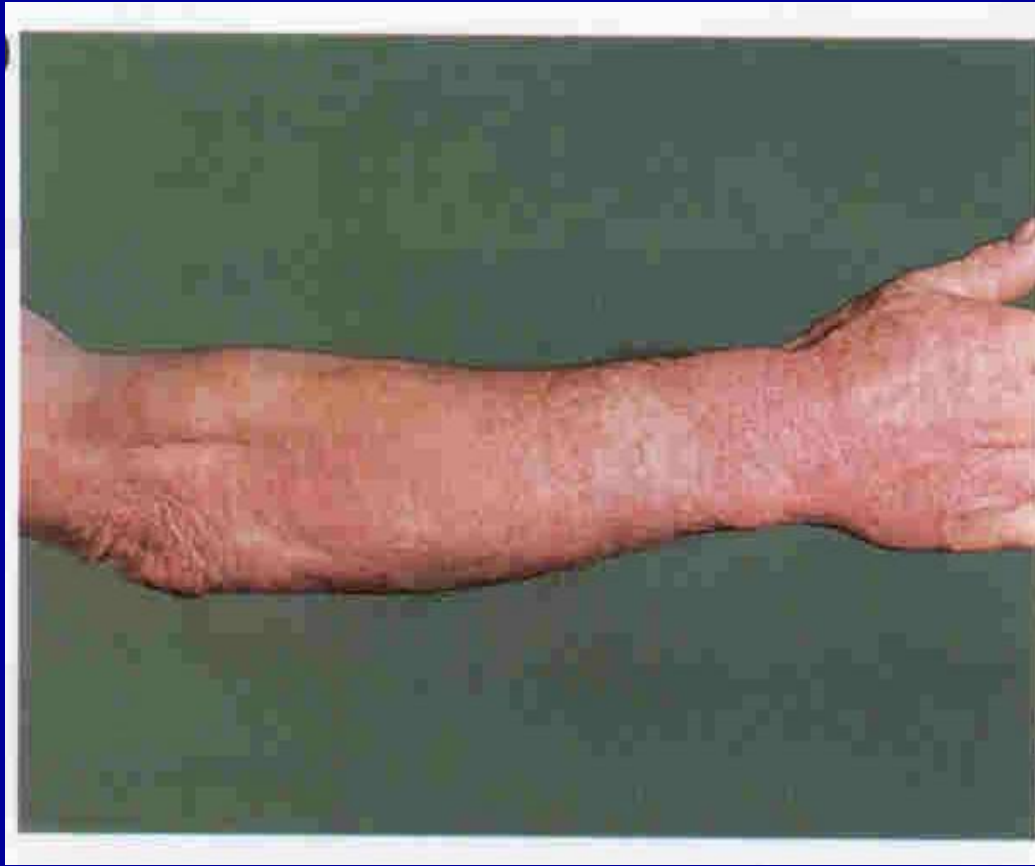
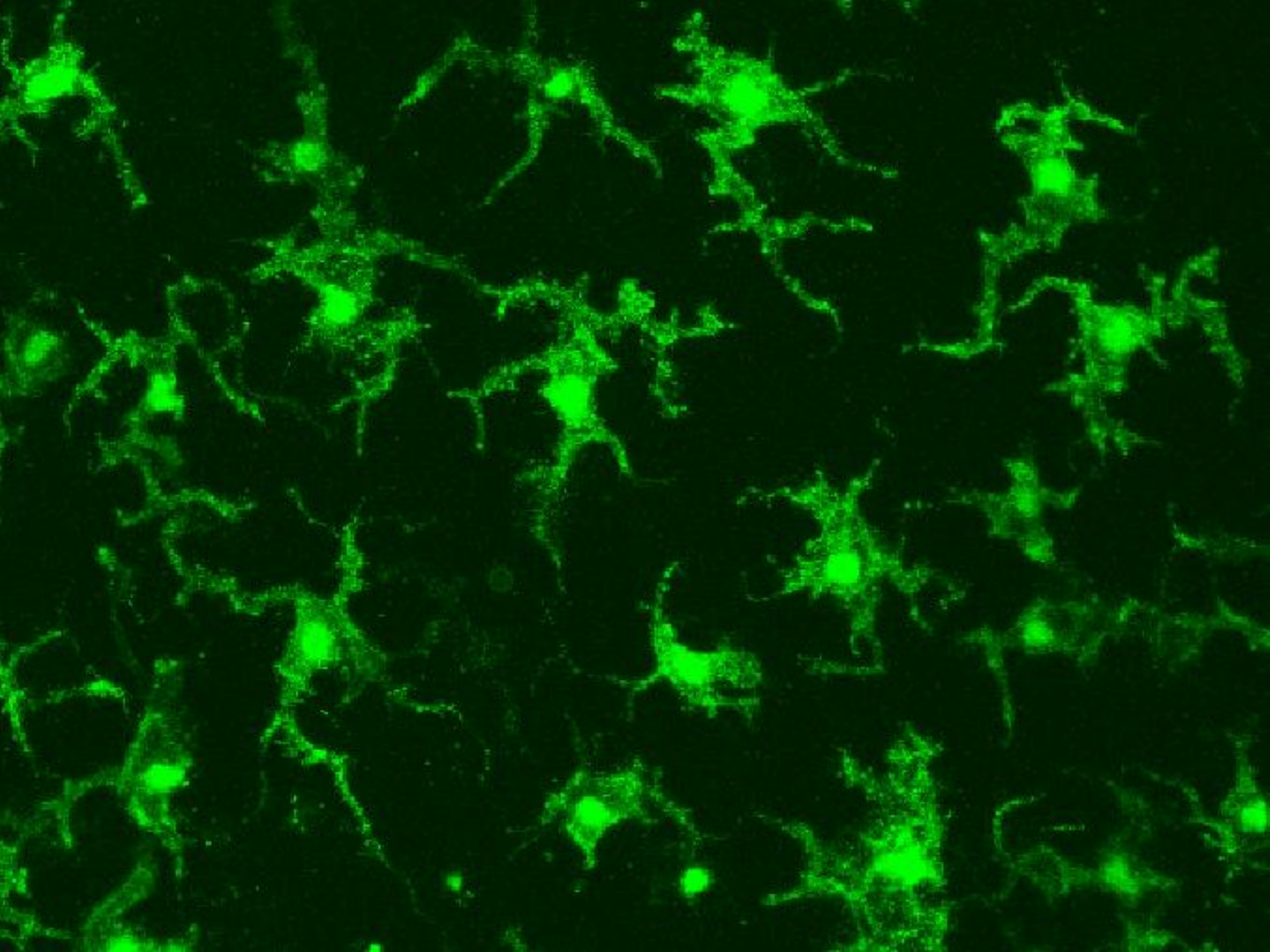


Introduction to Skin Sensitisation, the Local Lymph Node Assay and Alternative Strategies

