc IT – clinical efficacy mainly on cutaneous symptoms, high adverse events

(Sastre et al., JACI 2003)

- 26 patients, rush sublingual IT – improved glove-use test, high adverse events

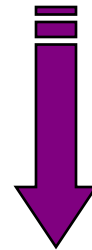
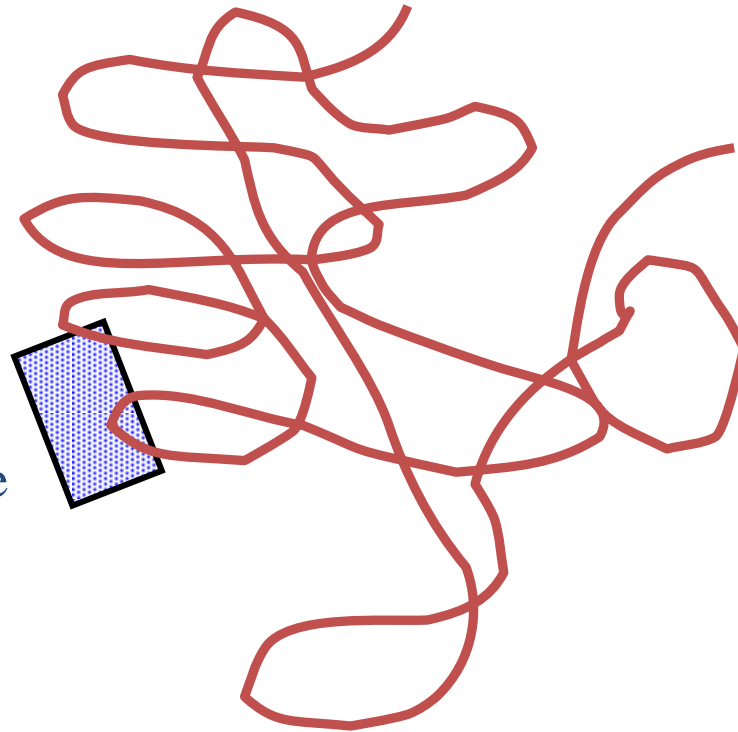
(Bahima et al., J Invest Allergol Clin Immunol 2004)

# Developing high dose allergen T cell targeted immunotherapy

# B and T cell epitopes of allergens

Allergen  
tertiary  
structure

B cell epitope



primary  
structure

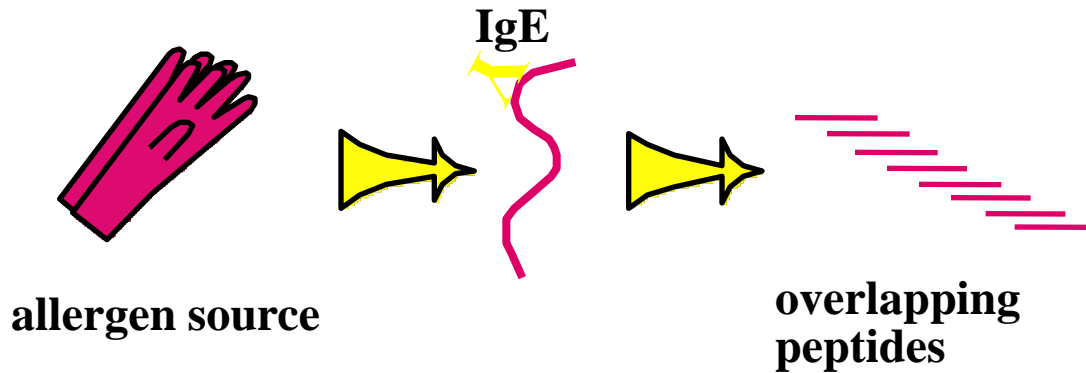


T cell epitopes

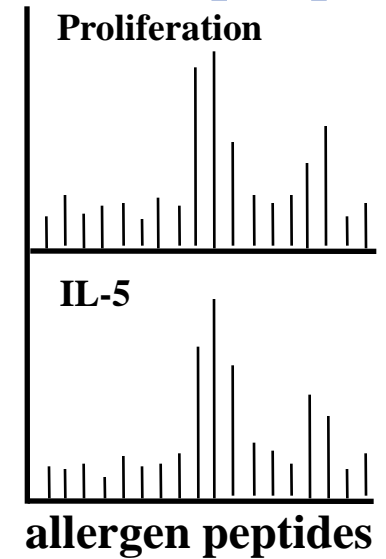
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# Hypoallergenic allergy vaccine strategy

