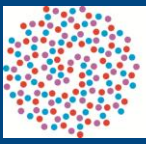


MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY

Simulating some aspects of lymphocyte biology

Auckland
Bioengineering
Institute
An Institute of The University of Auckland

THE UNIVERSITY
OF AUCKLAND
NEW ZEALAND

Outline

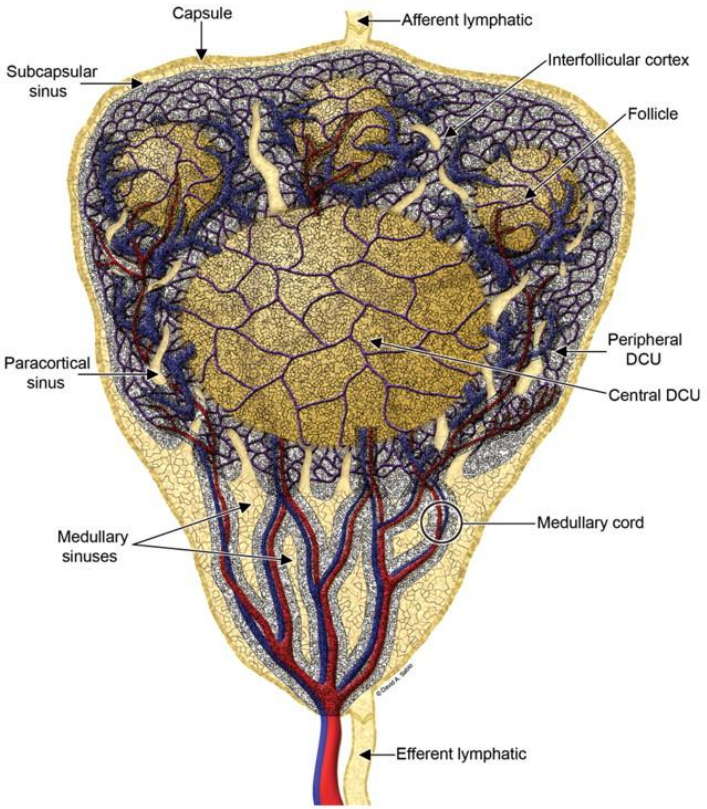
- Some immunological background
- Introduction to agent-based modelling (ABM)
- ABM for T cell immune response in the lymph node
- ABM for formation of the germinal centre in the lymph node follicle

T and B cell immune response

- Deep cortical unit (DCU) of the LN paracortex is where T cells meet evidence of pathogen (antigen) displayed on dendritic cells
- The very rare cognate T cells have receptors that can recognize and bind to the antigen.
- T cell receptor (TCR) stimulation initiates the adaptive immune response, driving T cell proliferation
- Activated T cells go forth and combat the pathogen

- B cells migrate to a follicle, where antigen is delivered by a variety of means
- After encountering antigen, cognate B cells link up with activated CD4 T cells (follicular helper)
- Activated B cells cluster to form a germinal centre, where they proliferate and mutate their antibodies (somatic hypermutation)
- Highest-affinity antibody is selected, and these B cells become plasma cells, secreting antibody

