

Mathematical modelling of skin sensitization: Guiding *in vitro* assay development for use in novel risk assessment methods

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Overview

- Background:
 - Why apply mathematical modelling to consumer safety risk assessments?
- The model:
 - An *in silico* model of skin sensitization induction
- Application within consumer safety risk assessment:
 - Focussing *in vitro* assay research / development and guiding data integration

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EU Cosmetics Directive

7th Amendment – March 2003

If the cosmetic product is to be marketed in the EU:

- alternative, non-animal tests must be used once validated
- animal testing and marketing bans on finished products
- animal testing and marketing bans on ingredients:
 - from March 2009: tests for acute (local) effects
 - from March 2013: more complex tests (including LLNA)
- threat to innovation and a major business risk
- challenge: market safe products without animal testing
- opportunity: apply new technologies in risk assessment

Unilever's R&D Activities: 2004 →



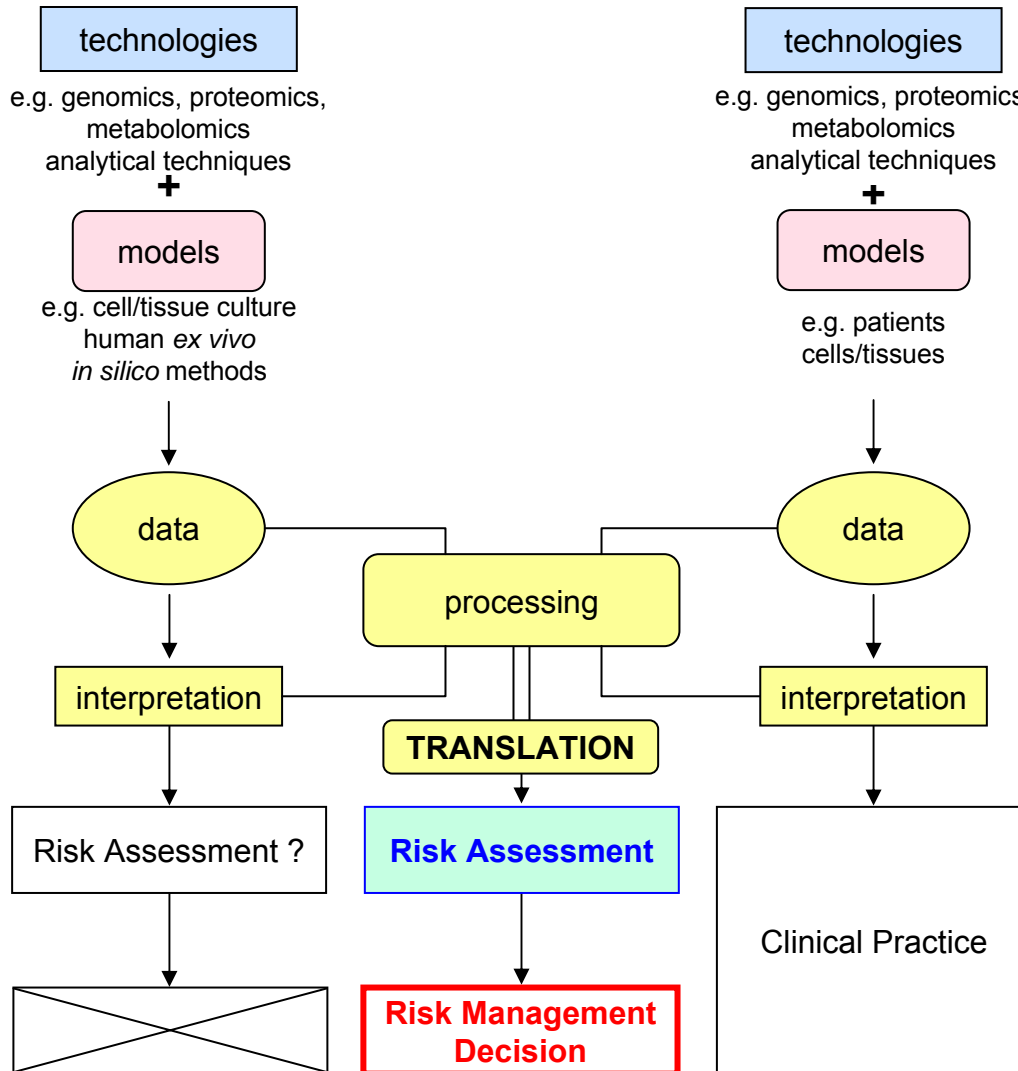
“Mulberry / ASAT Programme” *(Assuring Safety without Animal Testing)*

Objective: deliver safe new products without animal testing

- Developed and published “conceptual approach” – Fentem *et al.* ATLA, 2004
- Assessing feasibility of “conceptual approach” in practice
 - Invested in developing new capabilities
 - Evaluating applicability of new technologies and models for risk assessment: case study – skin allergy (sensitization)

Experimental Biology

Clinical Medicine



Skin Allergy: 'building blocks' of non-animal approach

■ Risk Assessment

- model development and experimental data generation driven by RA needs

■ Models

- experimental work on developing cell-based assays, peptide binding assays, and integrating dermal kinetics & metabolism

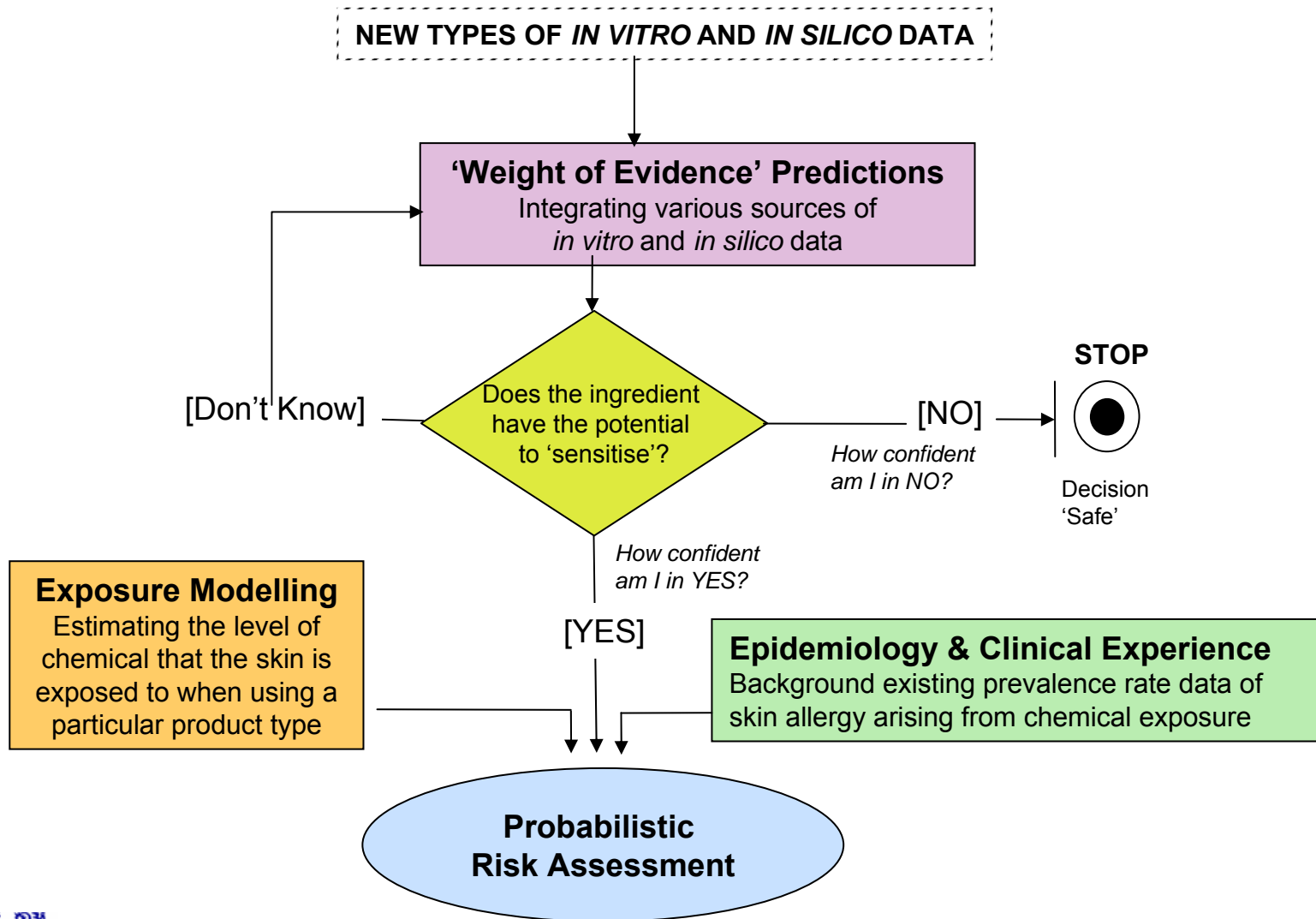
■ Technologies

- feasibility of omics and new informatics platforms explored through study of human skin inflammation

■ Data Integration

- tools developed to construct and analyse biological networks

New Risk Assessment Framework



Why apply mathematical modelling to consumer safety risk assessment?