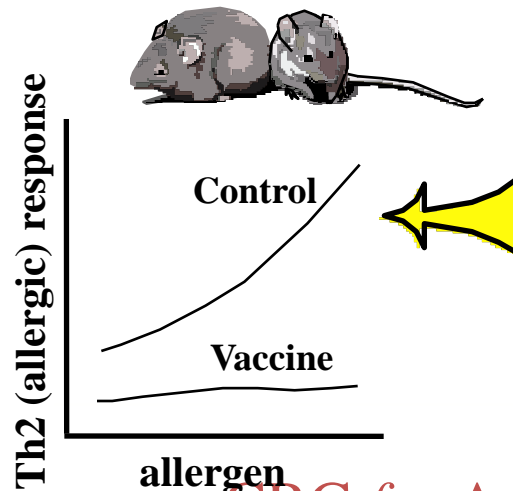
## 1. Identify allergenic proteins



## 2. Identify T cell epitopes



## 4. Test *in vivo*

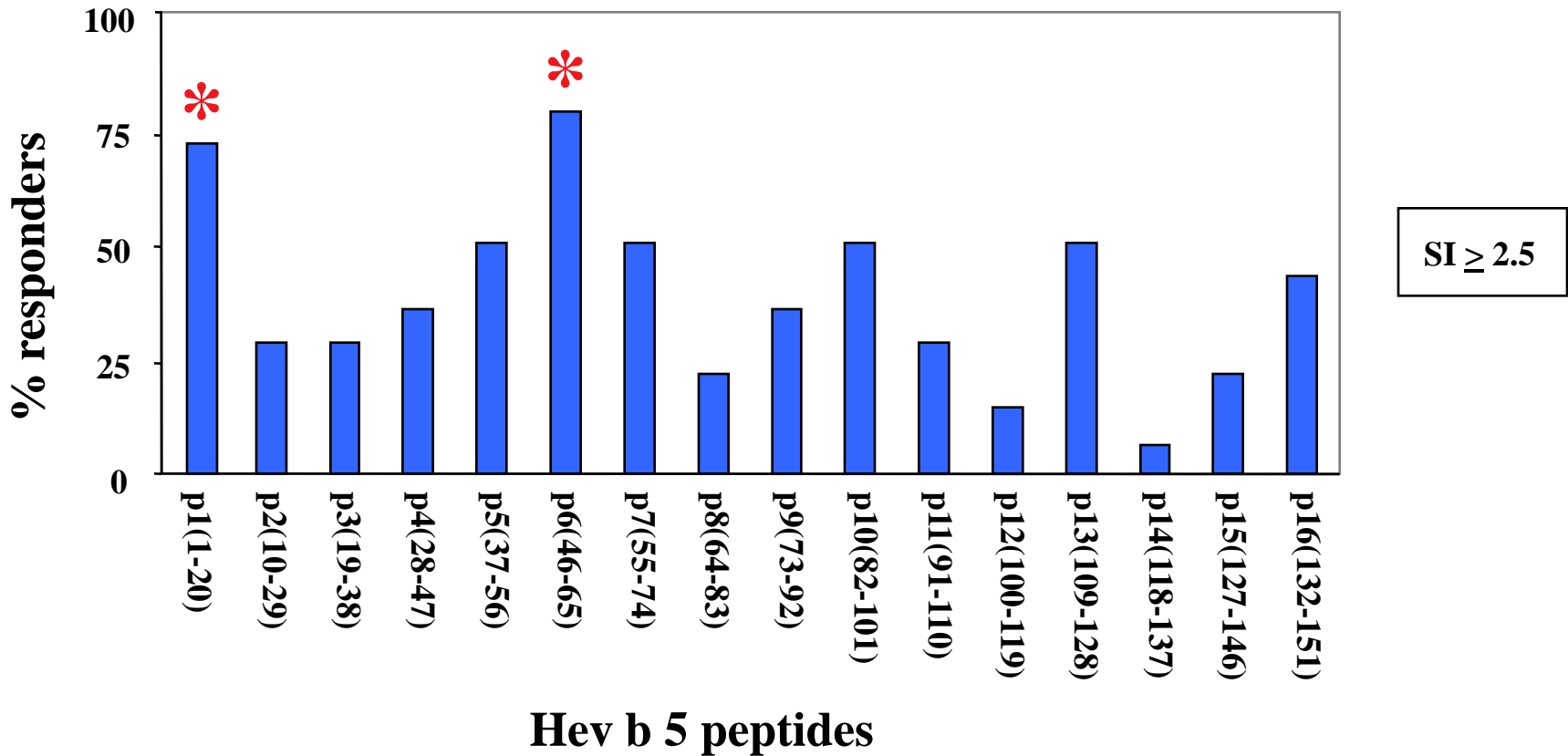


## 3. Prepare mutant protein or peptide vaccines