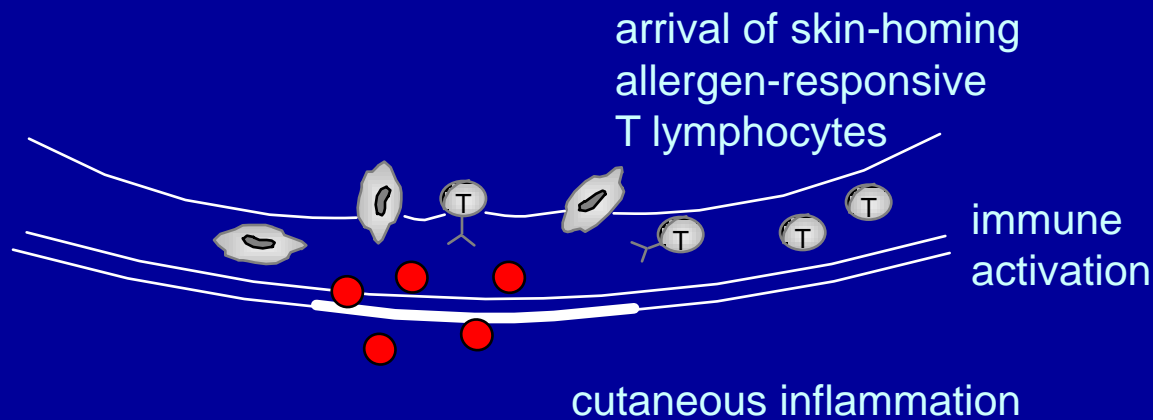
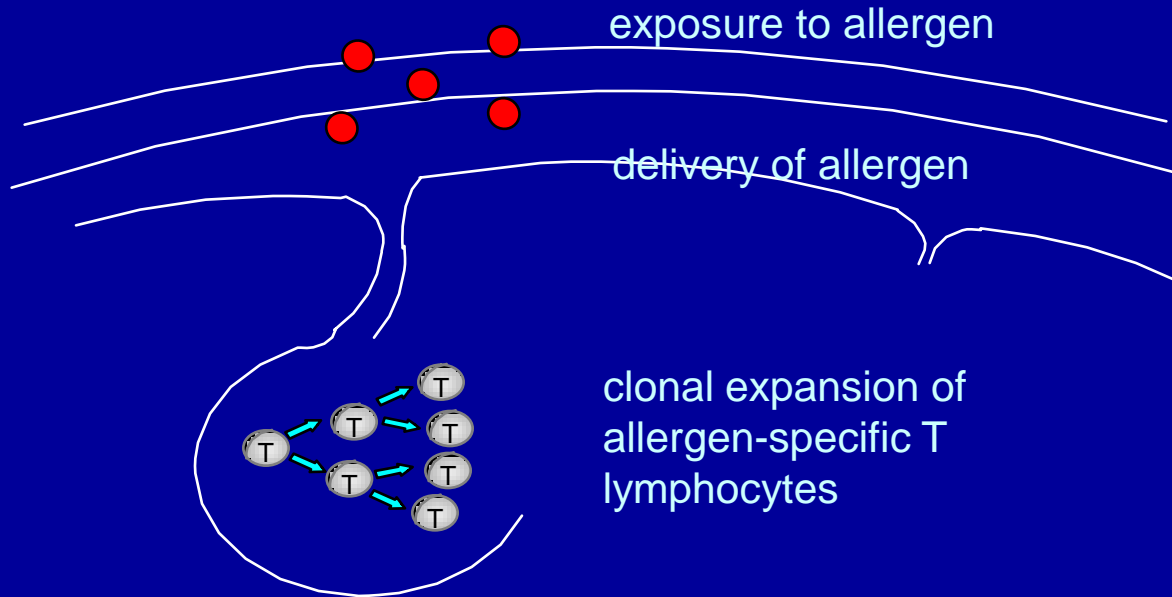
Ian Kimber
University of Manchester, UK