To focus research

- Creating a 'snap shot' of what we know and don't know about chemical-induced skin sensitization will allow effective targeting of investigative research



To guide assay development

- Evaluating the relative contribution of each biological pathway to skin sensitization induction will allow effective model and biomarker selection



To inform new risk assessment approaches

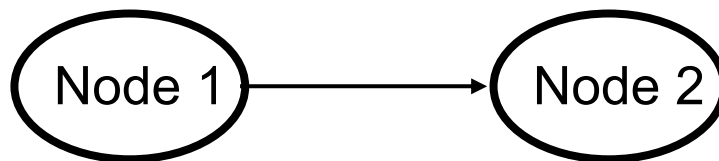
- The model represents a tool for guiding the integration and weighting of different forms of non-animal data

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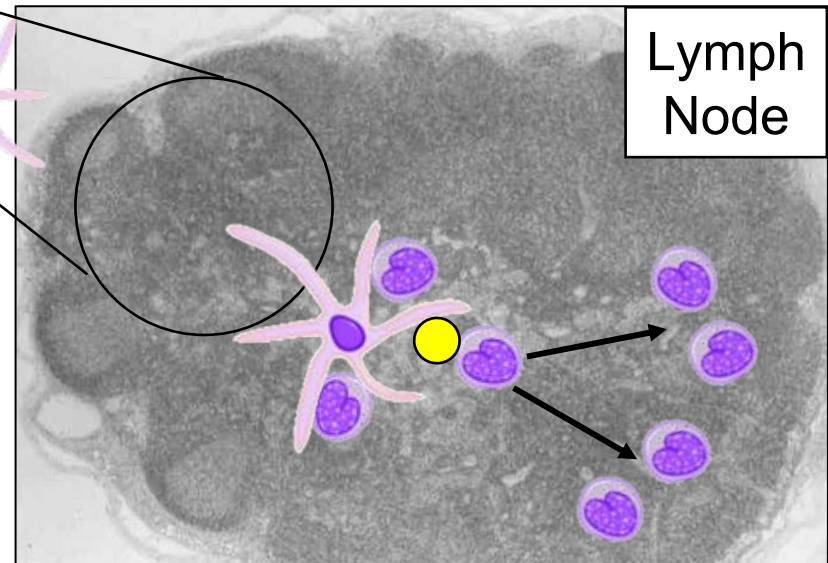
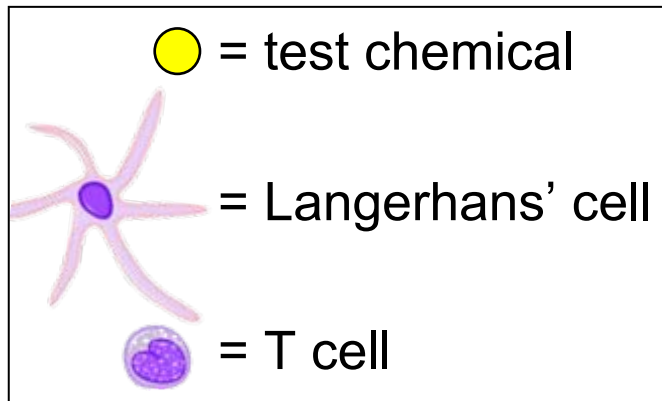
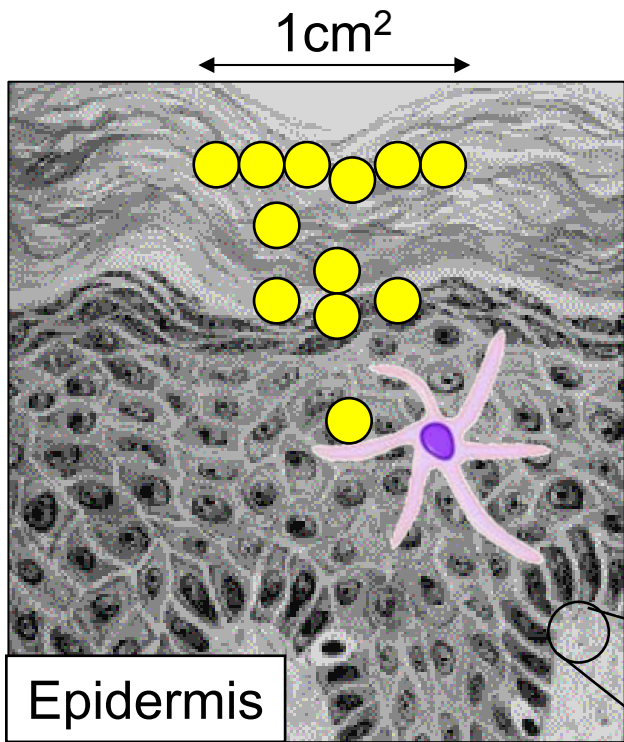
What is an mathematical model of skin sensitization?

- Computer-based representation of biology of sensitization induction described using mathematical equations
- Model limited in scope to cells/mediators/events known to have a role in skin sensitization (mouse/human data)
- Impact of 8 well-characterised sensitizers/non-sensitizers captured through effect on biological system
- Entelos Physiolab software used to visualise model
 - Nodes - Things (i.e. cells, mediators etc.)
 - Arrows - Link nodes, characterise effect of one thing (node) on another

