

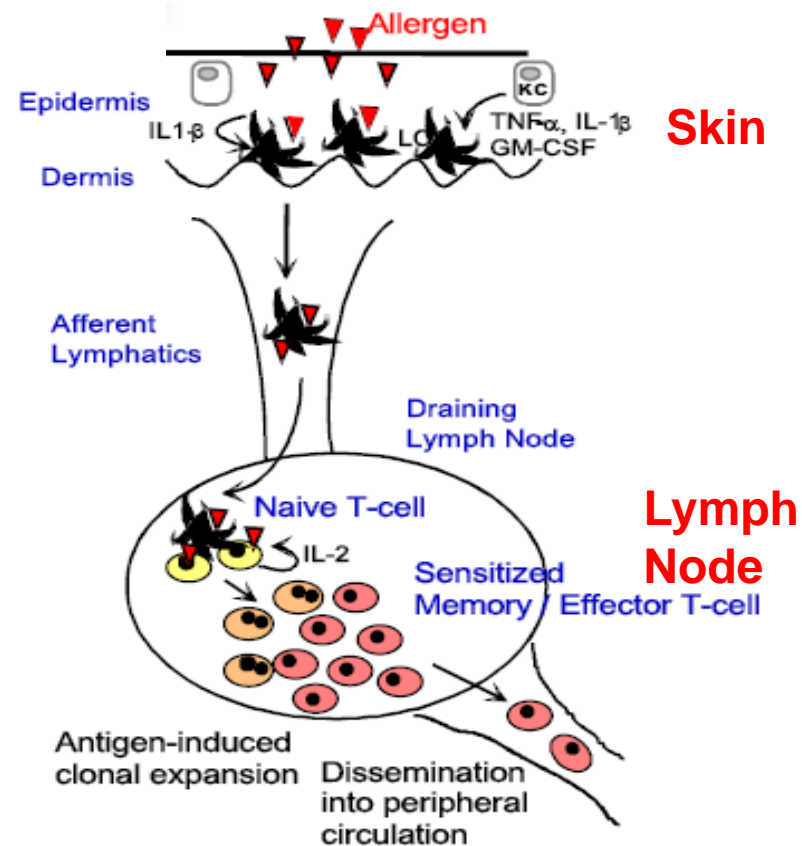


Conducting and Interpreting the Murine Local Lymph Node Assay (LLNA)

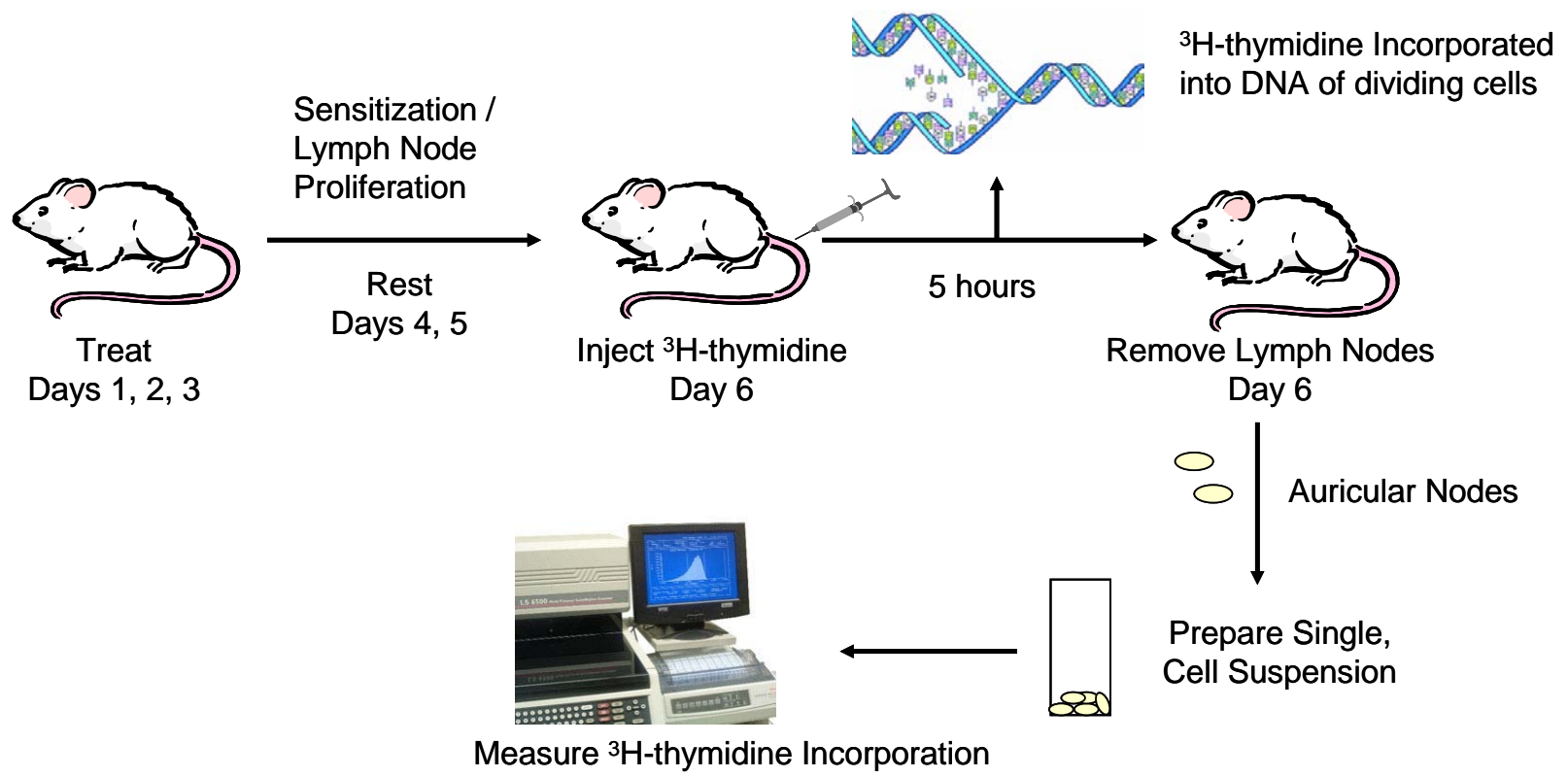
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Mechanism for Contact Sensitization