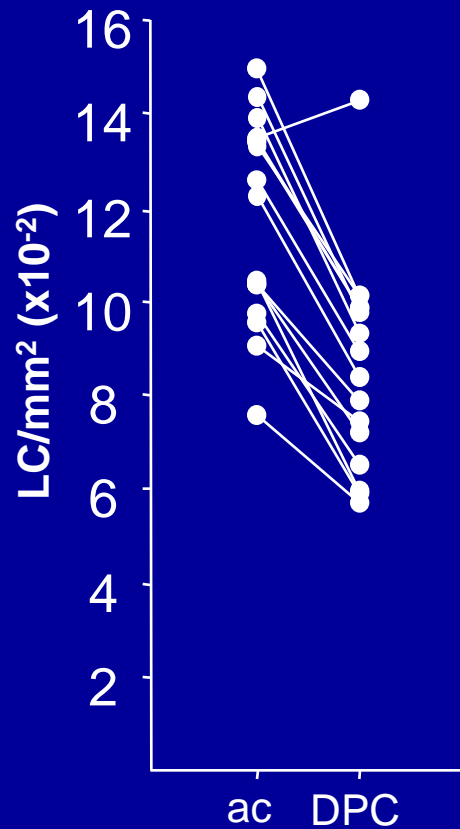




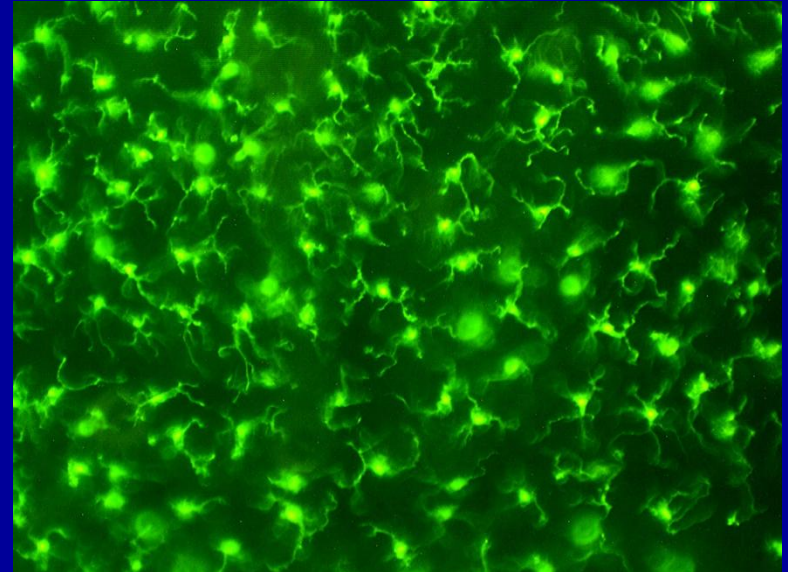
Contact allergy



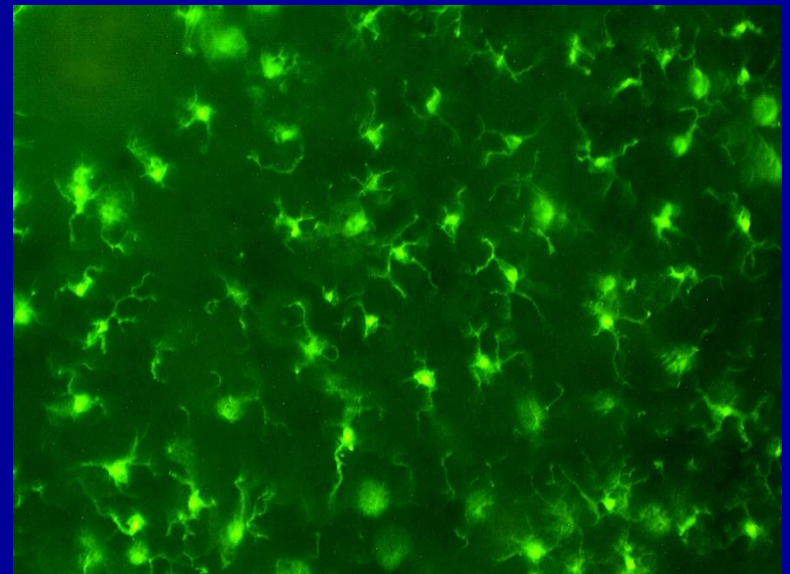
ALLERGEN-INDUCED LC MIGRATION IN MAN



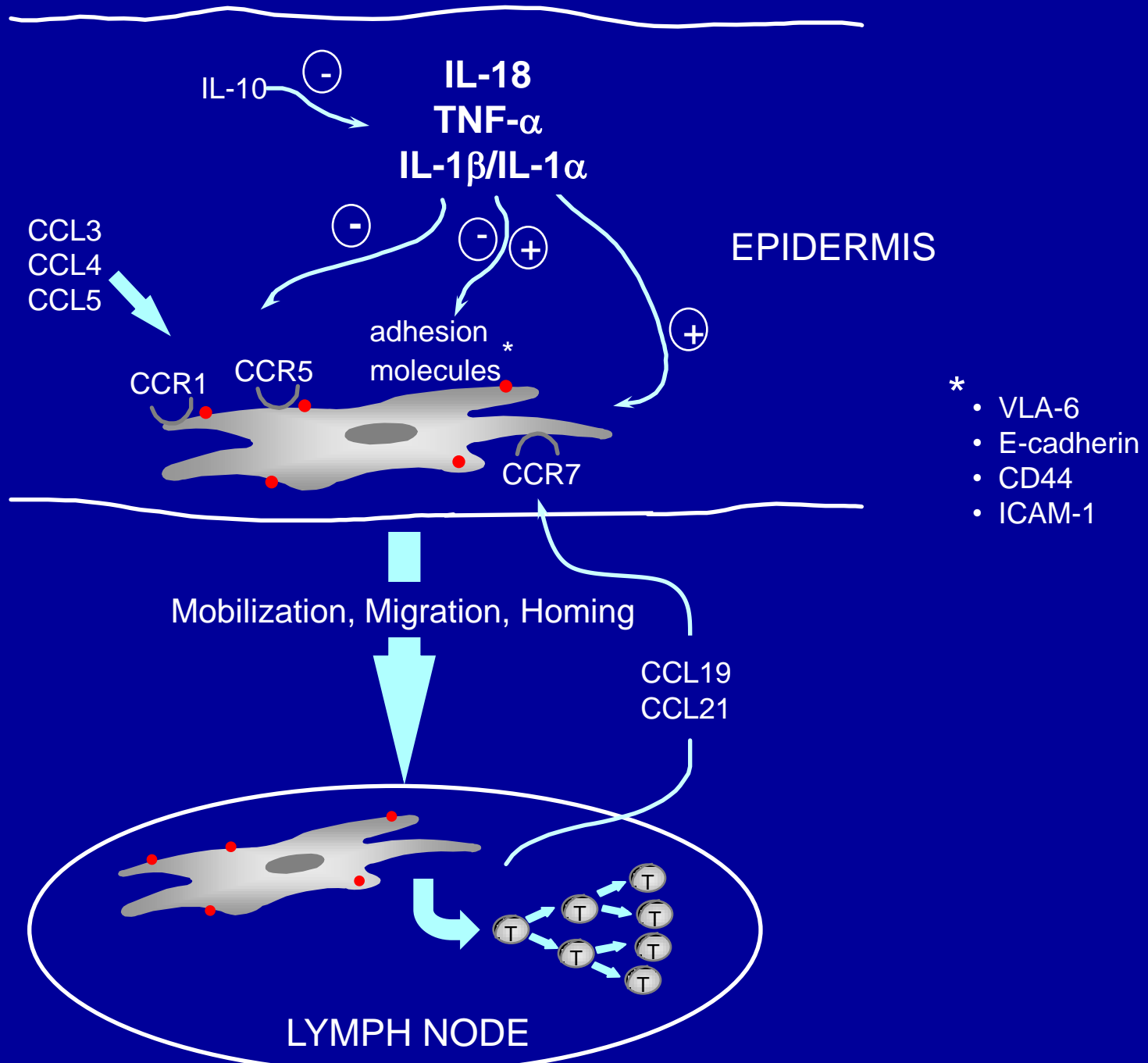
acetone



DPC

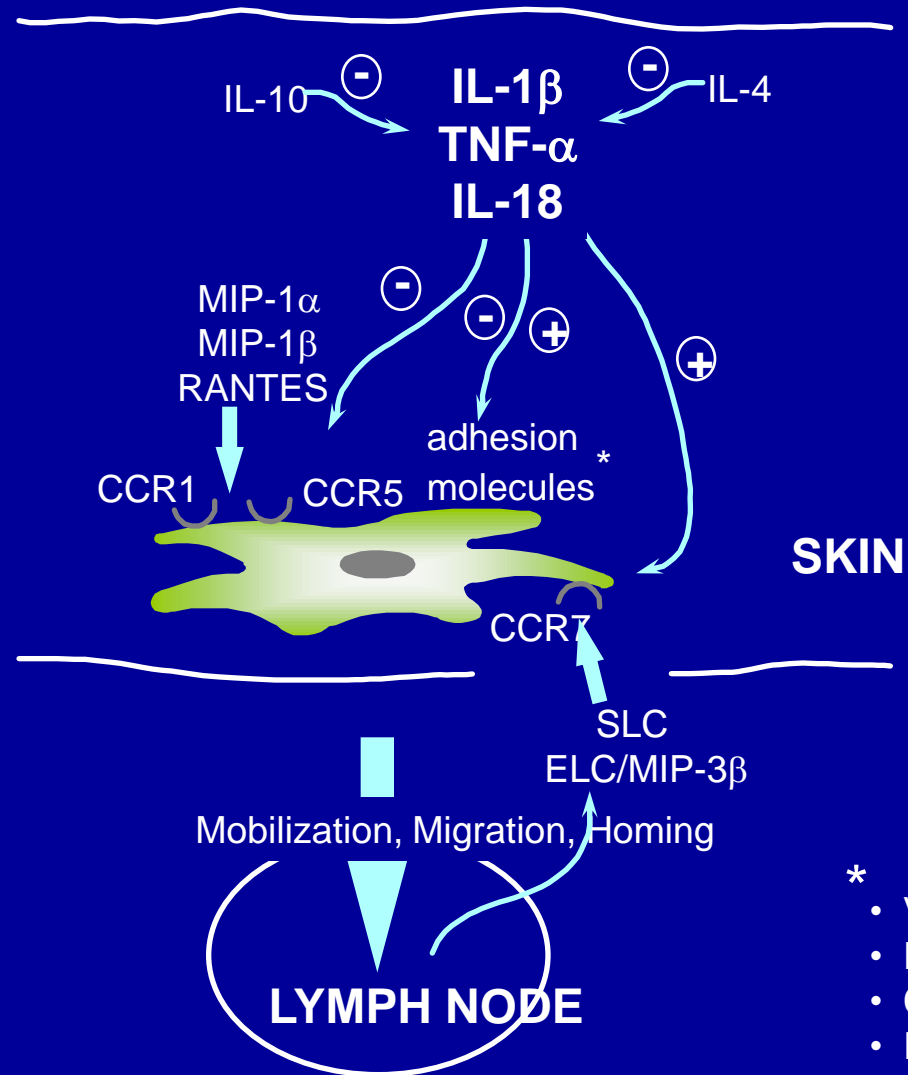
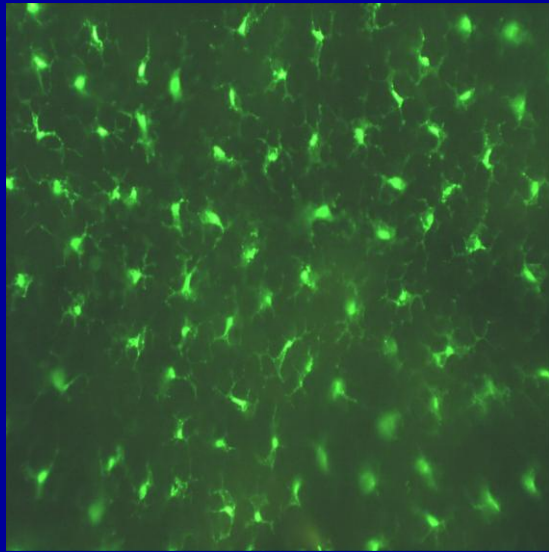