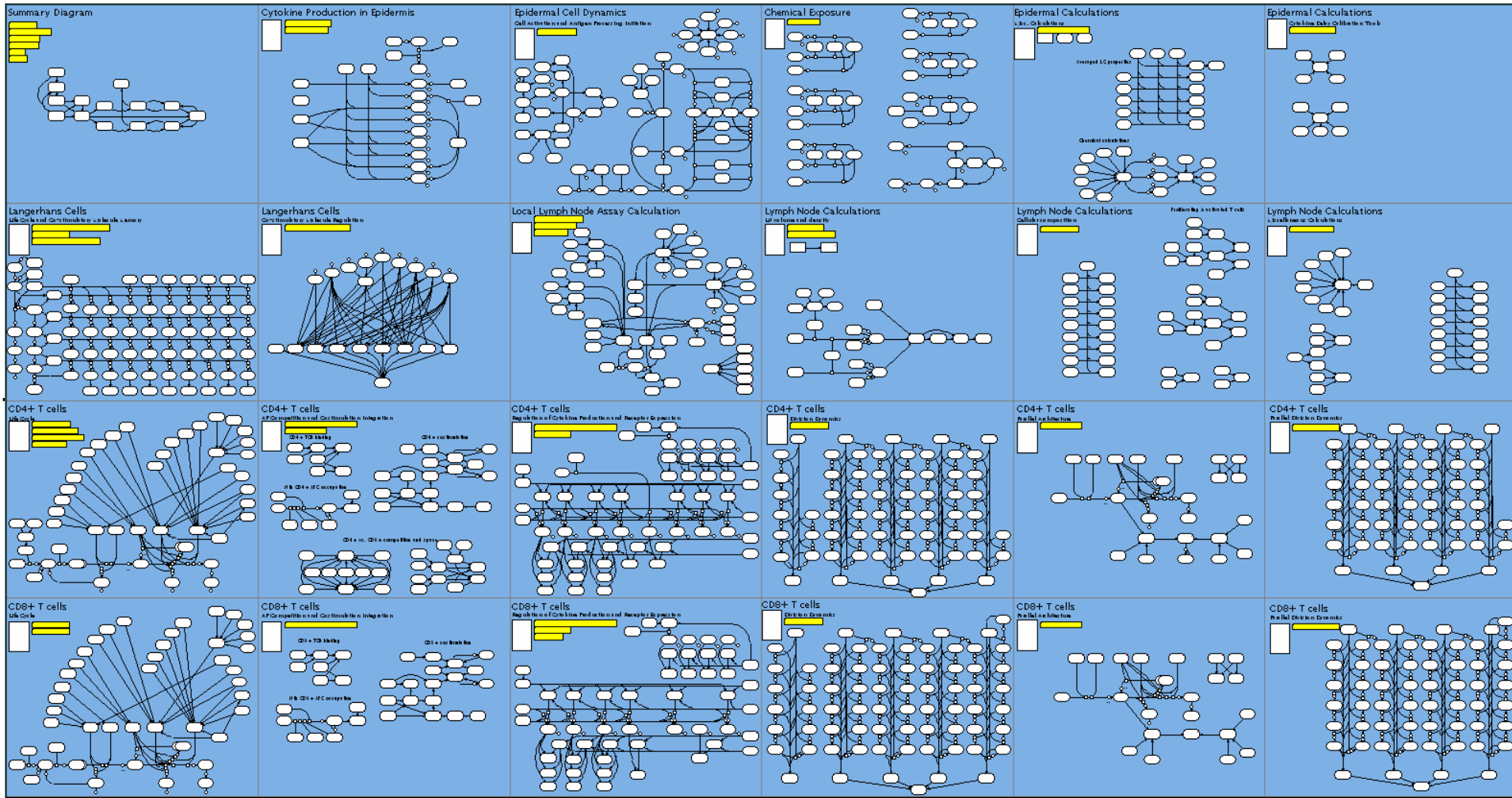


Biological scope of *in silico* model

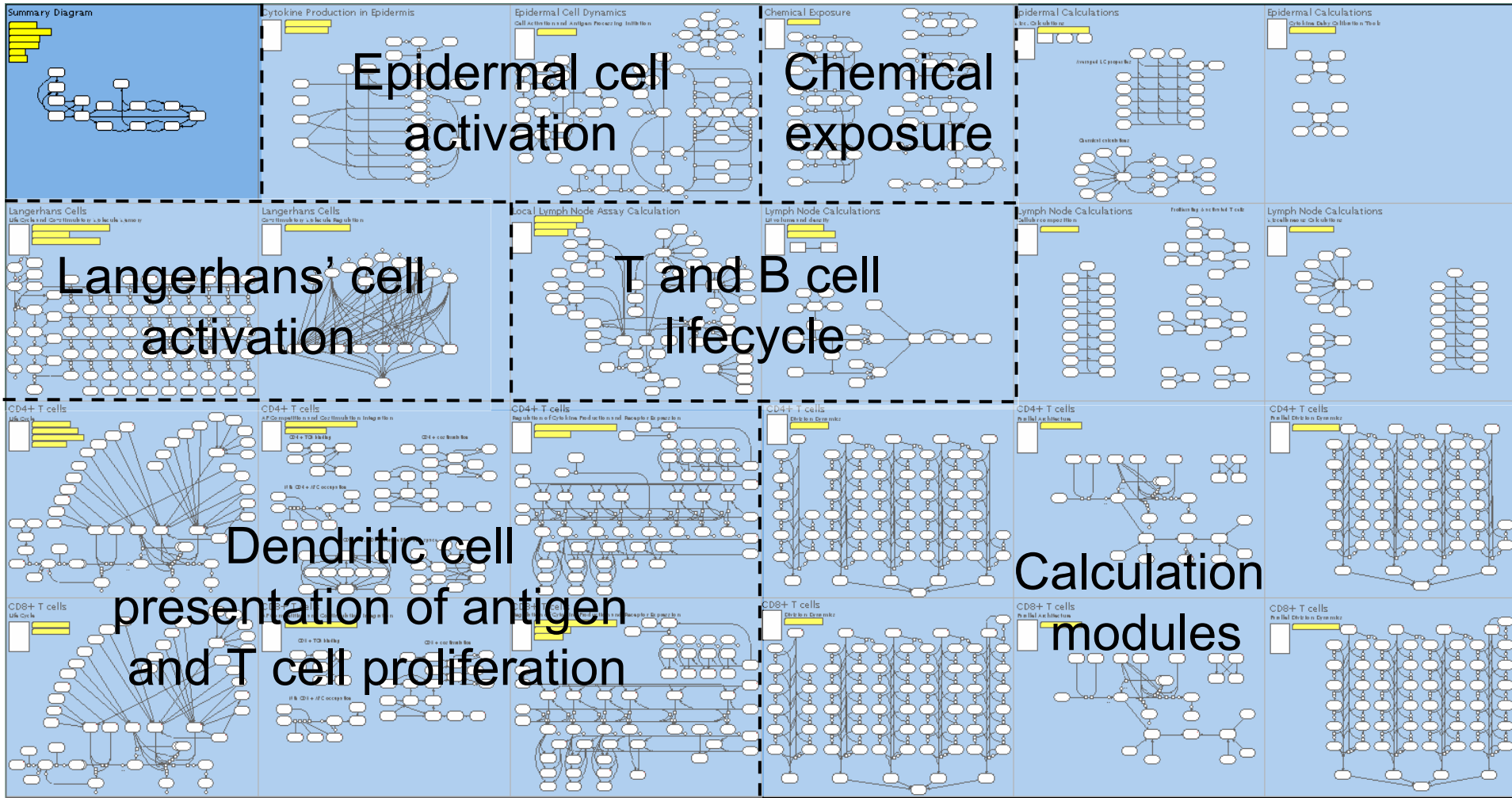
- Model covers biological pathways required for sensitization induction
- Two main biological compartments



In Silico Model Overview



In Silico Model Overview



Model Development and Sensitivity Analysis I

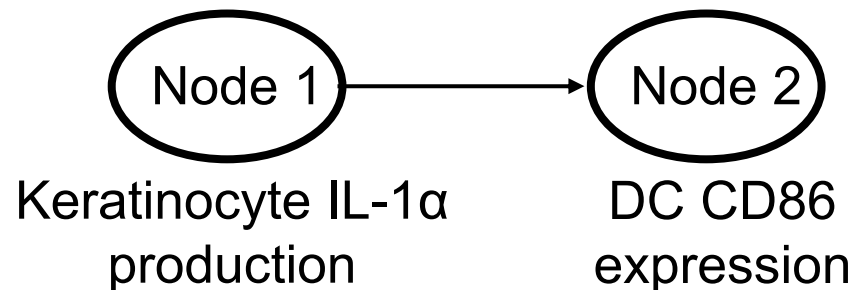
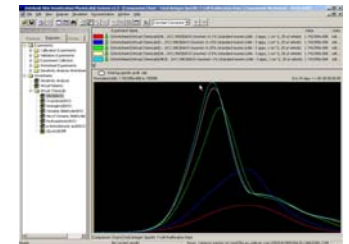
■ Qualitative Modelling

- Information from contemporary literature used to define cellular/molecular interactions