Human lymph node lobule



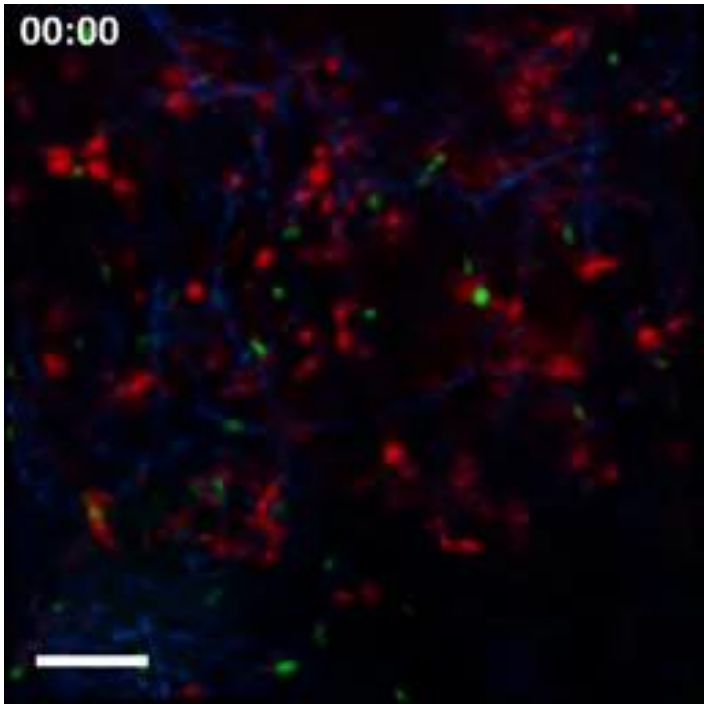
Cynthia Willard-Mack, Toxicol Pathol 2006; 34

T cell activation

T cells roam the lymph node paracortex, encountering dendritic cells displaying antigen.

Intravital microscopy

Green = T cell
Red = DC
Blue = fibro-reticular network

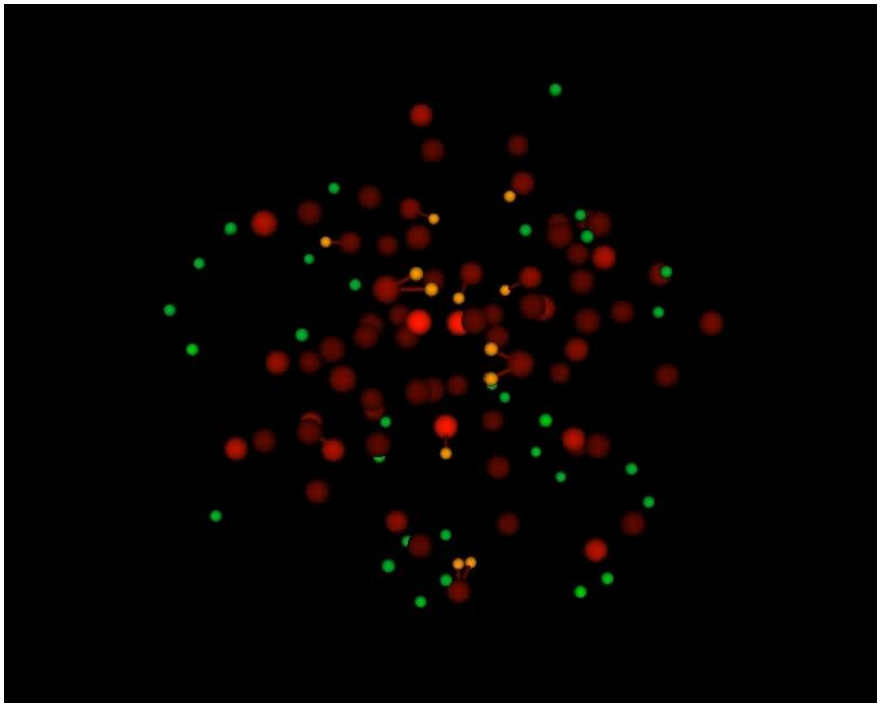


Scale bar: 50 μm

Mempel, Henrickson, von Andrian. Nature 427, 2004.

Simulation

Green = unbound T cell
Yellow = bound T cell
Red = DC (intensity scaled by antigen load)



Bogle & Dunbar, Immun Cell Biol 86, 2008.
Bogle & Dunbar, Immun Cell Biol 88, 2010.

Agent-based model concepts

Agent-based models are simulations to determine the global consequences of local interactions of members of a population.