Reduction: 27.3%^{**}

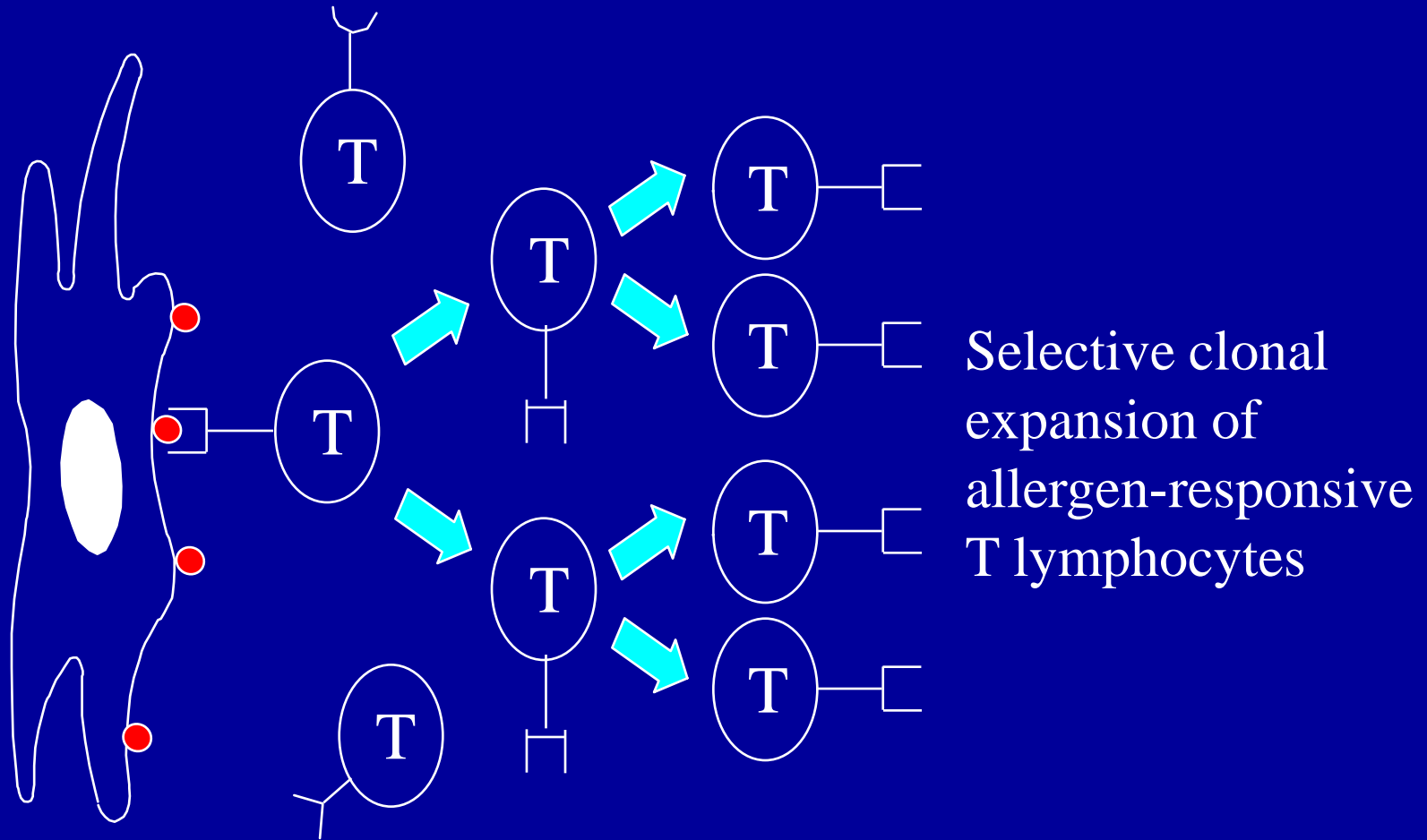


Toxicological Approaches to Skin Sensitisation

Skin sensitization : dependent upon multiple cellular molecular interactions



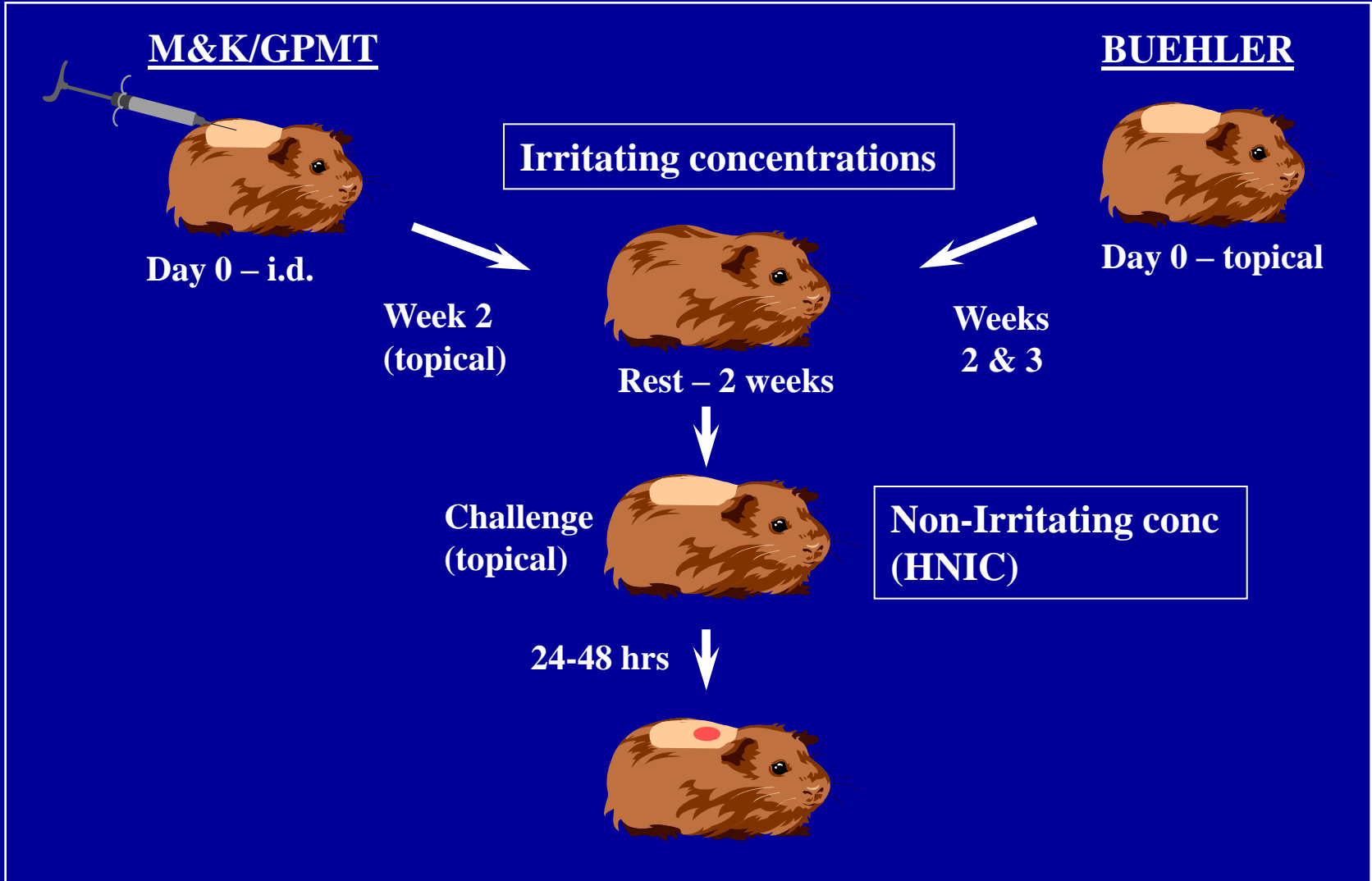
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Hurdles to Acquisition of Skin Sensitization

- Epidermal bioavailability
 - Local trauma – proinflammatory cytokine production
 - Protein reactivity
 - Immunogenicity

Guinea pig sensitization assays



Guinea pig scoring

Score	Skin response
0	No visible change
0.5 (SPE)	Very faint, non-confluent
1	Dispersed or blotchy erythema
2	Moderately diffused erythema
3	Marked erythema and oedema

Guinea pig sensitisation categories

Sensitization rate (%)	Grade	Classification
0	-	Non-sensitiser
1~8	I	Weak
9~28	II	Mild
29~64	III	Moderate
65~80	IV	Strong
81~100	V	Extreme

R43 Classification

M&K
≥30%

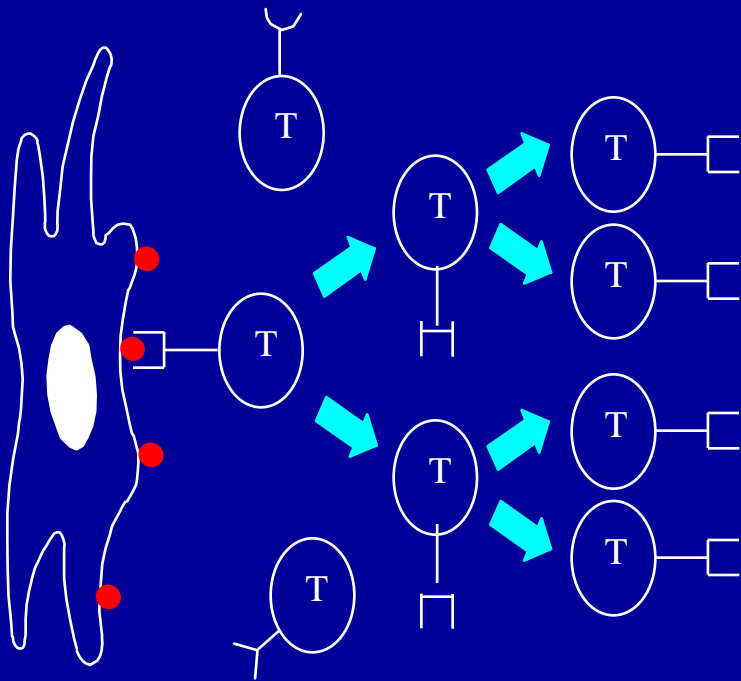
Buehler
≥15%

Guinea pig interpretation

- Elicitation model; valuable test; but.....
- Animal welfare
 - elicitation reactions;
 - GPMT uses injection, with Freund's adjuvant;
- Subjective scoring (irritation, colored substances);
- Not dose responsive.