contain T cell epitopes but non-IgE reactive

# Mapping T cell epitopes of latex allergens

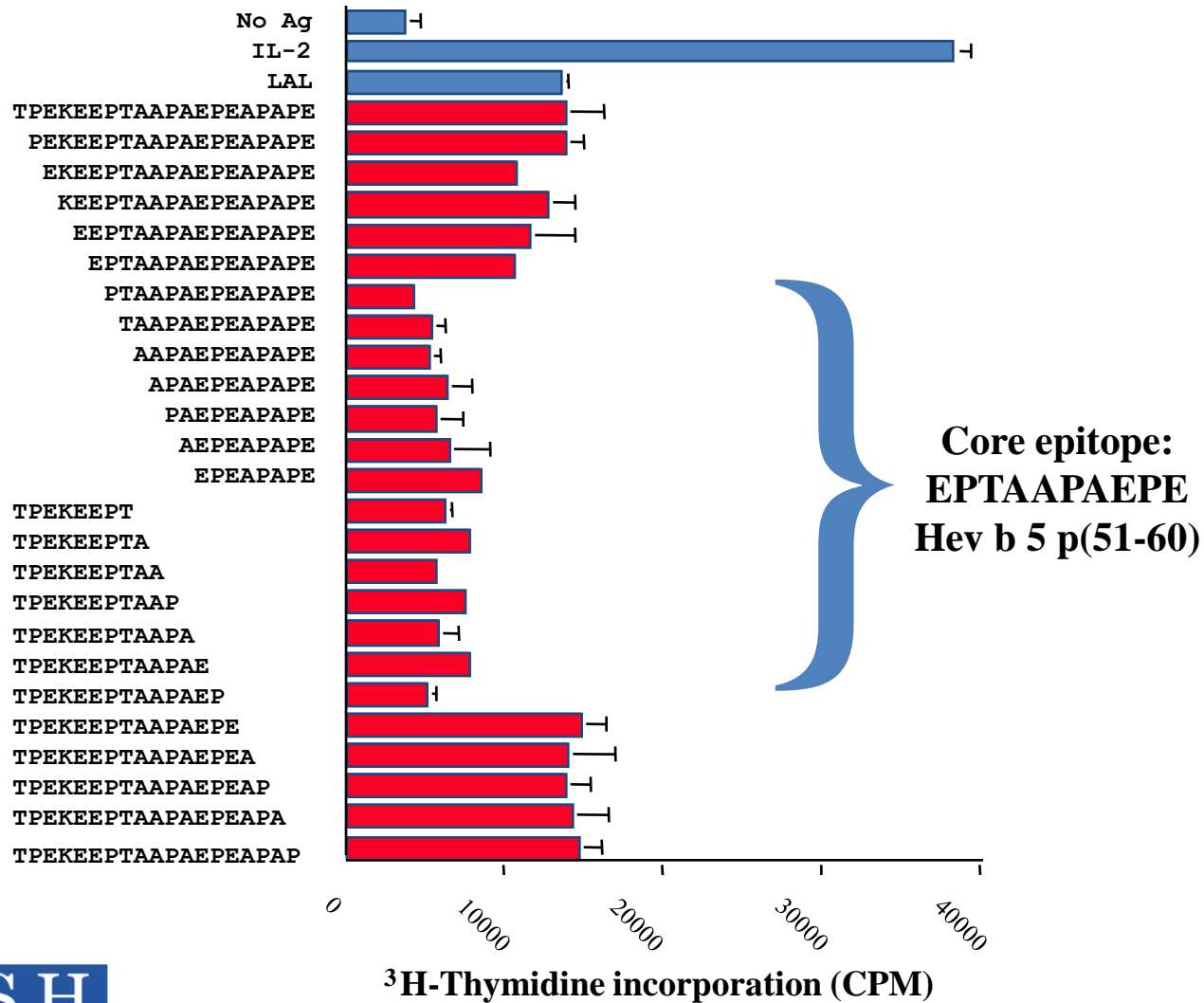
# Dominance of T cell response to Hev b 5 p(1-20) and p(46-65) in latex allergic subjects (n=14)