- An agent may be mobile
  - motion could be discrete (on a lattice) or continuous
- Agent state changes with time (evolves) according to the individual experience of the agent, and a set of rules
  - state vector components may take discrete or continuous values
  - interactions take place between nearby agents and/or between agent and environment
- Atomistic approach
  - deals directly with discrete, autonomous entities/individuals/agents rather than with averages or concentrations
  - net result determined by summing over the population, rather than by solving DEs
- Stochastic effects are often important
  - e.g. random walk motility of lymphocytes
  - results are in the form of probability distributions

ABM applications

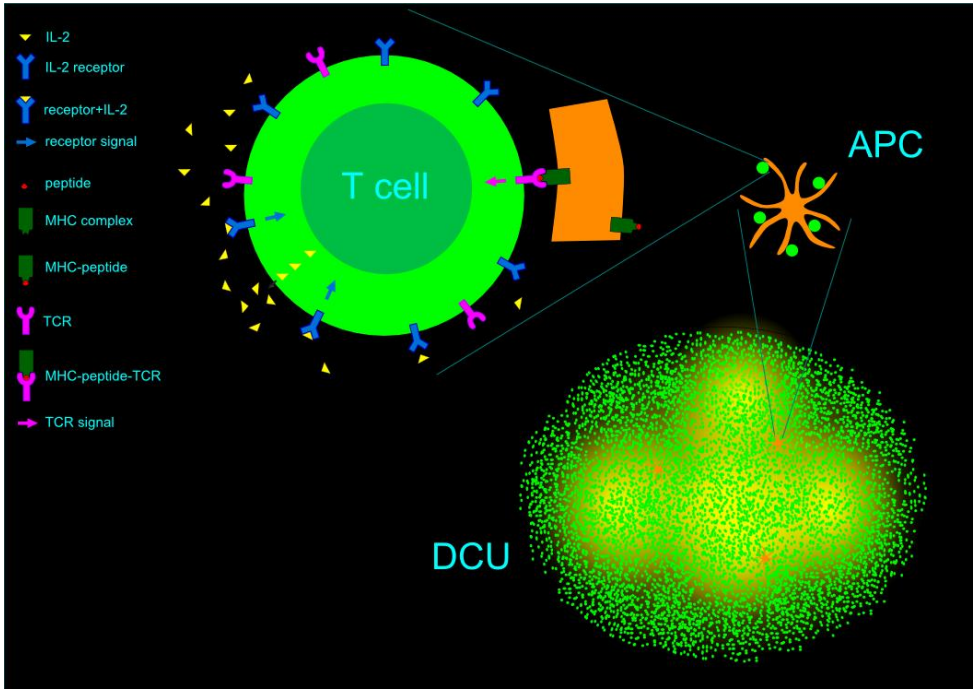
- Animal congregations
  - Flocking of birds (see Boids)
  - Fish schooling
  - Social insect behaviour
- Ecological modelling
  - Prey-predator dynamics
- Economic systems
- Biology at the cell level
  - Immunology (T cells, B cells)
  - Tumour modelling
  - Bone remodelling (osteoclasts, osteoblasts)
  - Skin (keratinocytes)

Why use ABM to simulate T cell activation?

- The crucial early stages of an immune response involve very small numbers of antigen-specific T cells (e.g. 1 in 10^4-10^5), invalidating concentration- or average-based methods.
- Each cell has its own unique “experience” of pMHC encounters and cytokine exposure.
- T cells differ in their TCR-antigen affinity, DCs carry different amounts of antigen.
- Probabilistic effects enter at every stage, starting with the inherent distribution of cell characteristics, and driven by the “random-walk” motility.