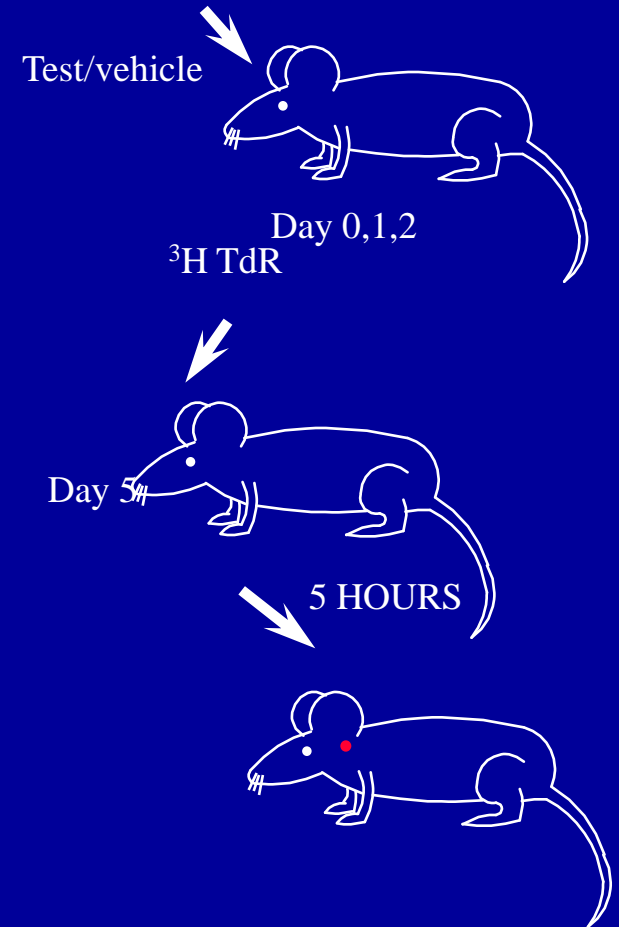
The mouse local lymph node assay (LLNA)

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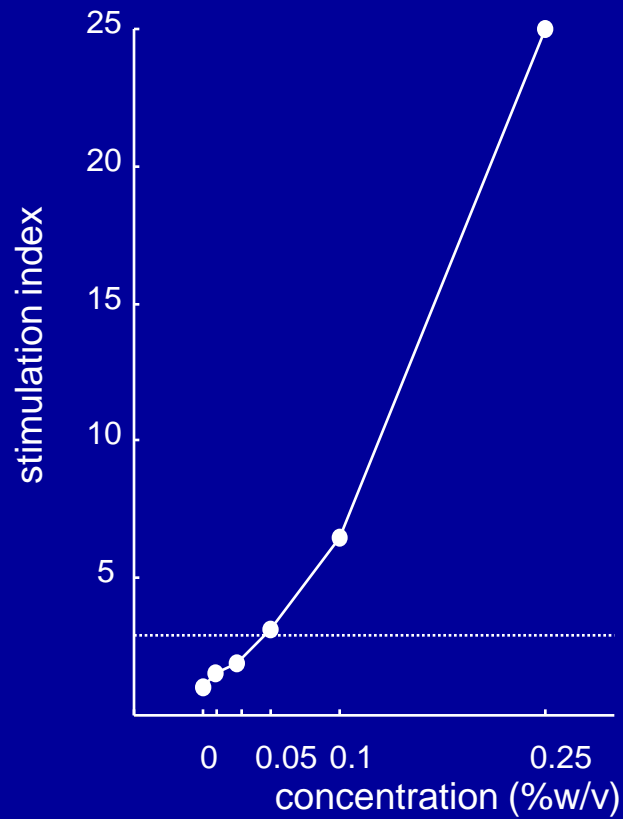
Selective clonal expansion of allergen-responsive T lymphocytes