De Silva et al., JACI 105:1017, 2000

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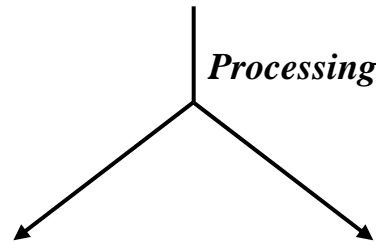
# Identification of Hev b 5 p(46-65) core T cell epitope



## Structure of Hev b 6.01



**Prohevein (Hev b 6.01)**



**Hevein (Hev b 6.02)**

**C-terminal fragment (Hev b 6.03)**

**IgE reactivity: 80 % positive**

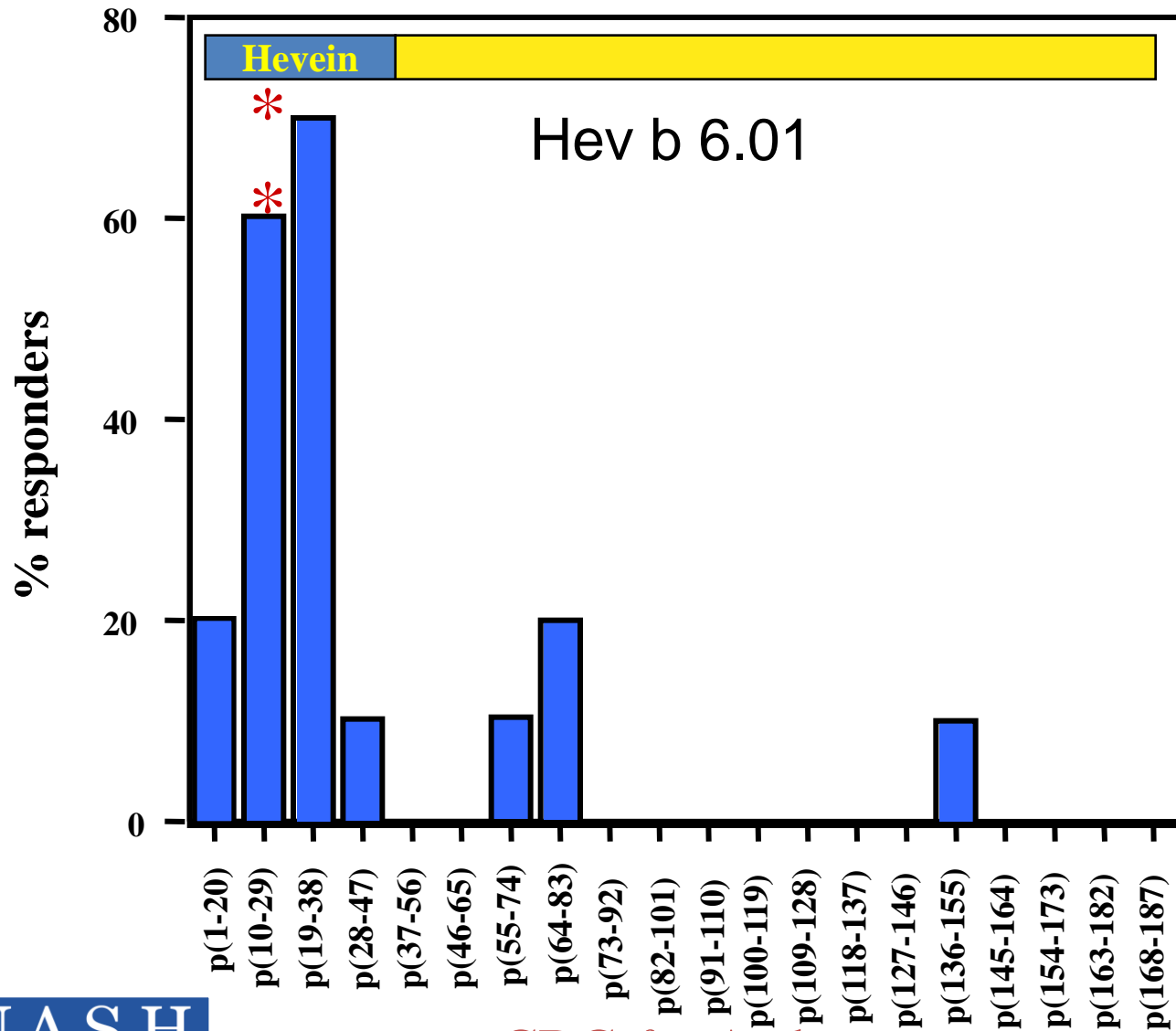
**20 % positive**

**Hevein 30 times as abundant as C-terminal fragment in rubber latex  
(Soedjanaatmadja *et al.*, 1995)**

*Rozynek et al., Clin Exp Allergy 28:1418,1998*

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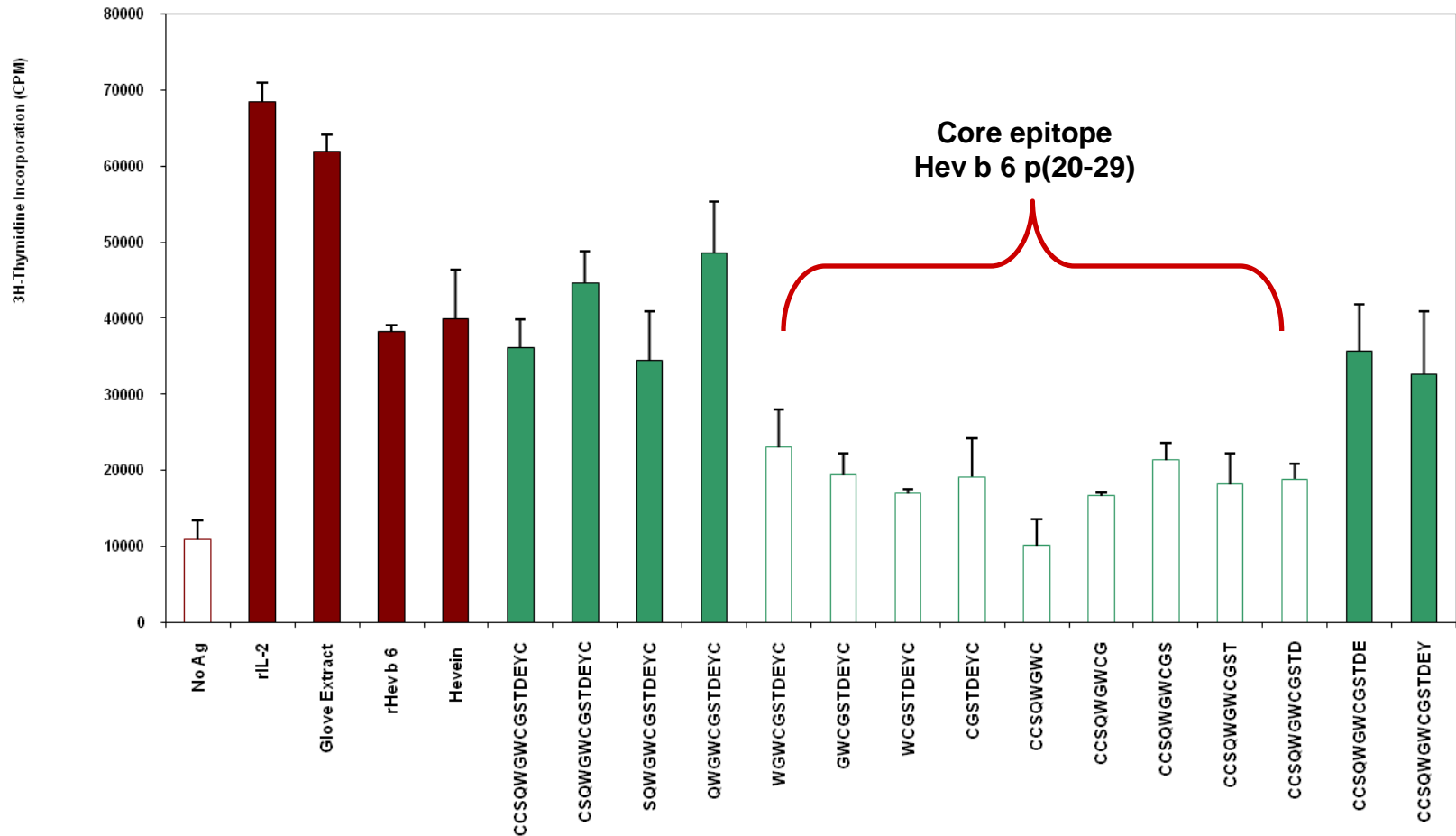
# Dominance of T cell response to Hev b 6.01 p(10-29) and p(19-38) in latex allergic subjects (n=10)



