APAC 3/5/01
SS #2

Laboratory of Immunobiochemistry

Research summary

LIB research program summary

- Allergen structure and function
  - Stability of lyophilized references
  - Glycosylation
  - Identification methods
- Immunomodulation
  - LPS
  - Epitopes
  - Cross-sensitization

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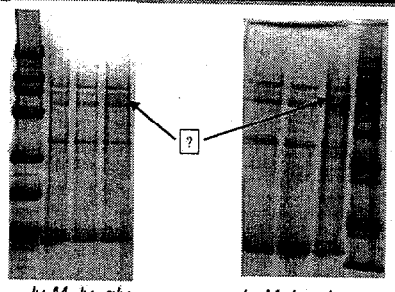
Stability of lyophilized references

		1	4	8	10	14
	D.pteronyssinus	0.70	1.08	1.12		
		0.71	1.42	0.98		
		0.92	0.94	0.91		
	D.farinae	0.82			0.90	
		0.85			0.89	
		1.05			0.92	
glycerin	Red top	0.85		1.01		
lyophilized		0.91		0.85		
lyo/mannitol		0.72		0.79		
	Meadow fescue	0.71		0.91		0.71
		0.75		0.92		0.71
		0.59		0.94		0.70
	Bermuda	0.92			0.80	
		0.81			0.71	
		1.07			0.72	

Cat pelt lyophilization - Fel d 1

	1	4	8	15
cat gly	12.81	13.75	13.18	12.03
cat lyo	11.22	14.20	13.46	12.01
cat man	11.44	13.88	14.01	10.56

SDS-PAGE of glycerinated and lyophilized Red top pollen extracts (1 and 8 mo)



ly-M ly gly ly-M ly gly

373551 02- FDA - (Jay Slater)

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stallographic Structure of e Venom Hyaluronidase

ockroach studies

- Establishment of a US standard
- Identification of significant allergens
  - current data are seroprevalence only
- Comparison of native and recombinant products

e of the IgE-specific Fab library

- Affinity purification of native allergens
- Identification of important allergens
- Identification of important epitopes
- Comparison of immunogenicity of native and recombinant products

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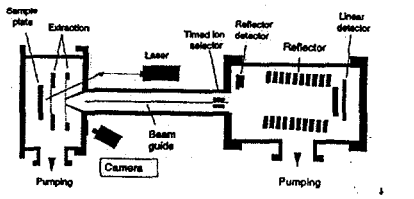
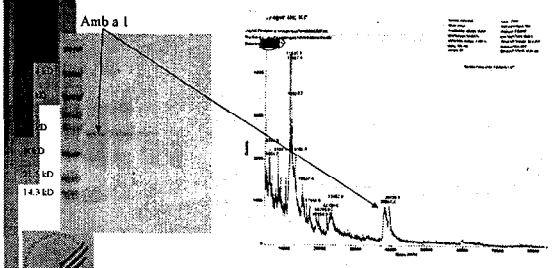
trix-assisted laser desorption/ionization e-of-flight mass spectrometry (MALDI-TOF)

Schematic of Voyager-DE PRO/STR



gweed extracts



ALDI-TOF for allergen aracterization

Can we develop a reproducible profile of natural allergen preparations?

- venoms - yes
- ragweed - yes
- cat - poor detection of Fel d 1 - may need further purifications prior to MALDI-MS analysis

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S and allergen responses

LPS is ubiquitous

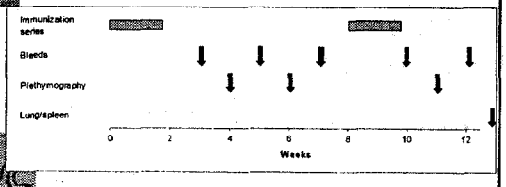
LPS elicits broad immunologic effects

- In mice - strong B cell mitogen
- In humans and mice -
  - adjuvant
  - Th1 and Th2 responses

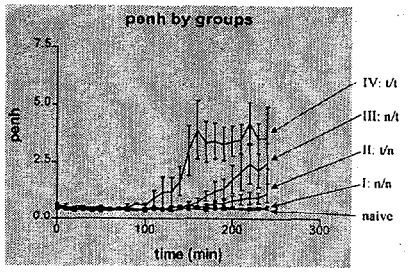
adjuvancy is not a consequence of B-cell mitogenesis (does not occur in athymic mice)

munomodulation: lipopolysaccharides

Group	1	2	3	4
antigen	nasal	tracheal	nasal	tracheal
LPS	nasal	nasal	tracheal	tracheal



albumin induces greatest penh responses in e receiving ovalbumin and LPS IT



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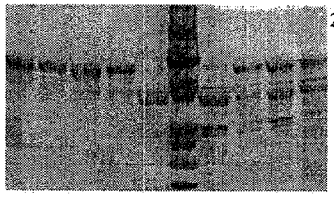
e-directed mutagenesis of Hev b 5

..CCA AAG AAT GAG ACA CCT GAG GTG ACC
.. P K_{15} N E T P E V T
CCA
P

AAG GCT GAG GAG ACG AAG ACC GAA GAA...
K_{23} A E E T K_{28} T E E ...
CCA CCA
P P

pression of Hev b 5 (P15, P23 d P28)



220000
97400
66000
46000
30000
21500
14300

W P28 P23 P15 MBP W P28 P23 P15

udies to be done with mutants

- Splice into His-tag expression vector; perform studies using both types of expressed protein
- Antibody binding studies
  - human antisera
  - mouse monoclonal antibodies
  - rabbit hyperimmune sera
- cell stimulation studies

B research program (I)

- Stability of lyophilized extracts
- Glycosylation
- US standard reference maintenance
- Regulation of recombinant allergens

B research program (II)

- Identification methods
- Epitopes
- LPS
- Lot release for current and future products
- Immunotherapy options
- Adjuvant safety and efficacy

stracts (February 2001)

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