LOCAL LYMPH NODE ASSAY

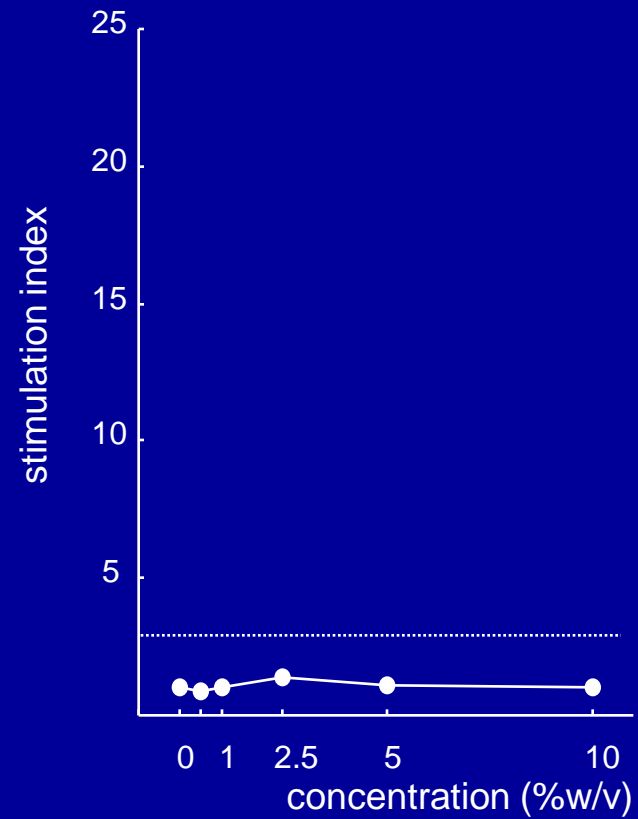


LLNA : Illustrative Results

DNCB



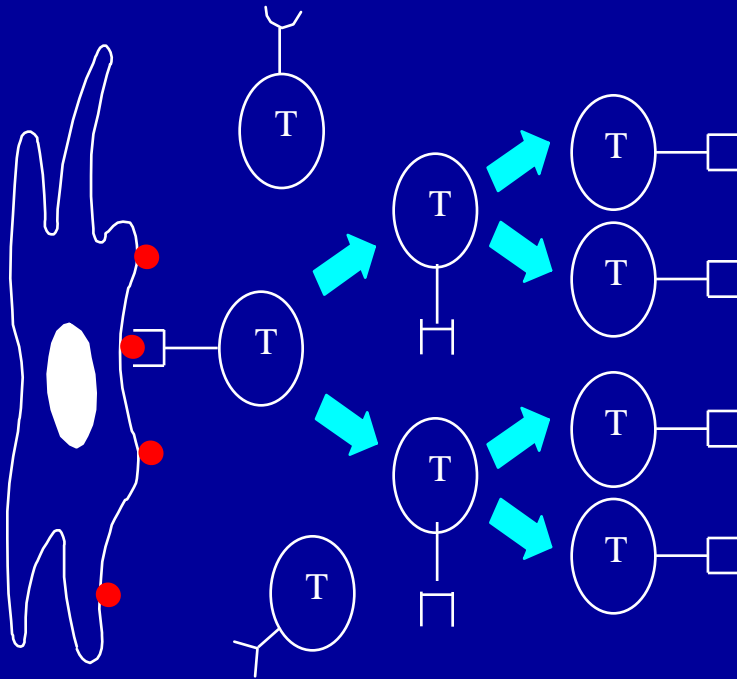
PABA



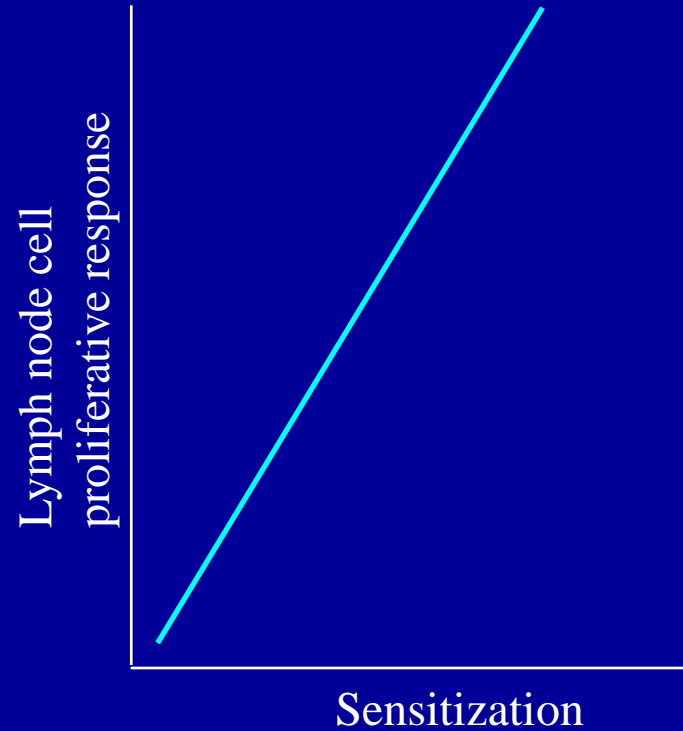
Hazard Characterization

Skin Sensitization Potency

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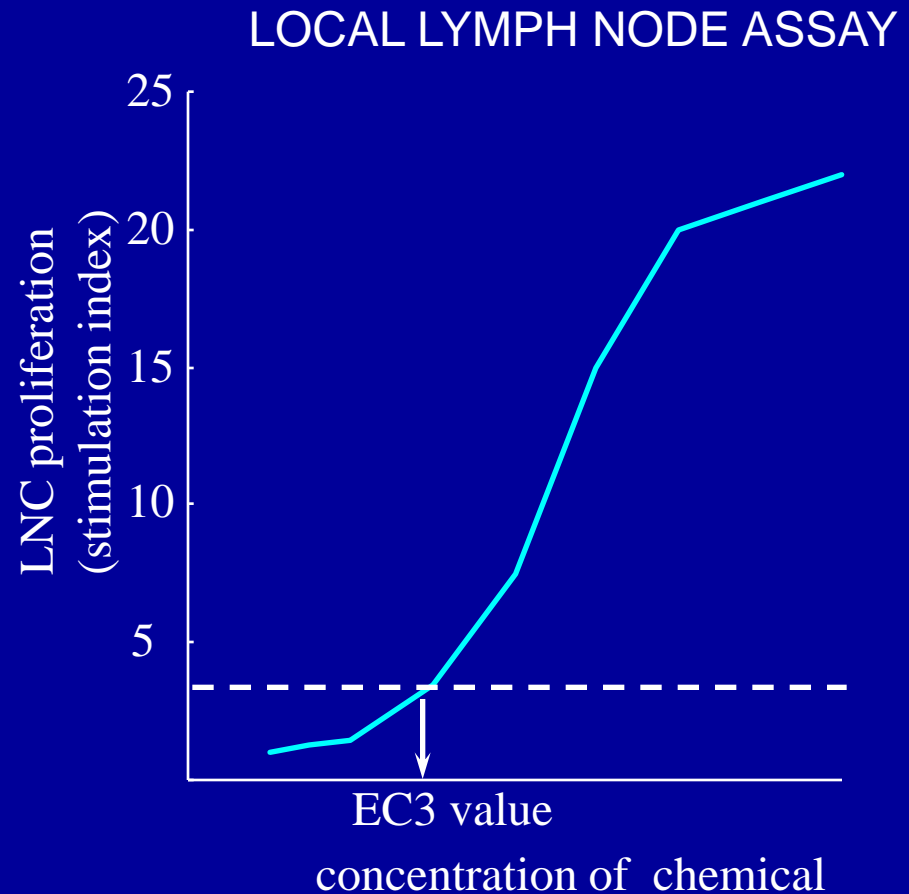
Selective clonal expansion of allergen-responsive T lymphocytes



The LLNA EC3 Model

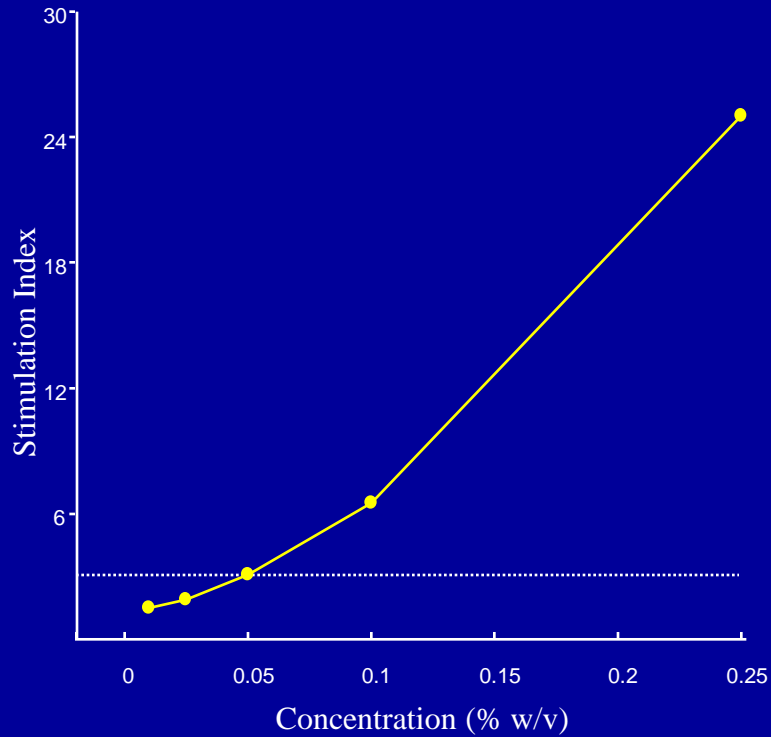
EC3 =

Application concentration of test chemical required to provoke a 3-fold increase in LNC proliferative activity compared with concurrent vehicle treated controls.



