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

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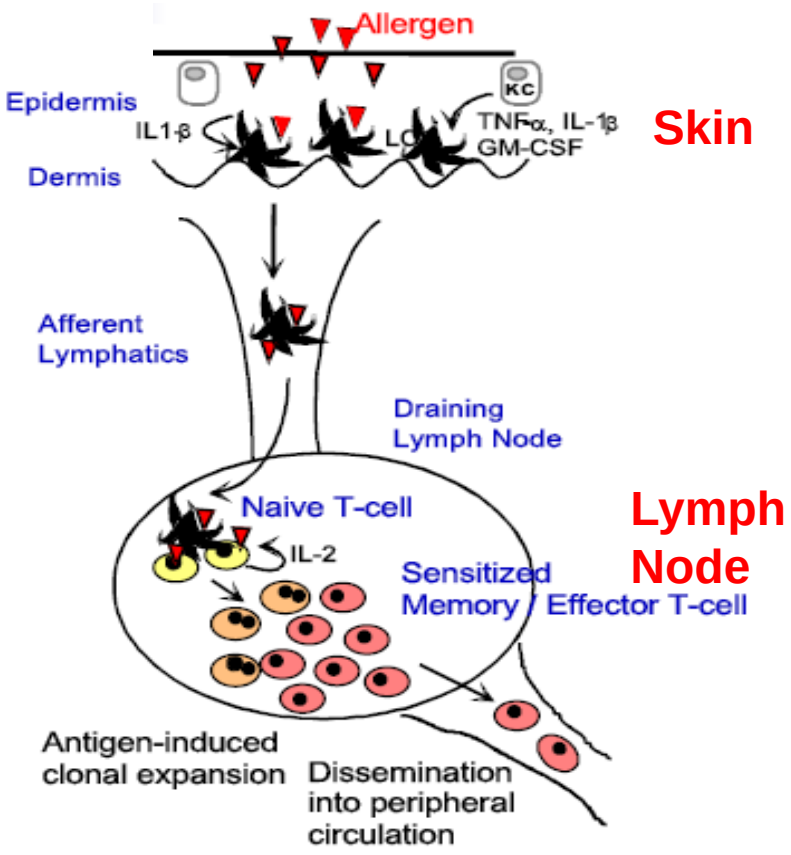
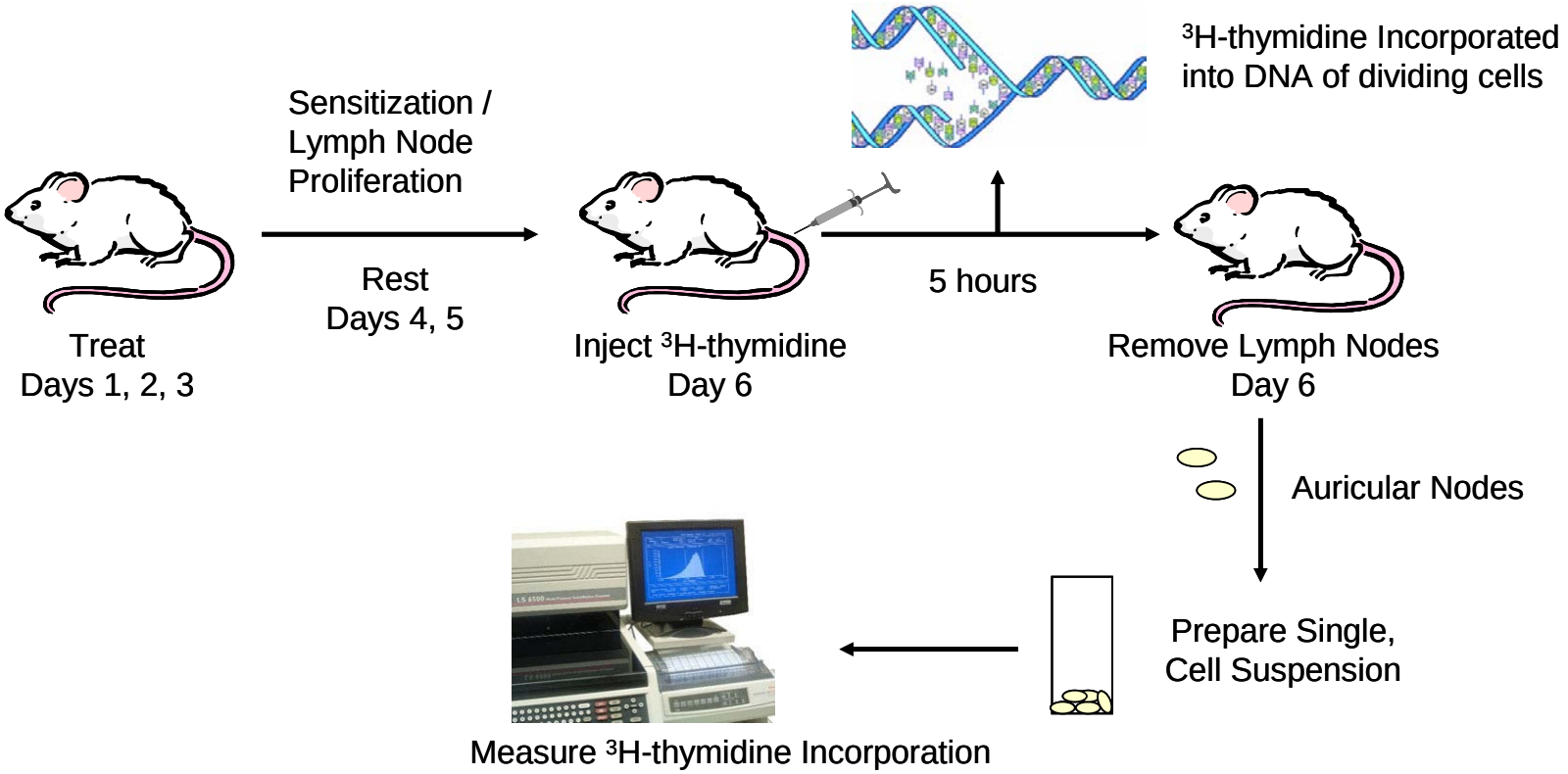
Mechanism for Contact Sensitization