SI  $\geq$  2.5

De Silva et al.,  
Clin Exp  
Allergy 34:1,  
2004

# Identification of core T cell epitope of hevein

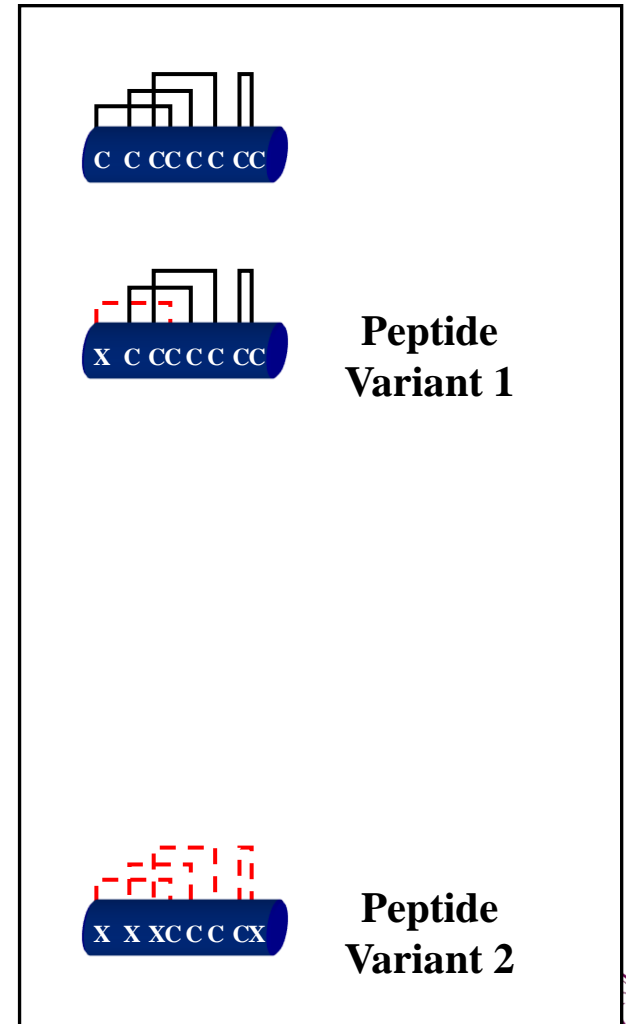
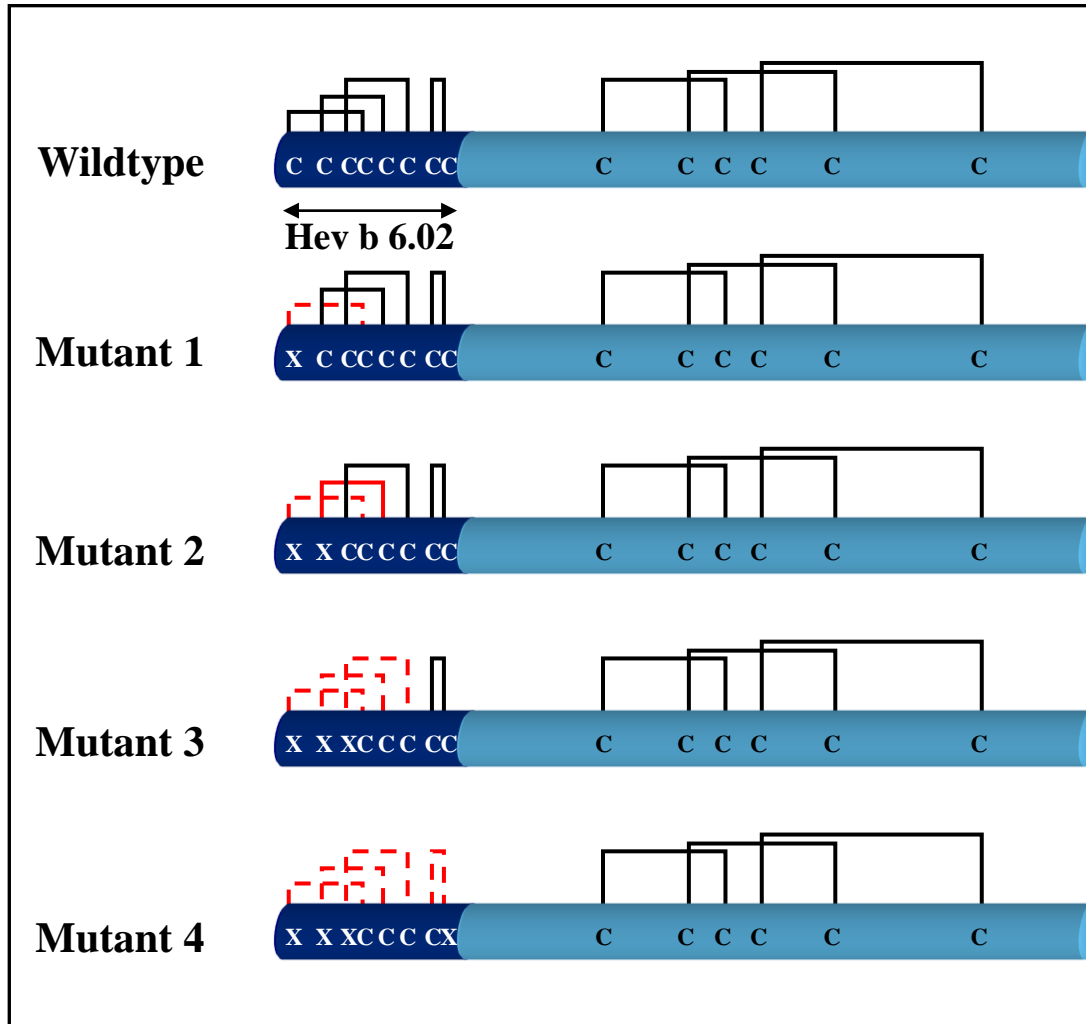