- Hapten (e.g., LMW chemical) penetrates the epidermis and forms a complex with a protein carrier.
- The hapten-carrier complex is processed by Langerhans' / Dendritic cells (LDC).
- LDC migrate to “draining” local lymph nodes and become antigen presenting cell (APC).
- **APC interacts with T cells leading to proliferation** and the generation of memory T cells.



LLNA Overview



Critical aspects of LLNA conduct

- Choice of vehicle
- Dose selection
- Test material application
- IV injection
- Lymph node harvest and processing
- Data analysis and interpretation



Choice of vehicle

- acetone:olive oil, DMSO-dimethyl sulfoxide,
- DMF-dimethylformamide, MEK-methyl ethyl ketone, PG-propylene glycol
- Pluronic L92 block copolymer surfactant
 - 1% in water – hydrophilic, aqueous-based product
- test substance, mixture/formulation
- solubility
- physical form
- reactivity

Dose selection

- pre-screen test to provide guidance for selecting the maximum dose level based on systemic toxicity and/or excessive local skin irritation.
- at least three (3) concentrations
 - 1 or 2 mice/group
- dermal, systemic toxicity
 - body weight loss
 - clinical observations (e.g., lethargic)



Dose selection

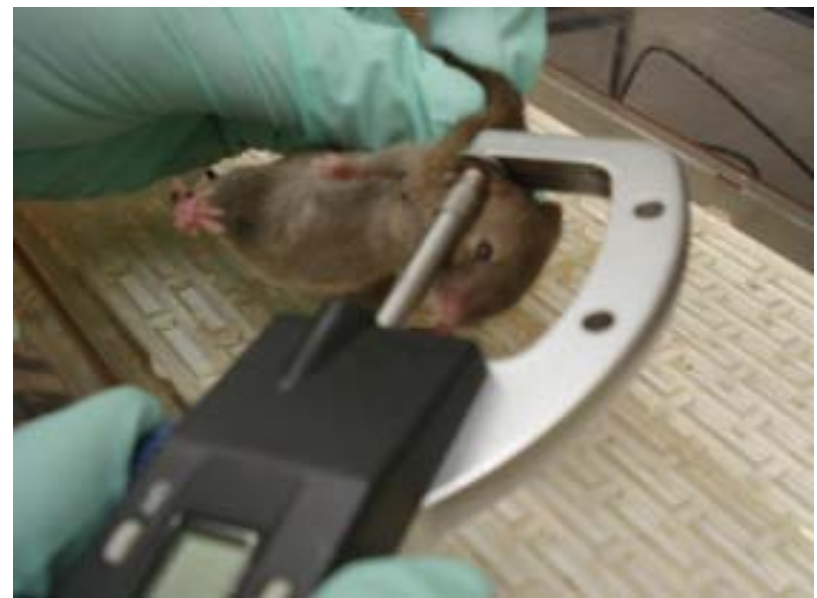
- irritation assessment
 - erythema ≥ 3

No visual effect	0
Slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Eschar	4

- remember characteristics of test substance

Dose selection

- irritation assessment
 - ear measurements (guideline $> 25\%$)
(e.g. digital micrometer or Peacock Dial thickness gauge)