Multi-scale processes of T cell immune response …

- Cell trafficking
- T cell motility
- TCR stimulation
- Signal integration
- Proliferation
- Cytokine secretion
- Chemotaxis

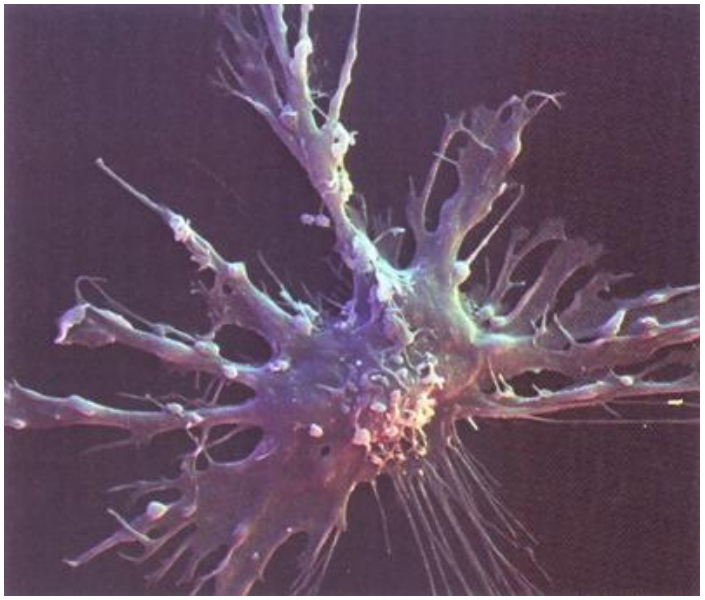


… require multi-scale modeling

Spatio-temporal issues

- T cells are tightly packed, occupying about 60% of the DCU space, the rest is fibro-reticular network, other cells, fluid
- T cell motility simulated on a 3D lattice with grid size 6.3 μm
  - one T cell per lattice site
  - spherical lattice region of available sites (blob)
  - from *in vivo* microscopy, mean speed ≈ 14 $\mu m \cdot min^{-1}$, motility coefficient $C_m \approx 80\ \mu m^2 min^{-1}$
- Random T cell – DC encounters

- DC soma occupies 7 sites
- DC "sphere of influence" diameter of 38 μm (116 sites)
- Probability of making contact with DC (when in the SOI) depends on current level of occupancy of the DC
- Duration of T cell-DC contact varies with activation state of T cell, from minutes to hours



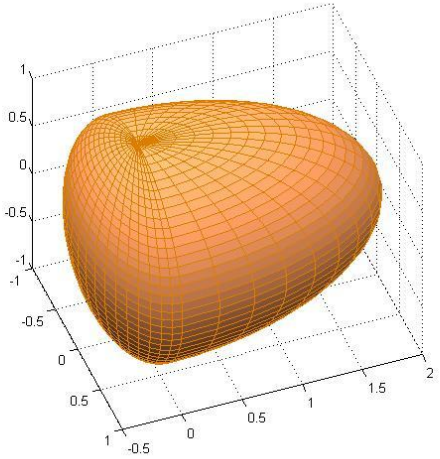
Dendritic cell

On-lattice lymphocyte simulation

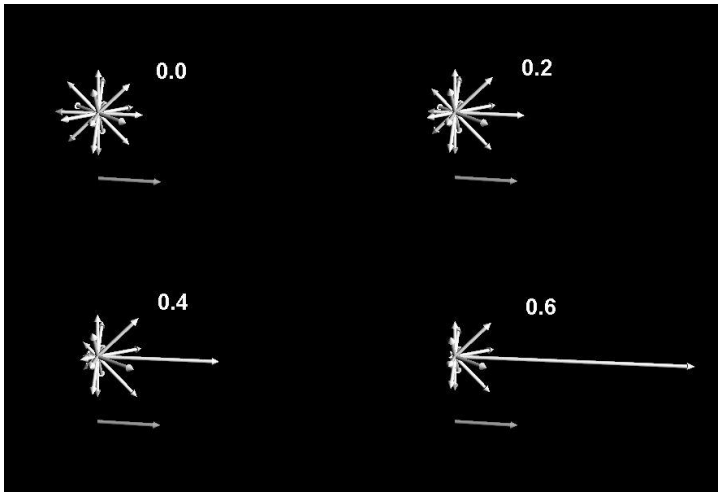
- The lattice is a computational convenience, it simplifies coding, is a cheap way to handle cell crowding
- Speed enables simulation of large number of cells (10^5-10^6), aided by parallel processing (OpenMP)
- Estimates of motility coefficient, mean speed are available from intravital microscopy
- Motion is simulated as a persistent random walk on the lattice, with specified probabilities of jumps in 26 directions
- Probabilities are set according to the previous jump direction, and adjusted according to occupancy of neighbour sites
- Observed motion parameters are reproduced
- Chemotactic influence can be incorporated by adjusting the probabilities
- Changing cell population is handled by adding or removing available lattice sites at the boundary