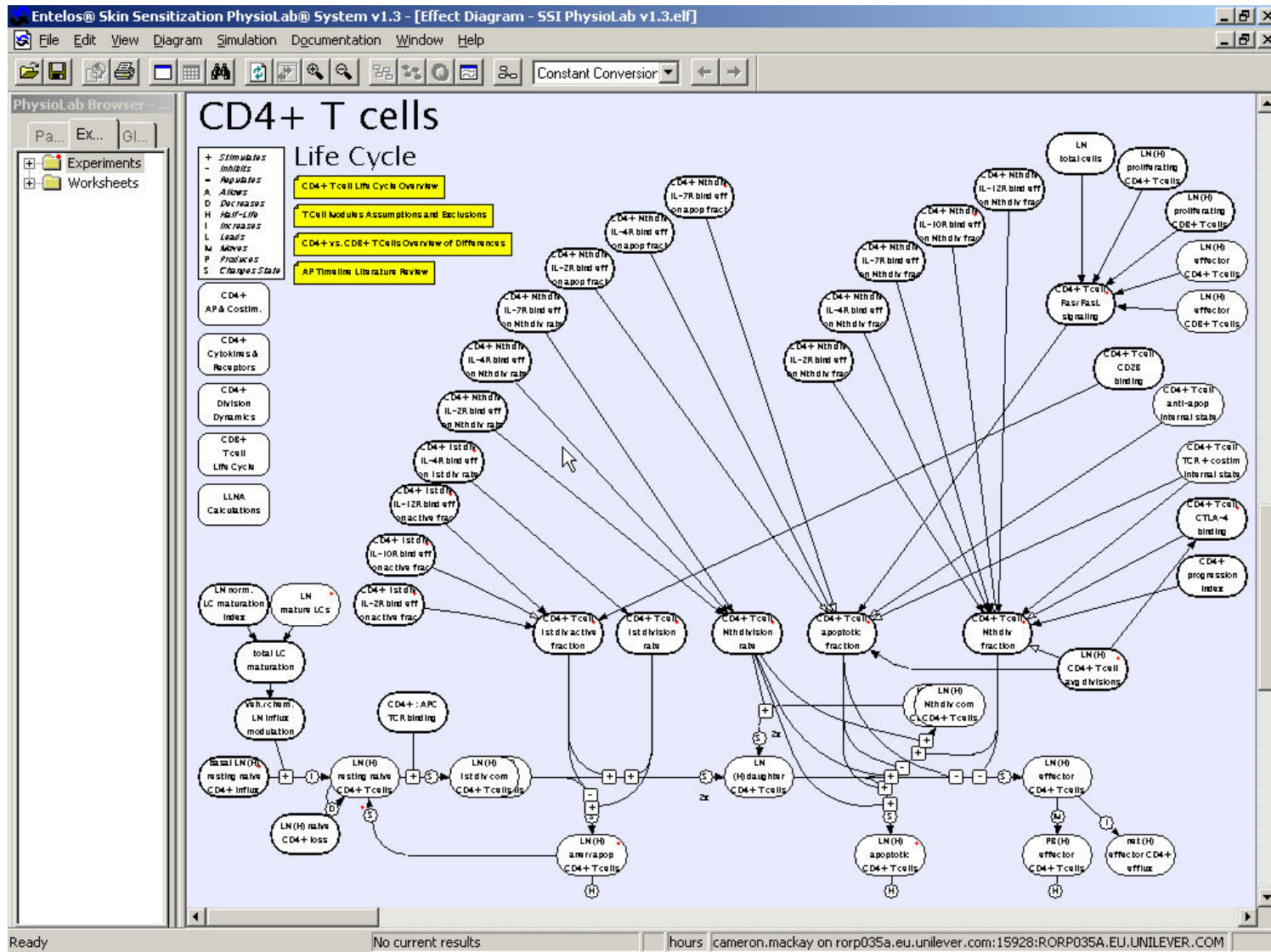


■ Quantitative Modelling

- Dynamic interactions of the biological system represented using mathematical equations & published experimental data



In Silico Model – Example



In Silico Model – Example

Entelos® Skin Sensitization PhysiLab® System v1.3 - [Effect Diagram - SSI PhysiLab v1.3.eif]

File Edit View Diagram Simulation Documentation Window Help

Constant Converter

PhysiLab Browser

Pa... Ex... Gl...

Experiments
Worksheets

CD4+ T cells Life Cycle

CD4+ T cell Life Cycle Overview
T cell Modulus Assumptions and Exclusions
CD4+ vs. CD8+ T cells Overview of Differences
AP Timeline Literature Review

CD4+ AP & Costim.
CD4+ Cytokines & Receptors
CD4+ Division Dynamics
CD8+ T cell Life Cycle
LLNA Calculations

LN norm. LC maturation index
LN mature LCs
total LC maturation
Vibrio cholerae LN influx modulation
resting naive CD4+ influx
LN(H) naive CD4+ loss
LN(H) resting naive CD4+ T cells
LN(H) 1st divy com CD4+ T cells
LN(H) 1st divy active fraction
LN(H) 1st divy rate
CD4+ T cell 1st divy active fraction
CD4+ T cell 1st divy rate
CD4+ Nth divy IL-4R bind eff on Nth divy rate
CD4+ Nth divy IL-7R bind eff on 2nd pop frac
CD4+ Nth divy IL-12R bind eff on Nth divy frac
CD4+ Nth divy IL-10R bind eff on Nth divy frac
CD4+ Nth divy IL-4R bind eff on Nth divy frac
LN total cells
LN(H) proliferating CD4+ T cells
LN(H) proliferating CD8+ T cells
LN(H) effector CD4+ T cells
LN(H) effector CD8+ T cells

Object: Diagram Note

Properties Notes

Note entries:

- Homann 2001a
- Jelley-Gibbs 2000a
- Kaech 2001a
- Kaech 2002b
- Mercado 2000a
- Murali-Krishna 1998a

Options

Jelley-Gibbs 2000a

Reference:
J Immunol 2000 Nov 1 ;165(9):5017-5026

Two distinct stages in the transition from naive CD4 T cells to effectors, early antigen-dependent and late cytokine-driven expansion and differentiation
Jelley-Gibbs DM, Lepak NM, Yen M, Swain SL

Trudeau Institute, Saranac Lake, NY 12983, USA

Ready No current results hours camer

OK Cancel Apply

In Silico Model – Example

The screenshot displays the Entelos Skin Sensitization PhysiLab v1.3 interface. The main window shows a diagram titled "CD4+ T cells Life Cycle" with various nodes and arrows representing biological processes. A sidebar on the left lists "Stimulators", "Inhibitors", "Regulators", "Allergens", "Decorations", "Molecules", "Locations", "Layers", "Adapters", "Products", and "Changes State".

An "Object: Diagram Note" window is open, showing a list of note entries:

- 1a
- 2b
- 000a
- ina 1998a

 The "Options" button is visible in the bottom right of this window.

Below the main window, a "Comparison Chart - Total Antigen Specific T-Cell Proliferation Rate" is shown. The chart plots "Normalized [A]i" from 1.742395e-008 to 150595 over a period of 0 to 10 days. The chart contains several curves in different colors (red, green, blue, black) representing different experimental conditions.

A citation window is also visible, containing the following text:

Ley-Gibbs 2000a
 e:
 2000 Nov 1 ;165(9):5017-5026
 distinct stages in the transition from naive CD4 T effectors, early antigen-dependent and late antigen-driven expansion and differentiation
 Ley-Gibbs DM, Lepak NM, Yen M, Swain SL
 Immunology Institute, Saranac Lake, NY 12983, USA

Model Development and Sensitivity Analysis II

■ Model Calibration

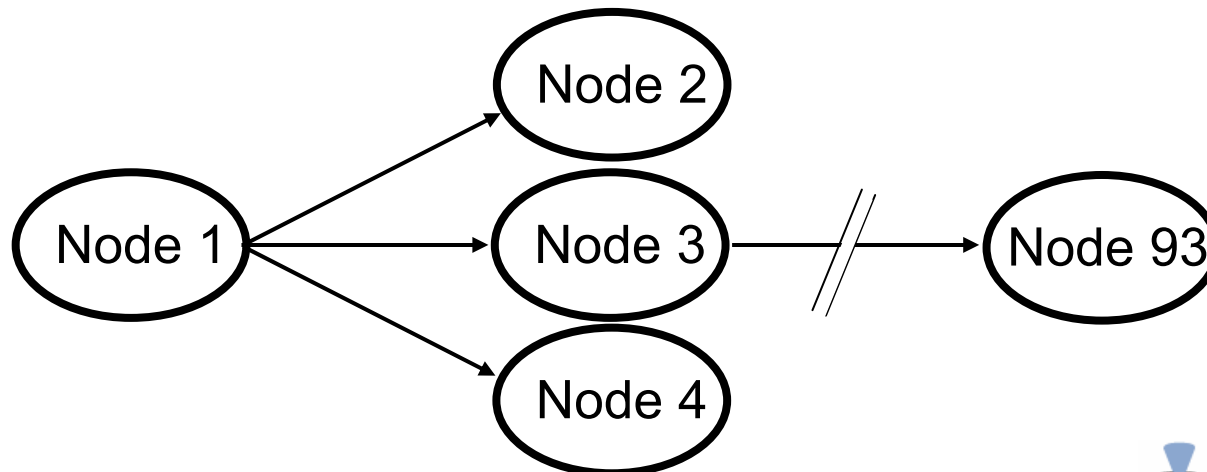
- Replication of published results from 35 key experiments within the model (e.g. Keratinocyte mediator release)

■ Model Validation

- Reproduction of system-level biological response (e.g. LLNA experiment)