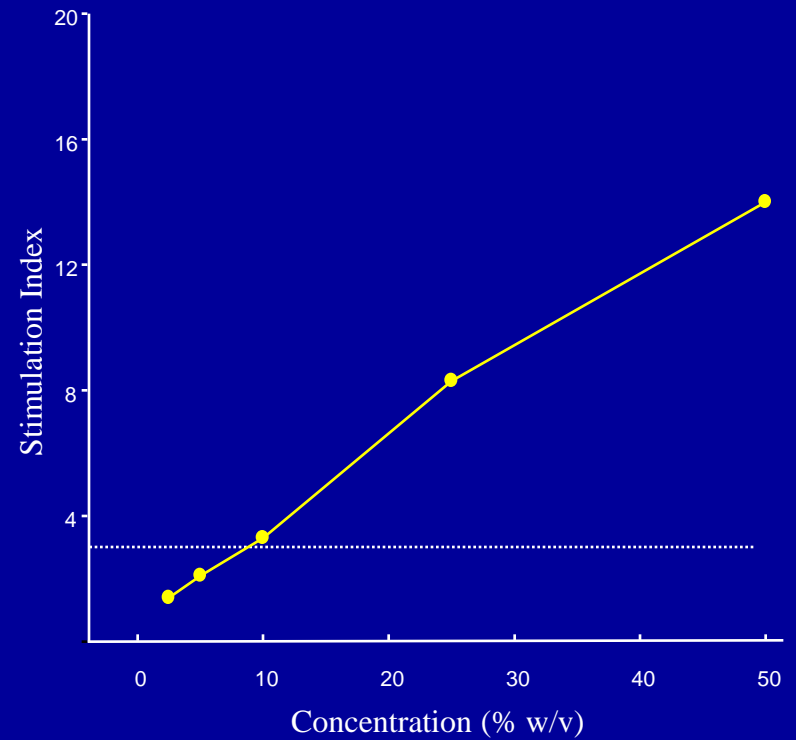
RELATIVE SKIN SENSITIZATION POTENCY

DNCB



EC3= 0.05%

HCA



EC3= 7.9%

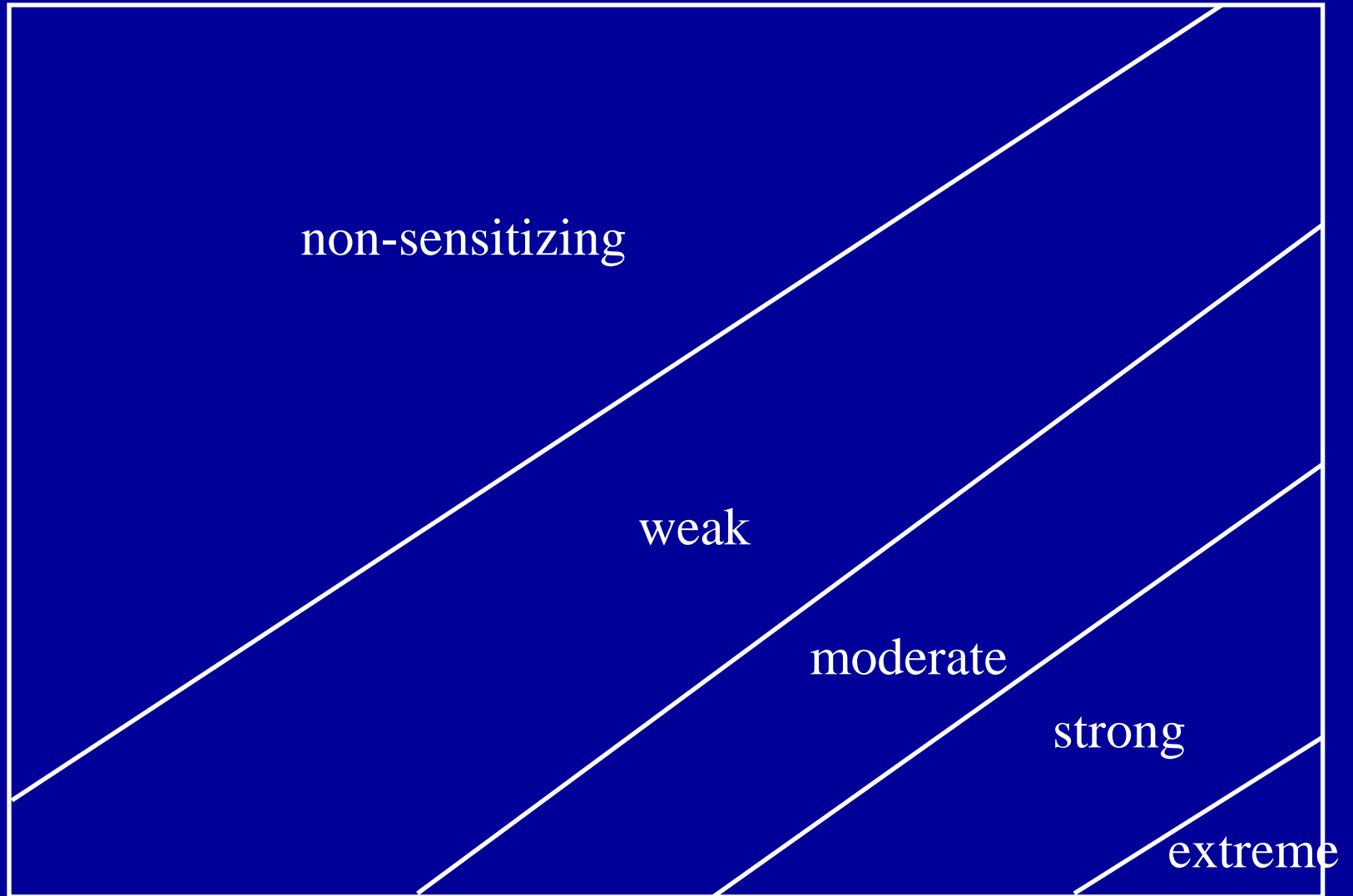
LLNA and relative contact allergenic potency

chemical	human classification	LLNA EC3 value
CMIT	strong	0.005%
DNCB	strong	0.08%
DPC	strong	0.05%
PPD	strong	0.06%
cinnamic aldehyde	moderate	2.0%
glutaraldehyde	moderate	0.2%
isoeugenol	moderate	1.3%
tetramethylthiuram disulfide	moderate	6.0%
citral	weak	13%
eugenol	weak	13%
HCA	weak	8.0%
hydroxycitronellal	weak	20%
ethyleneglycol dimethylacrylate	extremely weak	35%
isopropyl myristate	extremely weak	44%
propyl paraben	extremely weak	>50%
propylene glycol	extremely weak	>100%
glycerol	non-sensitizing	>100%
hexane	non-sensitizing	>100%
diethyl phthlate	non-sensitizing	>100%
tween 80	non-sensitizing	>100%

Proposals for classification of contact allergens according to potency

LLNA Category	EC3
Extreme	<0.1%
Strong	>0.1 to <1%
Moderate	>1 to <10%
Weak	>10 to < 100%
Non Sensitizers	>100%

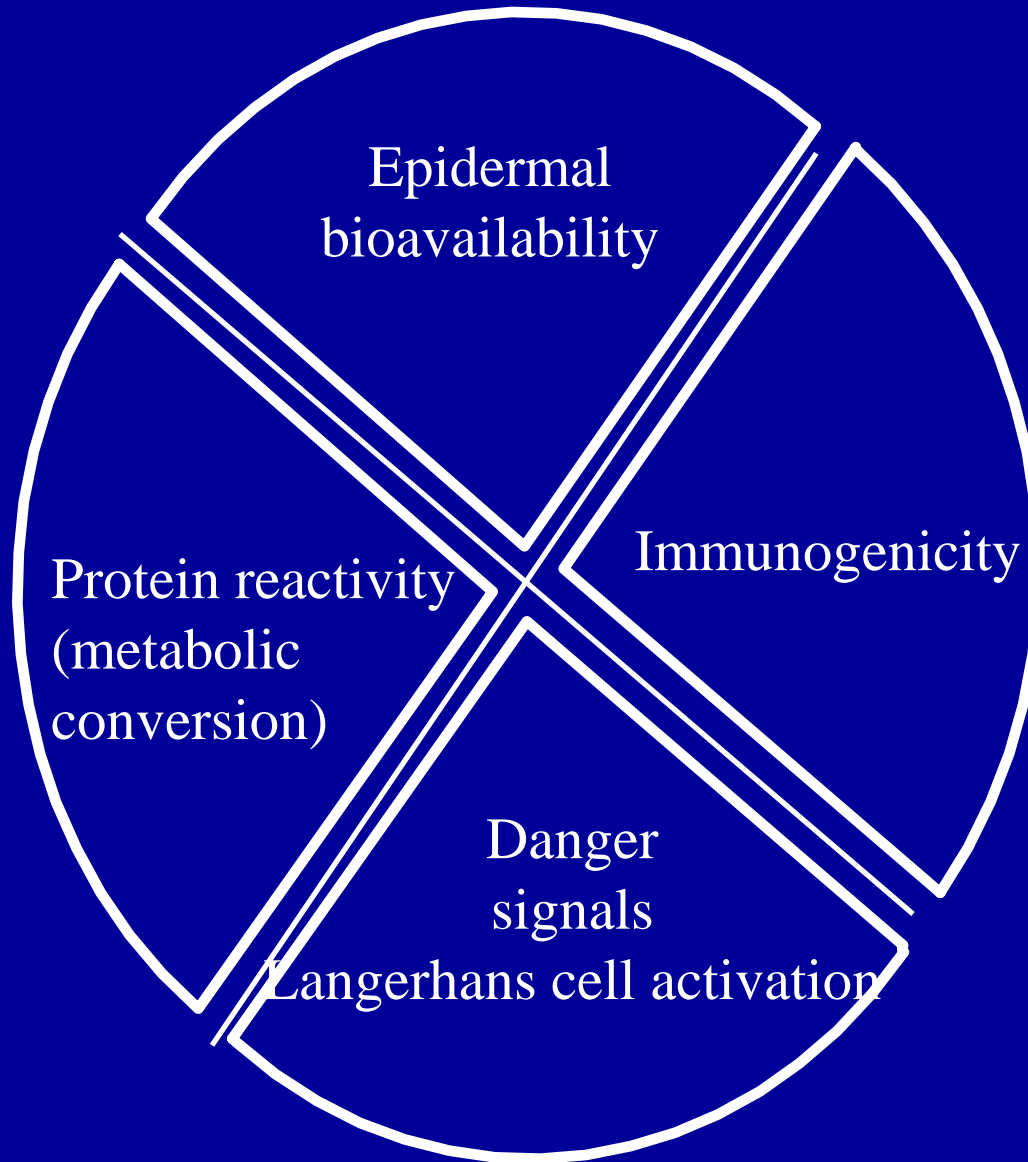
Categorization of Skin Sensitizing Chemicals