Motility model

- Motion on a packed lattice, available sites = number of cells
- Two cells can share a site, but this is discouraged
- Two parameters determine probabilities of jumps to neighbour sites (26)
- Persistence of direction needed to match observed mean speed and Cm



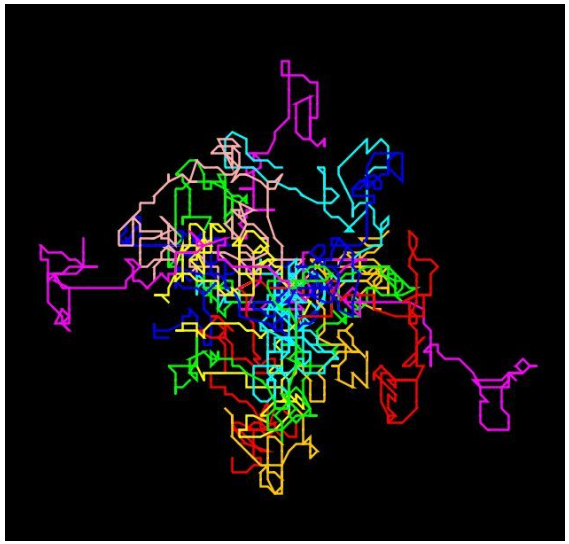
Composite ellipsoidal surface, half prolate and half oblate. The probability-weighted steps are proportional to the distances from the origin to the surface.

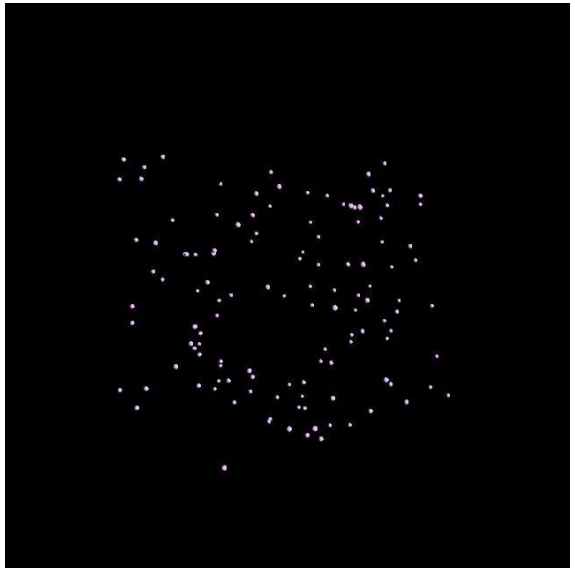


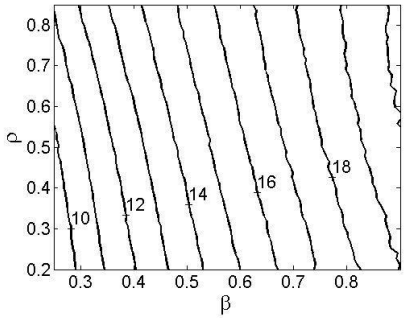
Probability-weighted steps for M18 model, persistence parameter ρ = 0.0, 0.2, 0.4, 0.6

Bogle, G and Dunbar PR, Immunol Cell Biol, August 2008.