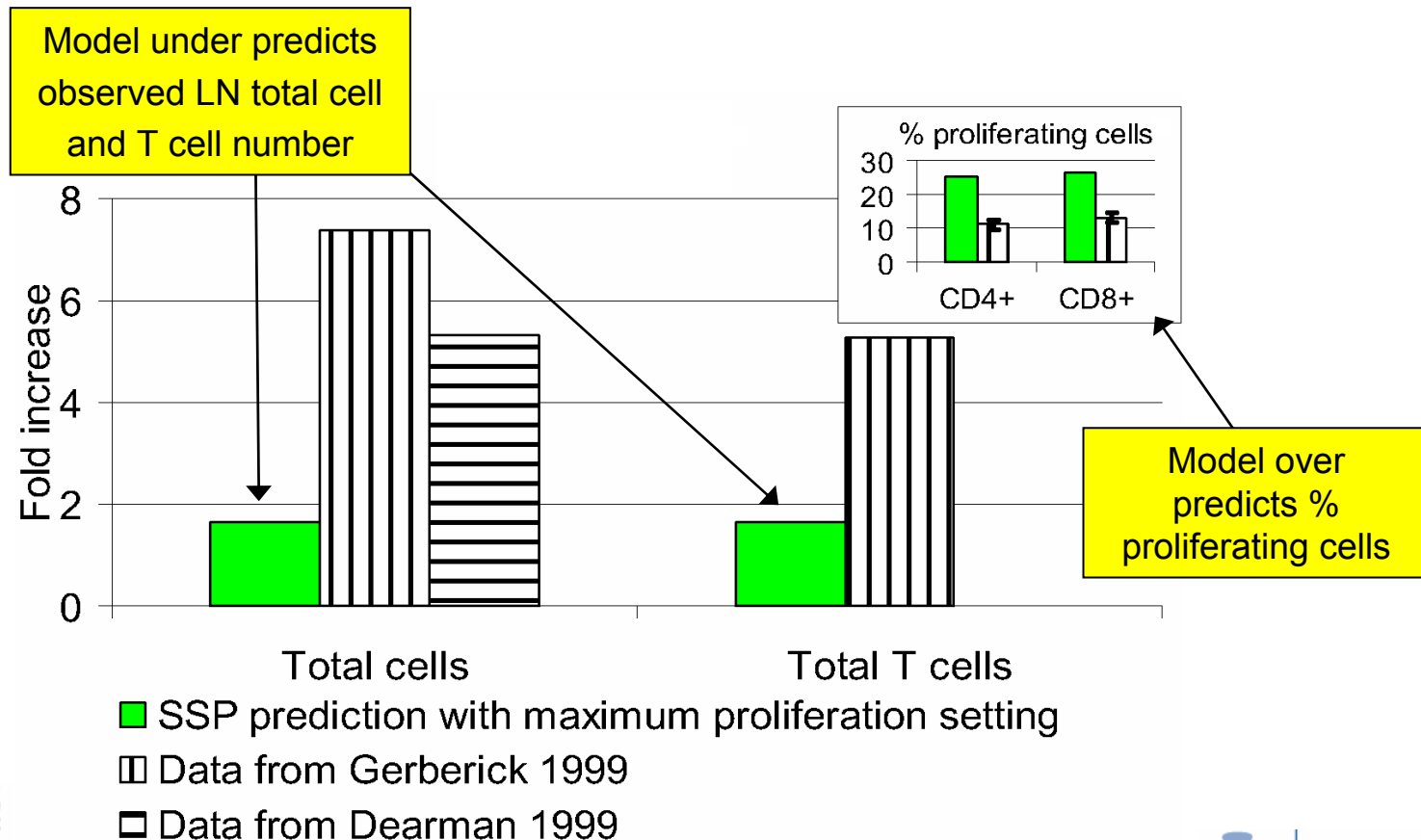
■ Sensitivity analysis

- Identification of pathways with largest influence on biological response (e.g. max Ag-specific T cell proliferation)



Model Insights

- During calibration phase, model was unable to reproduce published lymph node cell number data (example: 0.25% DNCB exposure in LLNA shown)



Modelling reveals new biological insights

- **Hypothesis 1:** T cells must undergo > 7 proliferations in sensitizer-induced responses.
- Model required > 20 proliferations to match data
 - Still over predicted % of proliferating cells
 - No experimental evidence to support this hypothesis and runs against infection data (approx. 5-6 proliferations)
- **Hypothesis 2:** Increased recruitment of lymphocytes to the lymph node supplements the total cell population
- Does experimental evidence support this hypothesis?

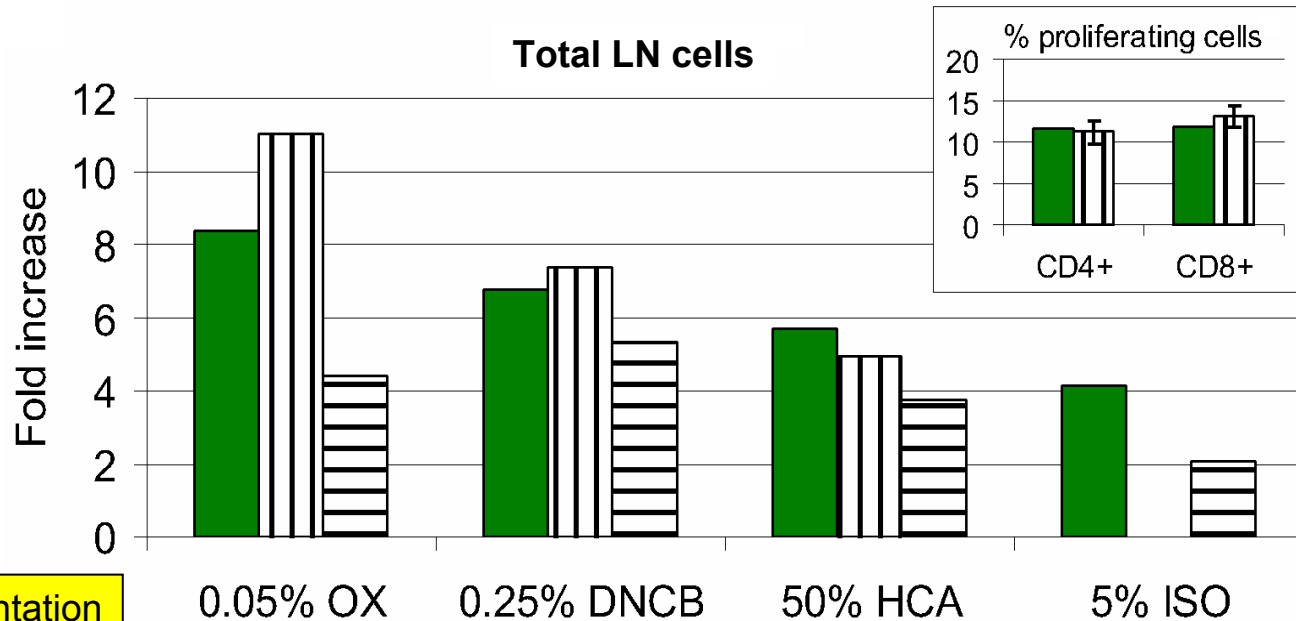
Modelling reveals new biological insights

- Tedla *et al.* 1998. *J. Immunol.* **161**. 5663-5672
 - DNFB (sensitizer) exposure on skin induces mouse LN chemokine production (MIP-1 α/β)
 - Peripheral leukocyte numbers depleted by 50% at 30mins after exposure

- Soderberg *et al.* 2005. *PNAS.* **45**. 16315-16320
 - TLR agonist intradermal exposure and Herpes Simplex virus infection caused massive recruitment of naïve lymphocytes to LN.
 - Most LN cells are non-proliferating (95%)
 - Proposed mechanism is via vessel re-modelling: greatly increases LN cell turnover.

New Insight: Cell recruitment to the lymph node

Hypothesis 2 implemented



Model implementation with new hypothesis

- SSP prediction with enhanced recruitment setting
- ▨ Data from Gerberick 1999
- Data from Dearman 1999

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Model Sensitivity Analysis

■ Aim:

- To evaluate relative contribution of individual pathways to overall biological response (e.g. Max Ag-specific T cell proliferation)

■ Method:

- Controls – assigned control dose for prototypic weak/moderate/strong sensitizers
- Experiments – vary model parameters to up/down-regulate biological pathways

■ Results:

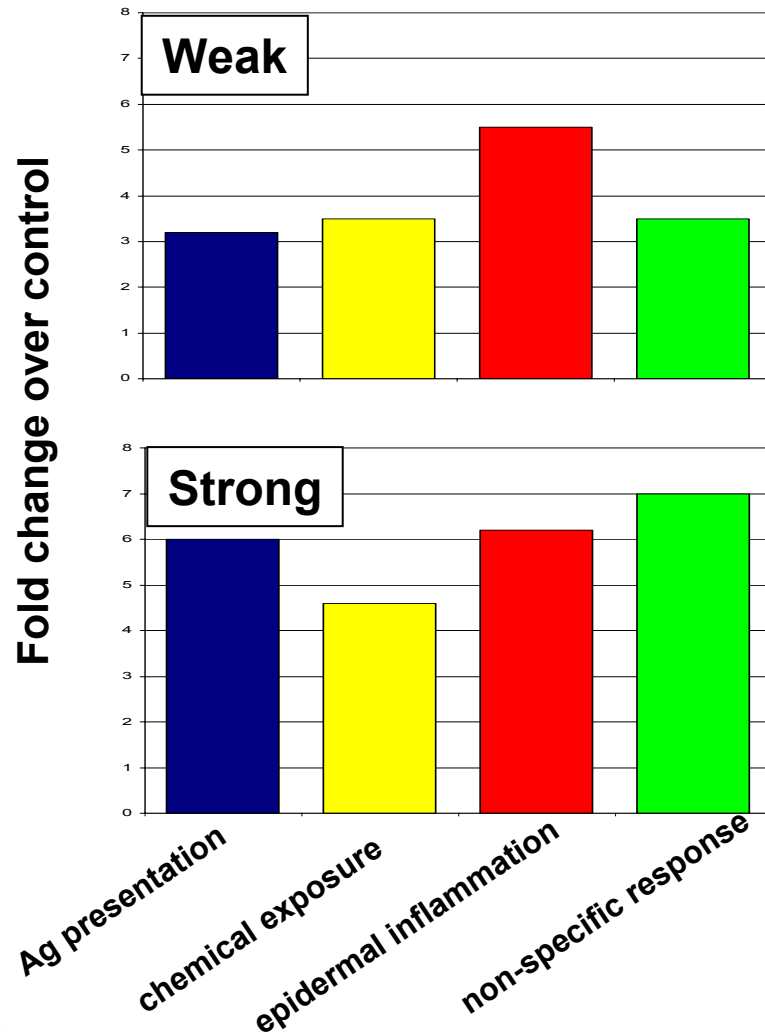
- Record model predicted outcomes under control and perturbed conditions – approx. 30,000 simulations performed
- Calculate fold change in outcome relative to control
- High fold change = high influence of pathway on response

Measure outcomes and modulated pathways

Modulated pathways:

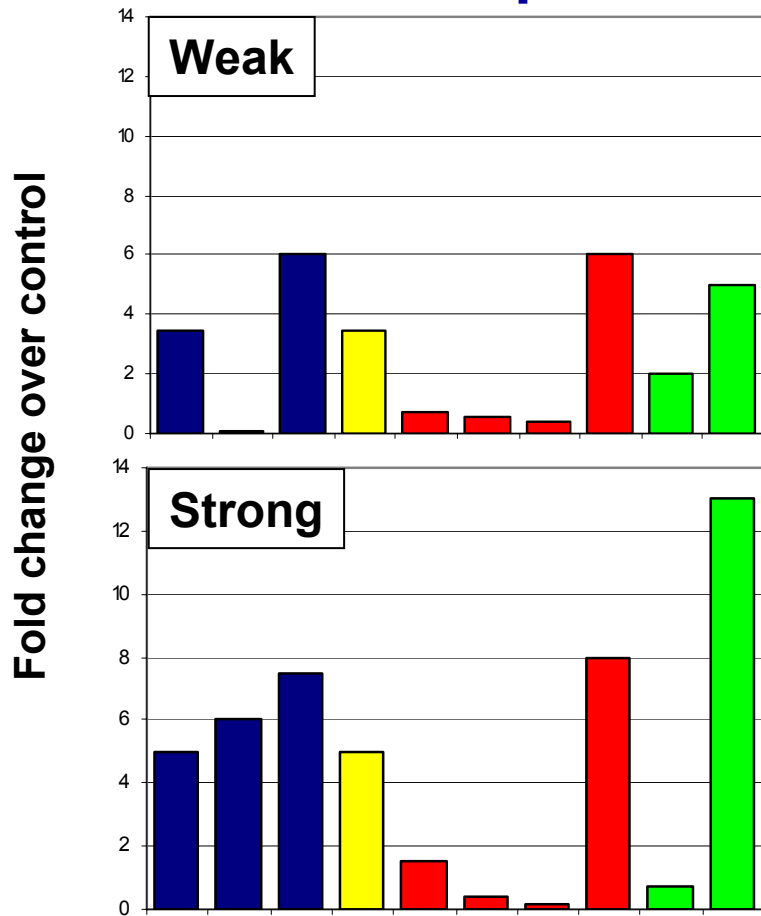
Category	•Subcategory	•Parameter/Location
Chemical exposure		<ul style="list-style-type: none"> •haptanated protein half-life •binding efficiency
Epidermal inflammation		<ul style="list-style-type: none"> •epidermal LC mat/mig induction •epidermal IL-1a production •epidermal IL-1b production •epidermal TNF-a production •epidermal IL-8 production •epidermal IL-10 production •epidermal GM-CSF production •epidermal cytokine production (all together)
Non-specific response		<ul style="list-style-type: none"> •veh/chem LN influx modulation •LN mature LCs
Antigen presentation		<ul style="list-style-type: none"> •space/LC •LN norm. mature LC MHC I •LN norm. mature LC MHC II •LN norm. mature MHCI and MHCII together •LN norm. mature LC B7-1 •LN norm. mature LC B7-2 •LN norm. mature LC anti-apop •LN norm. mature LC IL-12 prod •total LN LC phenotype (all markers together)

Relative pathway contribution: Maximum Antigen-specific T cell proliferation



- Max Ag-specific T cell proliferation selected as ideal measure of skin sensitization induction
- Epidermal inflammation and (Ag) non-specific effects have a significant influence over Ag-specific T cell proliferation
- All categories are significantly influential across sensitizer strength


Relative pathway contribution: Maximum Antigen-specific T cell proliferation



- Epidermal inflammation (e.g. TNF α release) has most significant effect over Ag-specific T cell proliferation
 - Due to role in induction of LC migration to LN

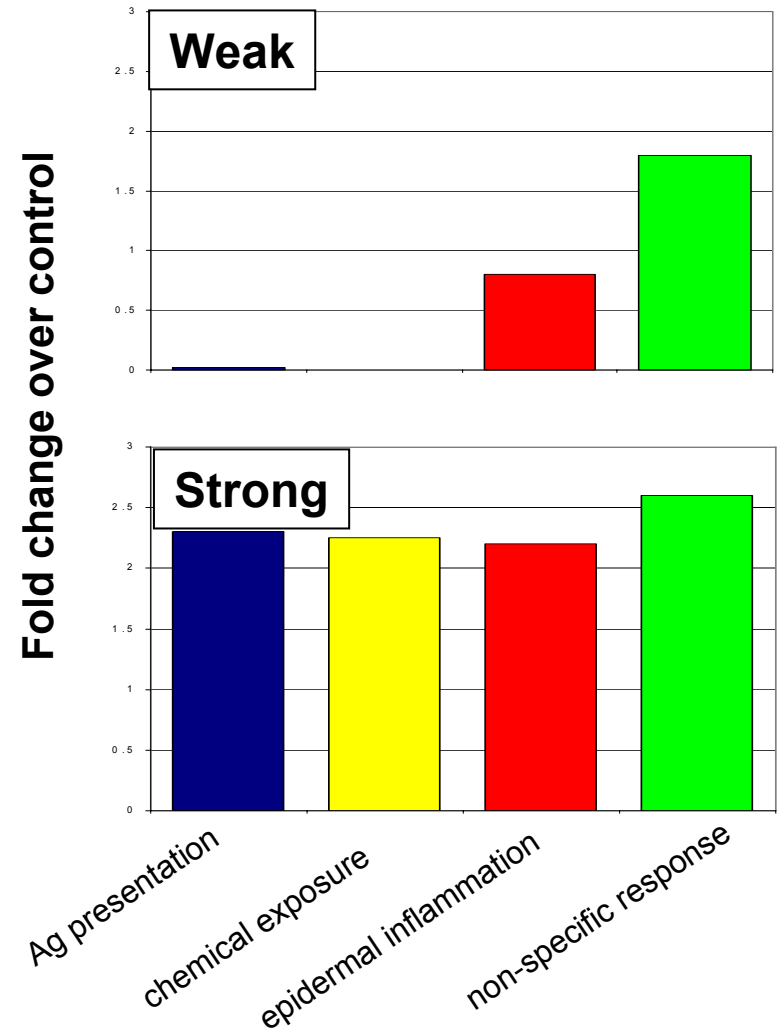
- Number of mature LN LCs and LC phenotype also have a significant effect on Ag-specific T cell proliferation

LC MHC I MHC II
 Space per LC
LC phenotype
 Binding efficiency
 Epidermal IL-10
 Epidermal IL-1 α
 Epidermal IL-1 β
Epidermal TNF- α
 Recruitment influx
mature LN LC



Relative pathway contribution: LLNA Stimulation Index

- LLNA SI measures both Ag- and Ag-non-specific T cell proliferation
- Weak Sensitizers
 - Epidermal inflammation and (Ag) non-specific responses dominate the LLNA SI
- Strong sensitizers
 - Chemical exposure and antigen presentation pathways become more important