# Production of T cell stimulatory hypoallergenic mutants

# Hev b 6.01 mutant recombinant and synthetic peptide molecules

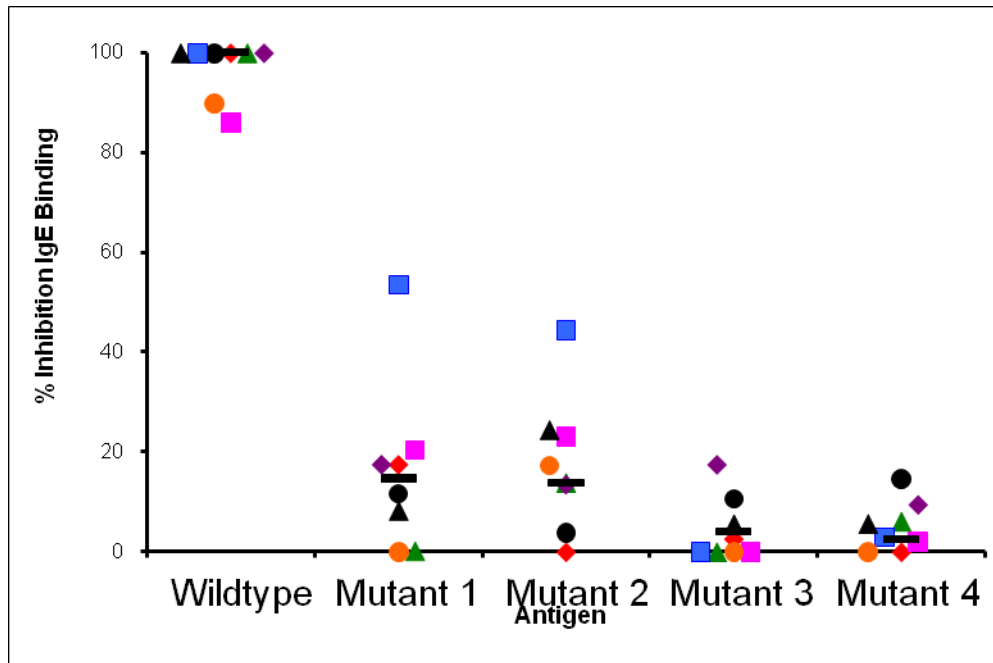
rHev b 6.01 proteins

Hev b 6.02 peptides

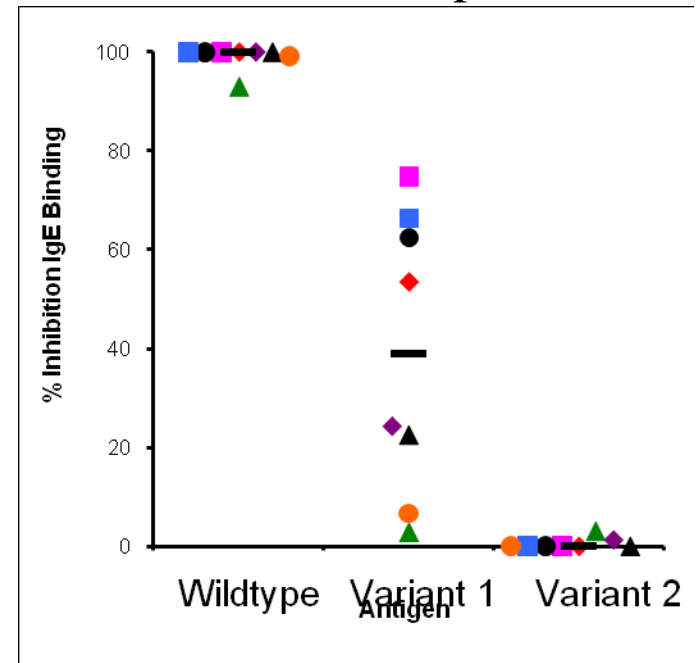


# rHev b 6.0 mutants and peptide variants have reduced IgE binding - inhibition ELISA

rHev b 6.01 Proteins



Hev b 6.02 Peptides

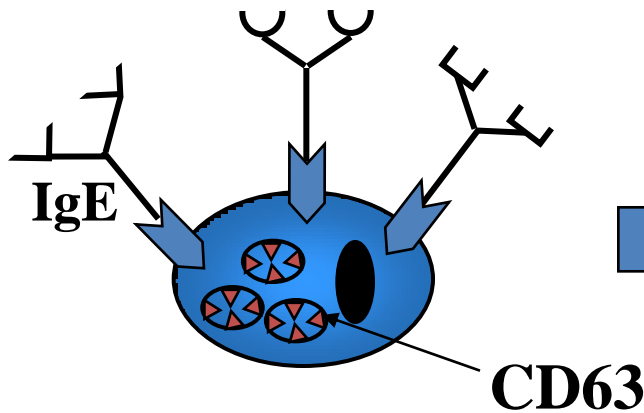


Drew et al., J Immunol 173: 5872, 2004  
*CRC for Asthma*

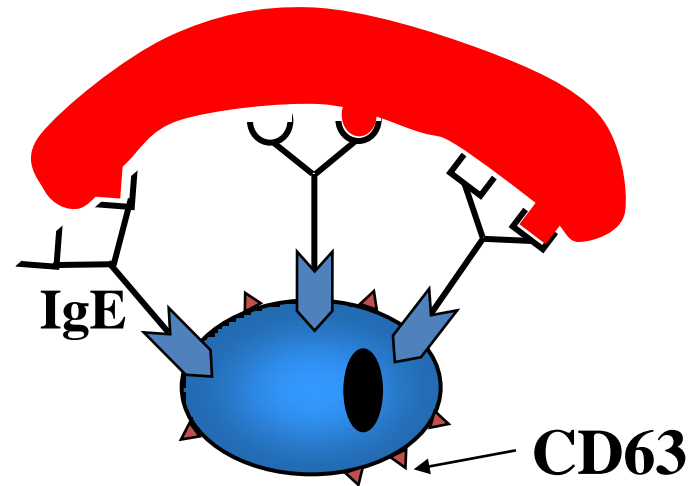
# Functional assays for hypoallergenic mutants

- Basophil activation assay by flow cytometry
- Allergen-specific T cell proliferation assay