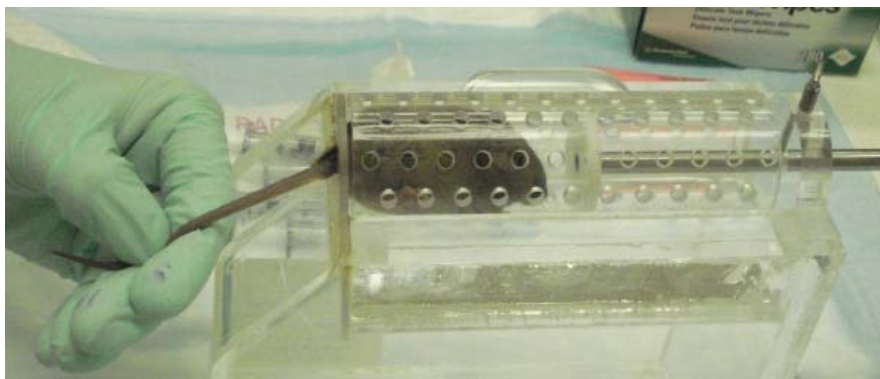


Test material application

- timing of dose preparation (reactivity)
- solubility, suspension
- dorsal surface, prevent roll-off
 - vehicle selection, aqueous-based

Tail vein i.v. injections

- warm mice
 - shoebox cages, warm gauze, incubators
- wire rack, Plexiglas tube
- 25 gauge needles
 - single use
- distal injections



Lymph node harvest and processing

- bifurcation of jugular vein
- blood contamination
- tissue pellet





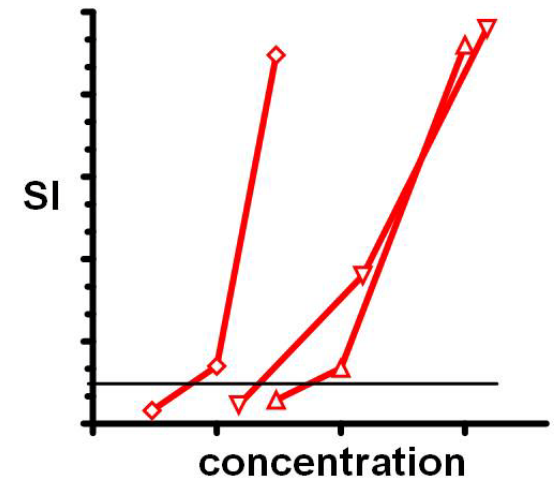
Data analysis

- Stimulation Index (SI):
Result individual mouse ÷ Average of VH controls

<u>Treatment</u>	<u>Animal</u>	<u>DPM</u>	<u>SI</u>	
VH (PG)	1	297.5	1.2	
	2	129.6	0.5	
	3	367.7	1.5	
	4	215.6	0.9	
	MEAN	252.6	1.0	
	S.D.	102.9	0.4	
0.2%	5	516.5	2.0	
	6	885.0	3.5	
	7#	3424.2	13.6	# statistical outlier included
	8	714.6	2.8	
	MEAN	1385.1	5.5	
	S.D.	1602.2	6.3	
1%	9	5367.6	21.2	
	10	4437.5	17.6	
	11	6554.0	25.9	
	12	7248.1	28.7	
	MEAN*	5901.8	23.4	
	S.D.	6035.4	23.9	
5%	13	14535	57.5	
	14	16474	65.2	
	15	19309	76.4	
	16	6922	27.4	
	MEAN*	14309.9	56.6	
	S.D.	14253.7	56.4	

Data analysis

- **Positive cut-offs**
 - ^3H -thymidine: dpm ≥ 3 fold
 - ATP bioluminescence ≥ 1.8 -2.5 fold
 - BrdU ELISA ≥ 1.6 -1.9 fold
- If positive, an effective concentration (EC) can be calculated by interpolation between two test concentrations eliciting SI values above and below cut-off.
 - Potency



Calculations - EC

$$EC_p = XL + [(P-YL)/(Yh-YL)](Xh-XL)$$

- Where, P = positive SI cut-off

YL = SI value below P 1.7

XL = chemical concentration that elicits YL 5%

Yh = SI value above P 6.5

Xh = chemical concentration that elicits Yh 10%

EC3 Calculation:

$$EC3 = 5\% + [(3-1.7)/(6.5-1.7)](10\% - 5\%)$$

$$EC3 = 6.4\% \text{ (moderate)}$$

Statistical analyses, and interpretation of data

- outliers (statistical, biological, historical)
 - vehicle controls
- strength of dose-response
 - mid-dose effect
- statistical significance
- borderline results, weight of evidence

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Questions?