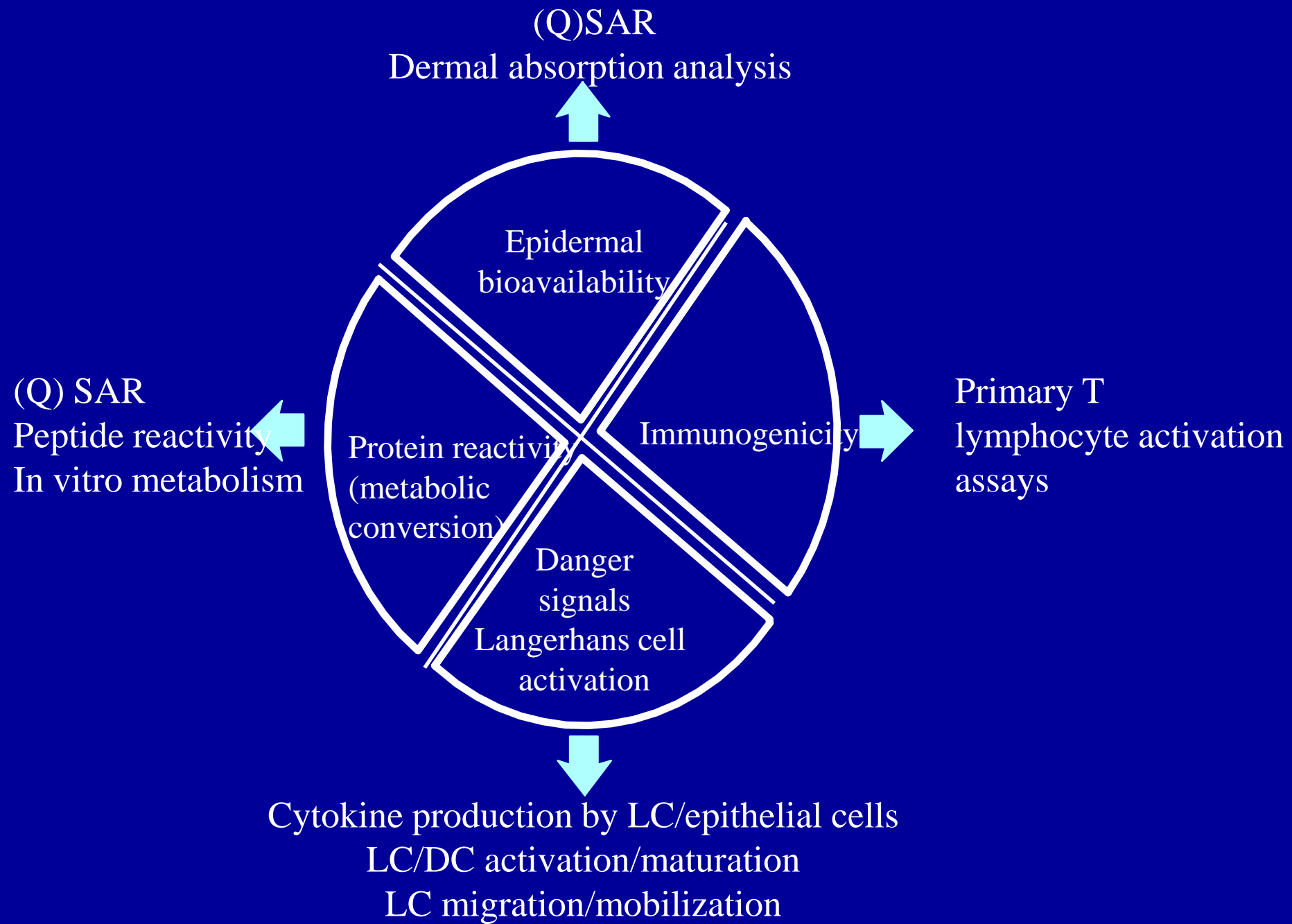
Alternative Approaches to Skin Sensitization Testing

The Challenges

Hazard Identification



Hazard Identification



Hazard Identification : The Issues

- Sensitivity/selectivity/accuracy
- Metabolic activation/inactivation
- Mechanistic relevance/appreciation
- Chemical delivery to in vitro assays
- Integration to provide an holistic picture

Questions

- mechanistic relevance of chemical-induced changes in DC phenotype?
- Does T lymphocyte activation in vitro equate with sensitizing activity?
- Does native or inducible protein reactivity necessarily reflect skin sensitization potential?
- Does absence of epidermal bioavailability necessarily equate with absence of skin sensitizing activity?

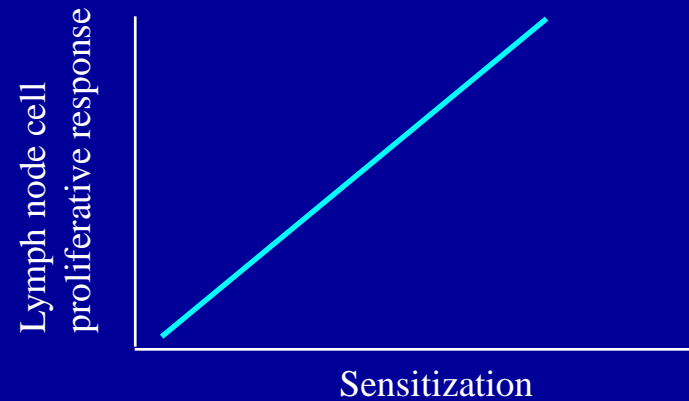
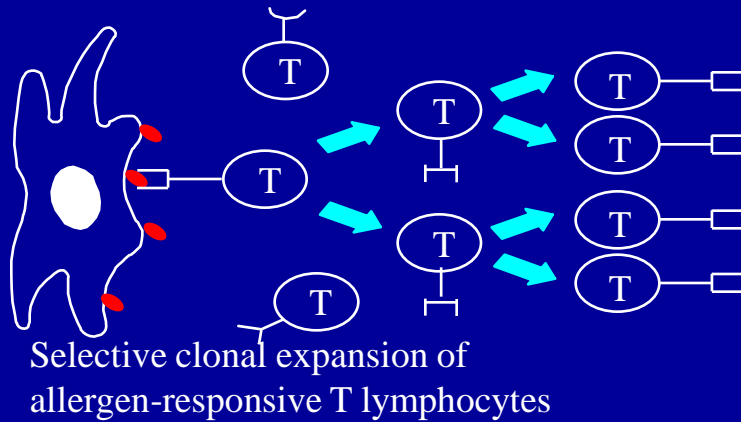
Hazard Characterization

The importance of potency in QRA

Contact allergens differ by up to 5 orders of magnitude with respect to their relative skin sensitization potency

Assessment of Relative Skin Sensitization Potency in an Integrated System

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RELATIVE SKIN SENSITIZATION POTENCY