# CD63 is a surface marker for activated basophils



Unstimulated basophil

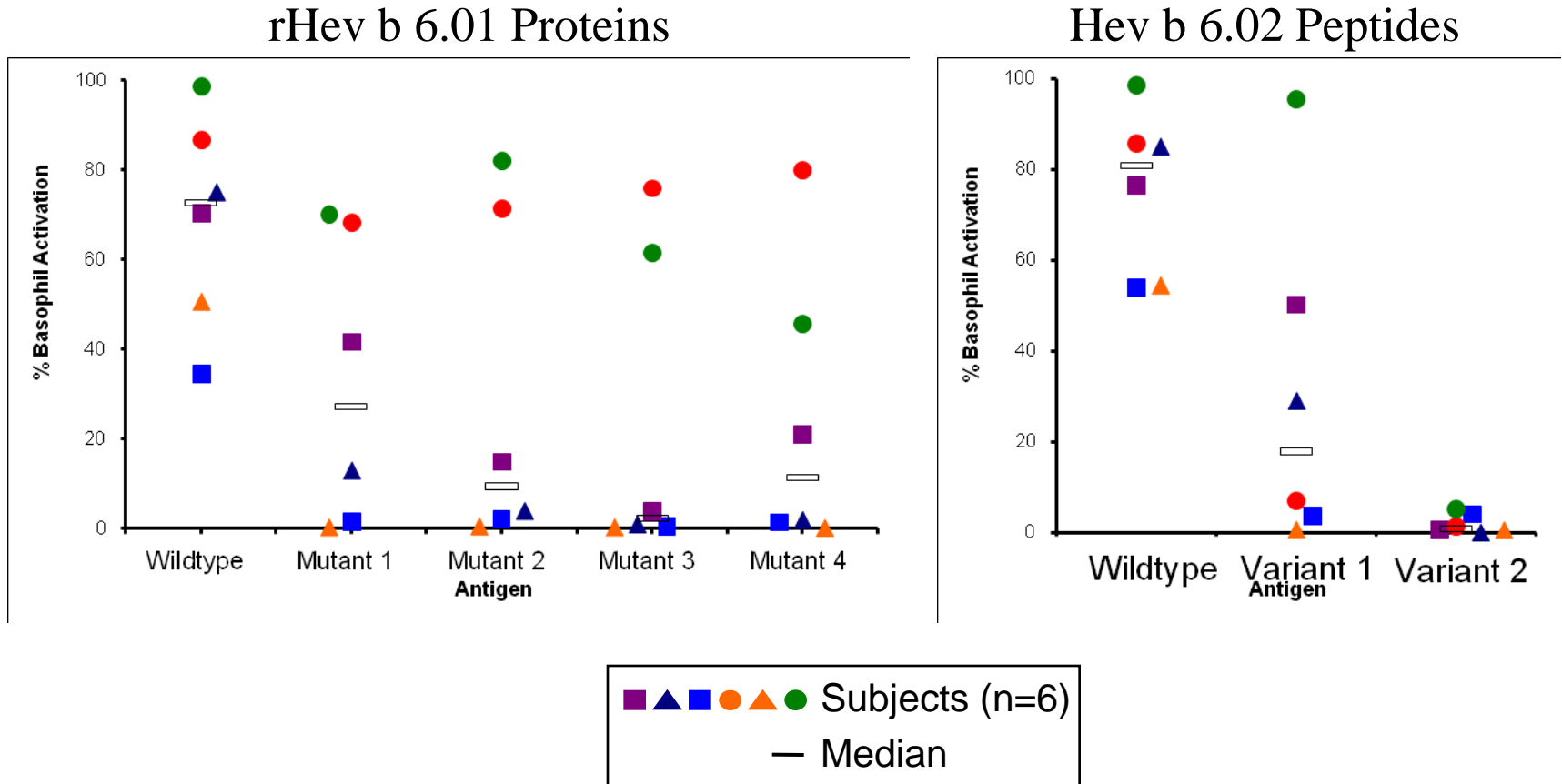


Activated basophil

*(Adapted from Valenta & Kraft, 2001)*

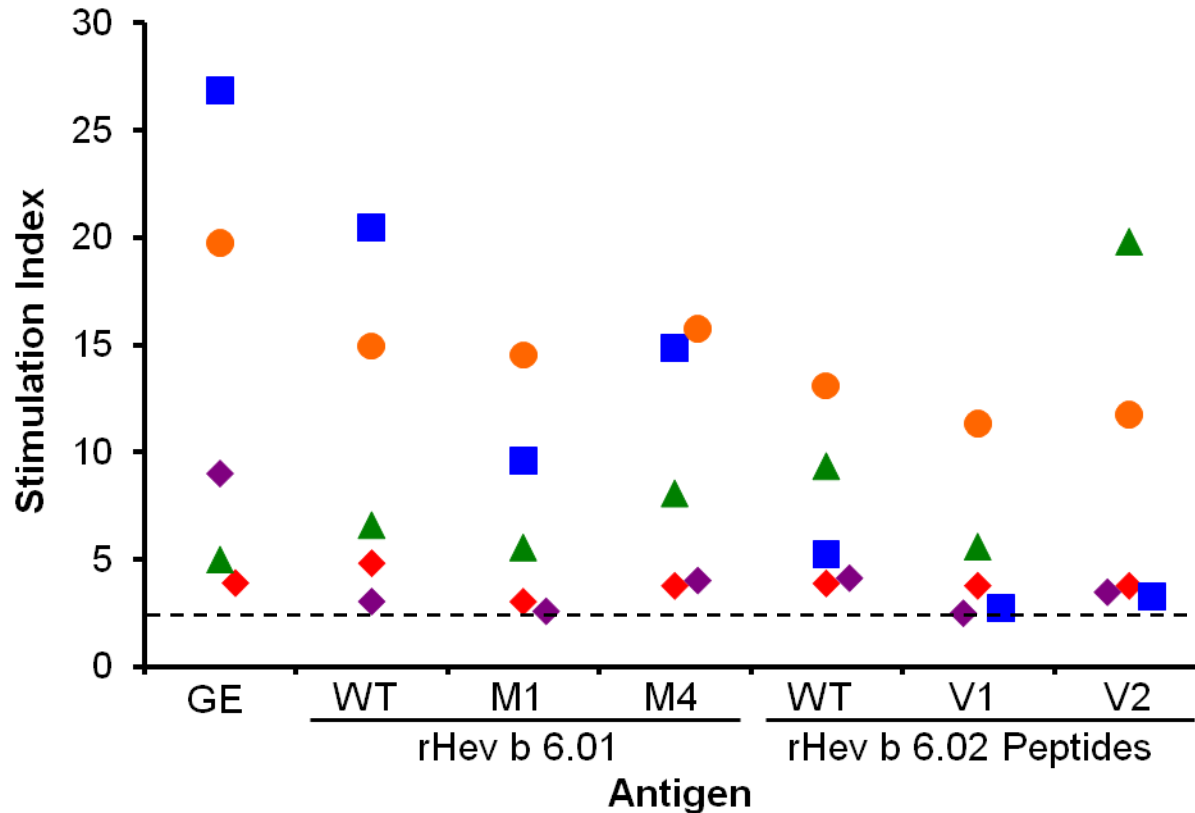
# Hev b 6.01 variants are poor activators of basophils

- no activation by Hev b 6.02 peptide variant 2



Drew et al., J Immunol 173: 5872, 2004

# T cells proliferate to variants of Hev b 6.01



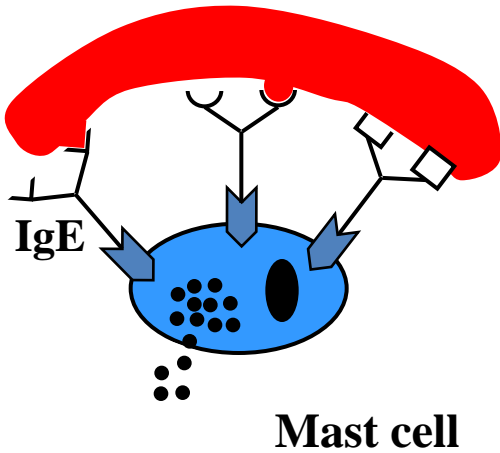
◆ ■ ▲ ● ◆ Subjects (n=5)