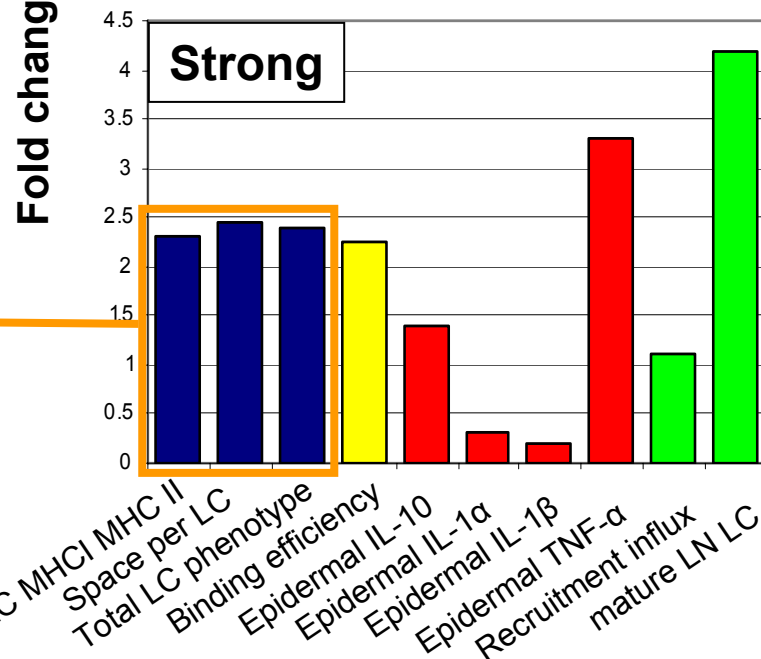
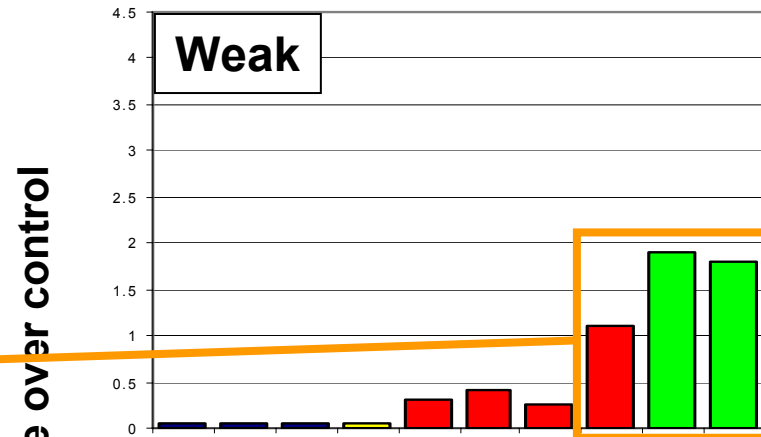
Relative pathway contribution: LLNA Stimulation index

■ Weak sensitizers

- LLNA SI is dominated by Ag-non-specific T cell proliferation (e.g. epidermal inflammation pathways)

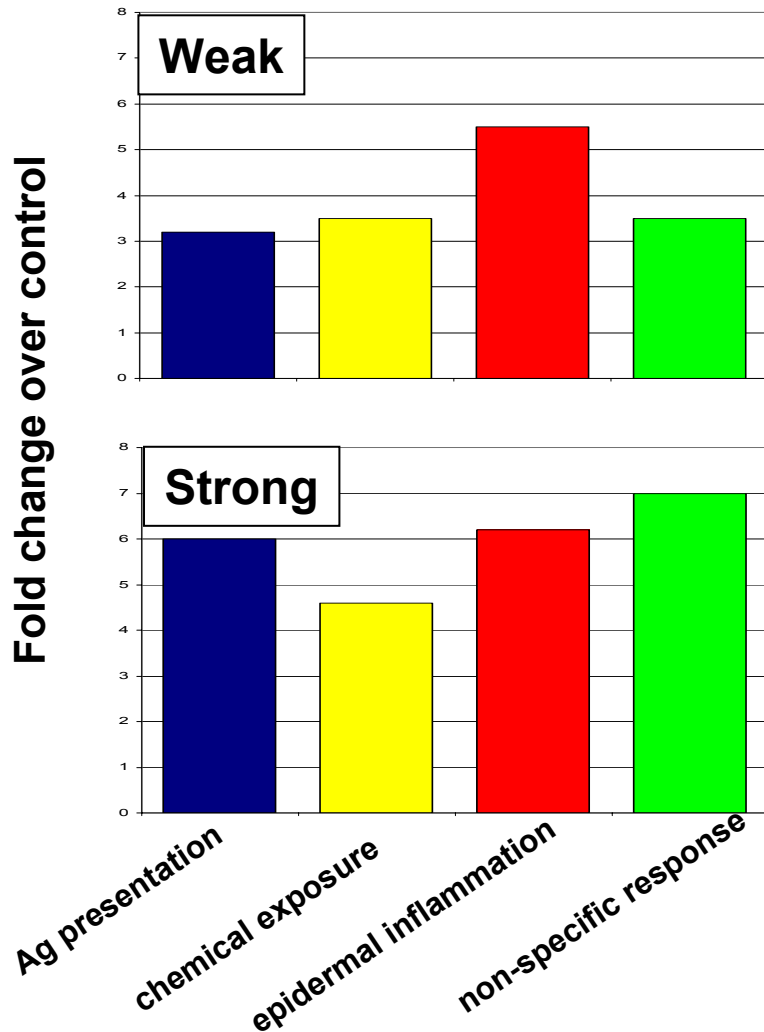
■ Strong Sensitizers

- LLNA SI includes a stronger contribution from Ag-specific T cell proliferation (i.e. Ag presentation pathways have strong influence on SI)



Insights for *in vitro* assay development

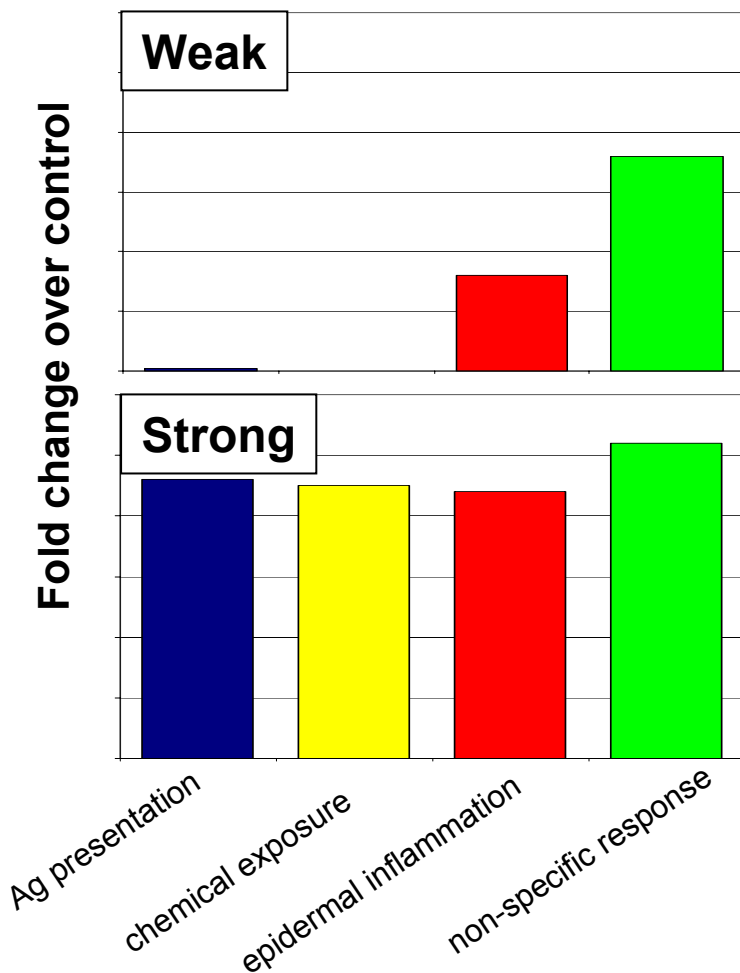
Max. Ag-specific T cell proliferation



- An array of predictive assays that cover all key categories should allow Ag-specific T cell proliferation to be confidently predicted
- Several model systems in development should be capable of generating these key pieces of data:
 - Chemical Exposure – Peptide Binding
 - Epidermal Inflammation – 3D Skin models
 - Ag presentation – DC activation or *in vitro* T cell proliferation
- (Ag) non-specific response may require *in silico* prediction or new assay type