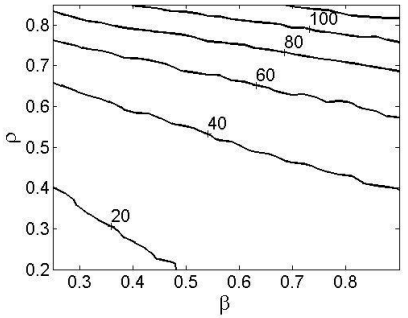
Motility results

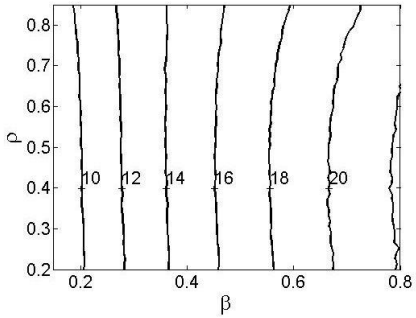




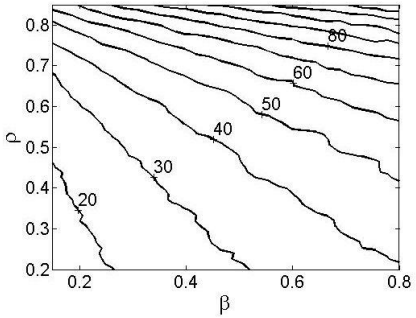


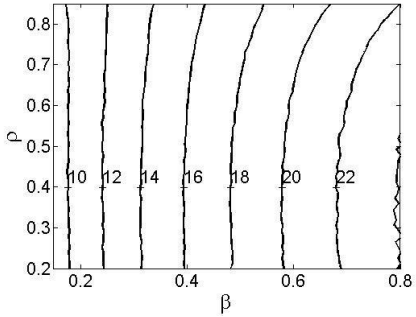
N6



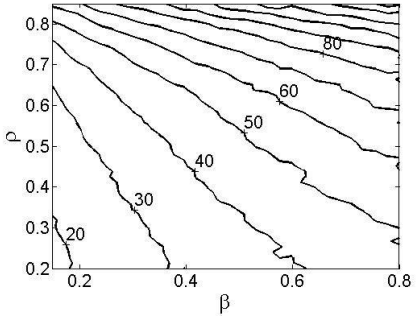


M18





M26



Mean speed

Coefficient of motility

Cell trafficking

- Hyper-vascularization, driven by inflammation signals, causes T cell influx to increase many-fold during an immune response.
- Inflammation -> VEGF secretion (stromal) -> vascularity
- T cell influx proportional to relative vascularity

$$D(t) = k_D N(t)$$

$$C_G(t) = \frac{M_G(t)}{D(t)}$$

$$\frac{dM_G(t)}{dt} = \alpha_G A(t) N(0) + \frac{dM_{Gbase}}{dt} - \delta_G M_G(t)$$

$$\frac{dV(t)}{dt} = \alpha_V H(C_G(t), \beta_V C_G(0), n_V) V(t) - \delta_V V(t)$$