- 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

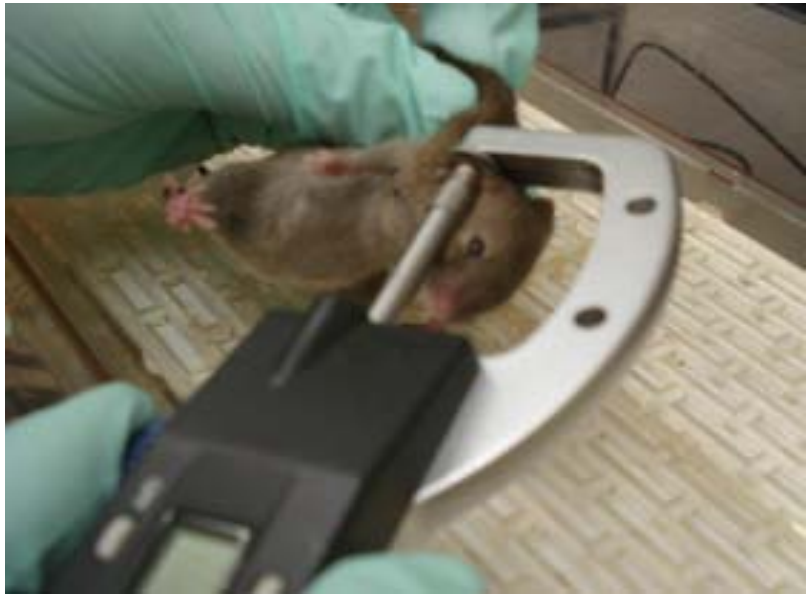
- irritation assessment
  - –erythema ≥ 3

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)