Insights for non-animal assay development

LLNA stimulation index



- Traditional *in vitro* assay validation (i.e. through direct correlation *in vivo* animal data) will not be possible
- Relative influence of different biological pathways on LLNA SI will vary across different chemicals
- Integration of data from multiple assays, delivering different types of hazard information, will be required

Next Steps

To focus research

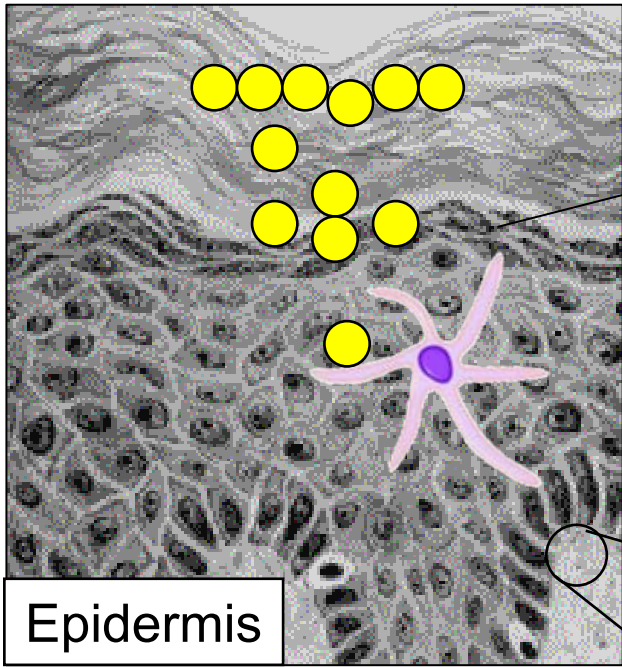
- Skin allergy research programme realigned to address key knowledge gaps
 - Future research data will be used to inform the model where possible



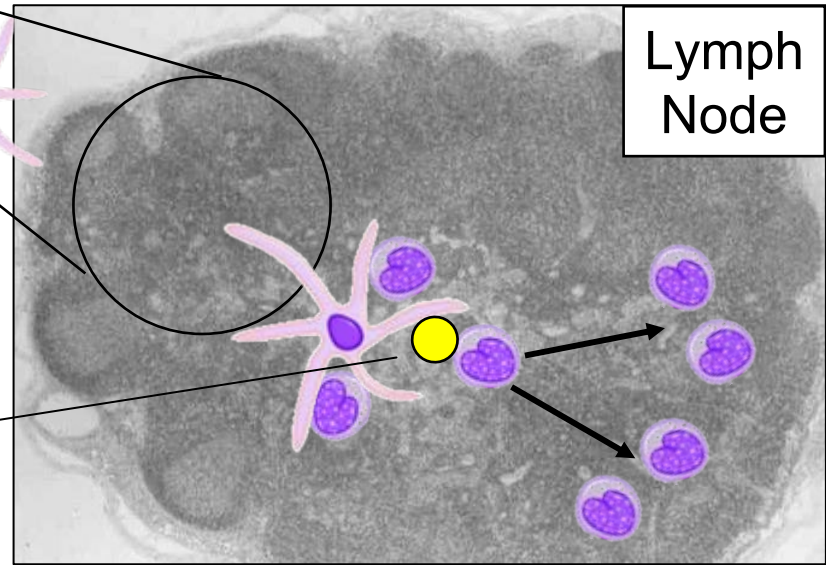
To guide assay development

- *In vitro* assay development research verified as broadly relevant by sensitivity analysis
 - *In silico* pathway analysis used to guide selection of experimental parameters





- How does the frequency and/or specificity of hapten: protein binding relate to sensitizer potency?
- Do sensitizers activate DC solely via indirect mechanisms (e.g. inflammatory signal release) or are direct mechanisms (e.g. receptor-mediated) also involved?



- How does sensitizer potency correlate to naïve, specific T cell clone frequency?
- What is the role of regulatory T cells and other lymphocyte subsets (i.e. B cells, NK cells)?

Next Steps

To focus research

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 - Future research data will be integrated into model where possible



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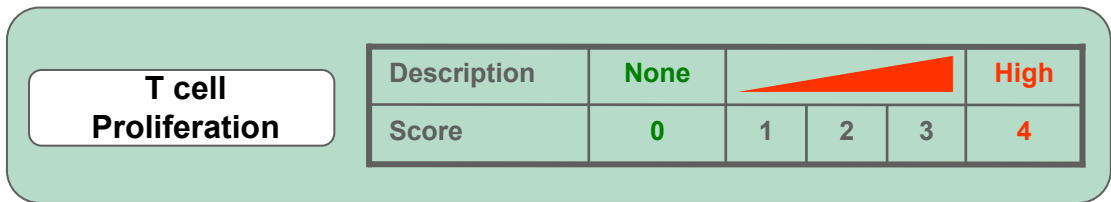
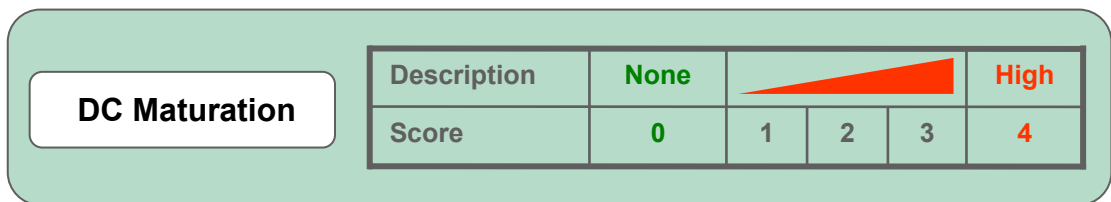
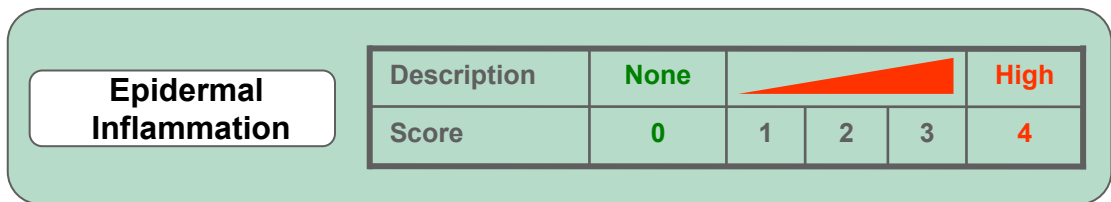
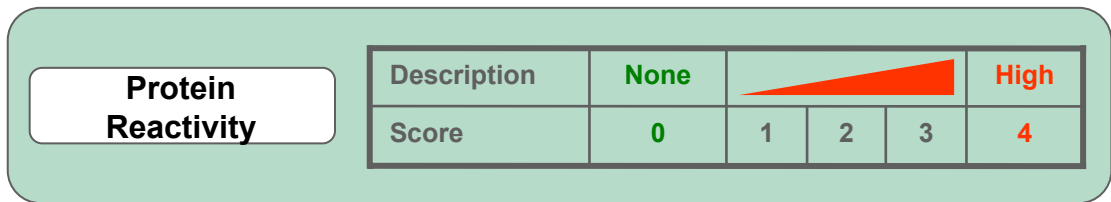
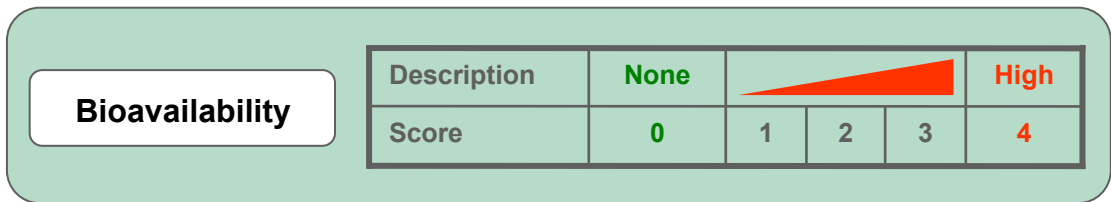


To inform new Risk Assessment approaches

- Model provides biological rationale for guiding integration of different forms of animal data
 - e.g. what is value of epidermal inflammation data?



Integration of different forms of non-animal data



'Weight of Evidence' Predictions
Integration of different forms of *in vitro* and *in silico* data

Does the ingredient have the potential to 'sensitize'?

Acknowledgements

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- Catherine Clapp
- Ian Jowsey
- David Lockley
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