WT, Wildtype  
M1, Mutant 1  
M2, Mutant 2  
V1, Variant 1  
V2, Variant 2

Drew et al., J Immunol 173: 5872, 2004

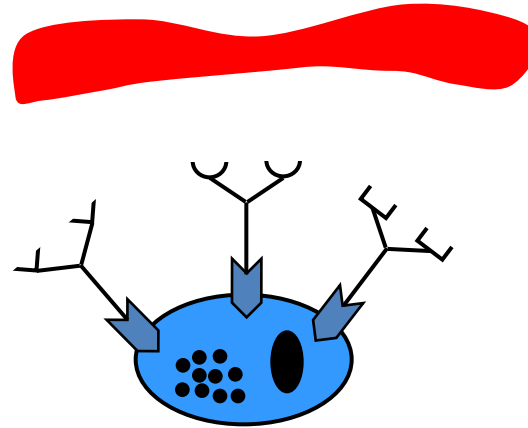
# Hypoallergenic preparations

Native allergen



Mast cell

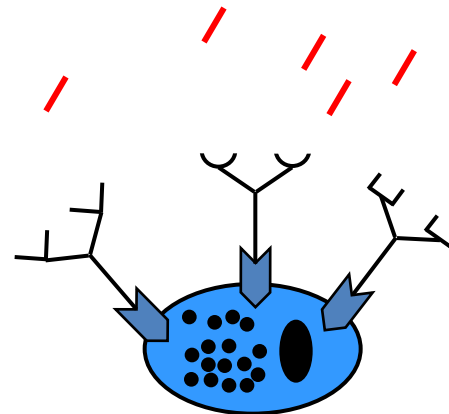
Mutant allergen vaccine



IgE epitope removal

No IgE binding

T-cell peptide vaccine



No IgE cross-linking

*(Adapted from Valenta & Kraft, 2001)*

CRC  


Latex allergen	IgE reactive glycan moiety	Allergen Status	CD4 <sup>+</sup> T-cell epitopes mapped	Hypoallergenic recombinant variants produced
Hev b 1		Minor	Yes	
Hev b 2	Yes	Minor/Major		
Hev b 3		Minor	Yes	
Hev b 4	Yes	Minor/Major		
Hev b 5		Major	Yes	Yes
Hev b 6.01		Major	Yes	Yes
Hev b 6.02		Major	Yes	Yes
Hev b 6.03		Minor	Yes	
Hev b 7		Minor		
Hev b 8		Minor		
Hev b 9		Minor		
Hev b 10		Minor		
Hev b 11		Minor		
Hev b 12		Minor		
Hev b 13	Yes	Minor		

# Summary

- T cell epitope based Hev b 5 and Hev b 6.02 peptide combination - potential latex immunotherapy
- Role of Hev b 2 and Hev b 13 needs clarification
- Hypoallergenic preparations for safe administration of higher doses with greater efficacy: down-regulation of Th2-polarised response and expansion of Tregs
- Need to identify most effective and safe method for vaccine administration (route, adjuvant, regimen)

# Allergen avoidance in Latex allergy

- Primary prevention works: “substitution of powdered latex gloves with low-protein powder-free NRL gloves greatly reduces NRL allergens, NRL sensitization, and NRL asthma in healthcare workers” Lamontagne et al, 2006
- Secondary Prevention – most latex allergic individuals can safely continue to work in low latex protein and powder free environment (Turjanmaa 2002)

# Prevalence and characterization of latex allergy in nursing staff at a major Australian hospital

- Questionnaires mailed to 1373 nurses.
- 920 returned (67%). 5 invalid.
- 230 nurses symptomatic (25%)
- 685 asymptomatic (75%)
- 100 from each group underwent skin testing and RAST

Drew et al, submitted

**Table 2 Immunological Testing**

Test	Percent Of Initial Questionnaire Grouped Subjects Positive On Testing (positive/tested)		Percent Of All Subjects Symptomatic & Testing Positive <sup>1</sup>	Percent Of All Subjects Non-Symptomatic & Testing Positive <sup>2</sup>	Percent Of All Subjects Testing Positive
	Symptomatic	Non-Symptomatic			
Stallergene Latex SPT	3.2 (3/93)	0 (0/99)	0.8	0	0.8
Latex Glove Extract SPT	0 (0/93)	1.0 (1/97)	0	0.8	0.8
Latex EAST	7.0 (7/100)	4.0 (4/100)	1.8	3.0	4.8
rHev b 5 ELISA	0 (0/100)	0 (0/100)	0	0	0
rHev b 6.01 ELISA	0 (0/100)	0 (0/100)	0	0	0

<sup>1</sup> Percent of Symptomatic Group Positive\*Number of Symptomatic Questionnaires/Total Questionnaires Returned Correct

<sup>2</sup> Percent of Non-Symptomatic Group Positive\*Number of Non-Symptomatic Questionnaires/Total Questionnaires Returned Correct

Drew et al, submitted

**Table 4** Hev b 6.02 Content Of Latex Gloves In Use At The Alfred Hospital

Glove Brand Name	Manufacturer	Country	Material	Powdered	Protein content (mg/g glove) <sup>1</sup>	Hev b 6.02 Content (ng/g glove)	
						mAb reactive (denatured) <sup>2</sup>	IgE reactive (native) <sup>3</sup>
Uniglove*	Uniglove	Malaysia	Latex	Yes	514	47568	180180
SafeSkin Satin Plus	Kimberly-Clark	Thailand	Latex	No	<47	873	13
Labtex Plus	Ansell Medical	Malaysia	Latex	No	<47	1462	554
<u>Gammex PF</u>	Ansell Medical	Malaysia	Latex	No	173	577	5
Conform	Ansell Medical	Malaysia	Latex	Yes	150	846	48
Gammex	Ansell Medical	Malaysia	Latex	Yes	705	679	4
<u>No Powder SensiClean</u>	Ansell Medical	Sri Lanka	Latex	No	290	875	13
DermaPrene Ultra	Ansell Medical	Malaysia	Neoprene	No	<47	<137	<1

\* high allergen latex glove no longer used at the Alfred Hospital

underline indicates gloves used to produce SPT reagents used in study

<sup>1</sup> limit of protein detection 47 ug/g glove. Assessed using Pierce BCA protein detection kit

<sup>2</sup> limit of mAb reactive Hev b 6.02 content 137 ng/g glove

<sup>3</sup> limit of IgE reactive Hev b 6.02 content 1 ng/g glove

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# Conclusions

- It appears rates of sensitization to latex are reduced compared with a decade earlier likely due to the introduction of high quality, powder free latex gloves.
- Levels of IgE-reactive Hev b 5 and 6 are either undetectable or extremely low in high quality medical grade gloves
- Molecular characterisation of latex allergens has enhanced diagnosis and offers hope of immunotherapy in latex allergy