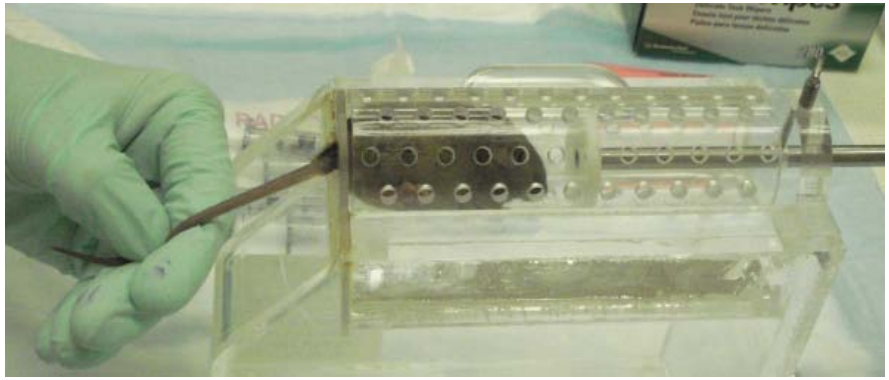


Test material application

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

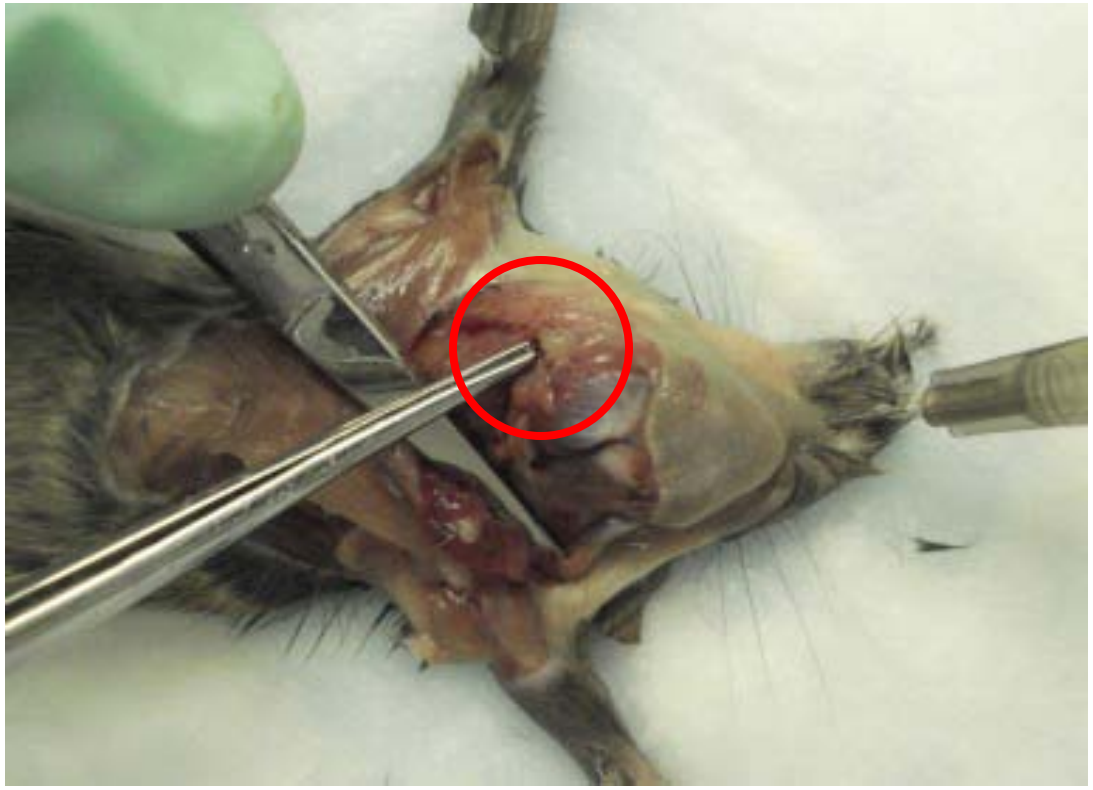
Tail vein i.v. injections

- 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

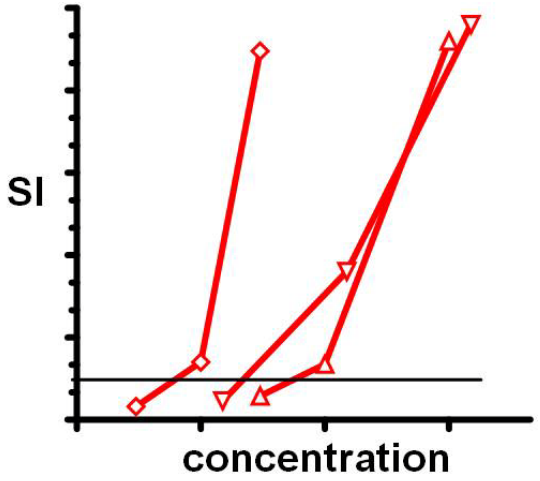
- Stimulation Index (SI): Result individual mouse ÷ Average of VH controls

Treatment	Animal	DPM	SI
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
	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**

 # statistical outlier included

Data analysis

- **Positive cut-offs**
  - ^{3}H-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% (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?