$$F_{in}(t) = F_{in}(0) V(t)$$

$$\frac{dN(t)}{dt} = F_{in}(t) - F_{out}(t)$$

$N(t)$ = number of T cells

$D(t)$ = fluid volume

$A(t)$ = inflammation signal

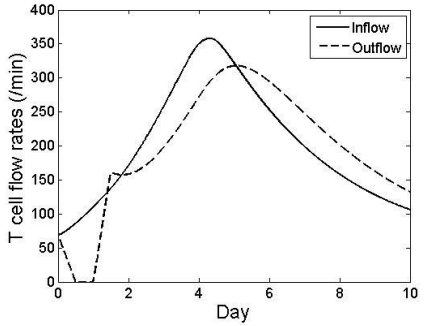
$M_G(t)$ = mass of VEGF

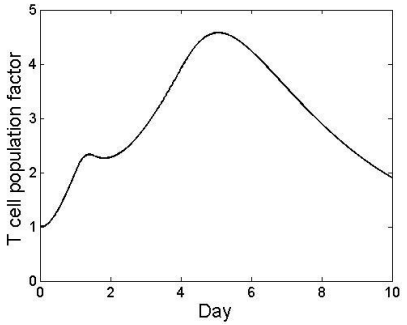
$C_G(t)$ = VEGF concentration

$V(t)$ = relative vascularity

$F_{in}(t)$ = T cell inflow rate

$F_{out}(t)$ = T cell outflow rate





Cell state evolution

- Cell state represented by stage of activation, integrated TCR signal level

  =>receptor expression, e.g. CD69, S1PR1, IL-2/IL-2R …
- A cognate T cell bound to a DC receives TCR signalling at a stimulation rate ~ (cell TCR avidity)*(DC antigen density)
- "Signal integration" hypothesis: TCR signal is summed over serial DC encounters
- DC binding duration varies with level of summed stimulation
- "Staged activation" – transitions through stages of activation occur as integrated TCR signal exceeds threshold levels
- A fully activated cell is committed to proliferation, and may undergo 15 or more cycles of division.
- Production of IL-2 and IL-2R (not currently simulated, although it is known that IL-2R signal influences the subsequent proliferation)
- Permission to exit the LN

Signal integration and stagewise activation

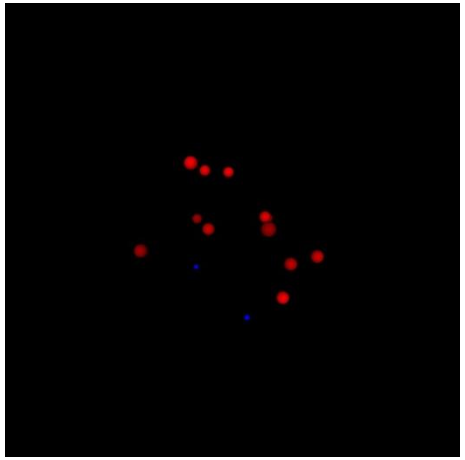
- Intravital observations have showed that cognate T cell interactions with DCs can be categorized into several stages. Miller et al (2004) have four stages, Mempel et al (2004) three stages:
  - Stage 1: (~6 hrs) transient, serial encounters (10-20min), upregulation of activation markers
  - Stage 2: (~14 hrs) stable binding events lasting hours, initiation of cytokine production
  - Stage 3: rapid motility resumed, cell proliferation, short pMHC contacts (10-20min)
- All evidence points to T cells integrating TCR signalling over serial DC contacts, with stage transitions occurring as signal thresholds are reached.
- Henrickson et al (2008) showed that the duration of DC binding depends on accumulated TCR stimulation, and highly stimulated cells divide sooner (i.e. stage timing is variable).

Model execution

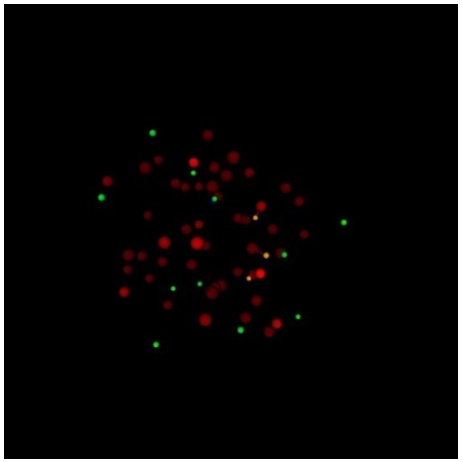
In each 15 second time step:

- VEGF secreted, HEV vascularization changes
- T cells enter, leave the blob
- T cells move
- T cells form and break DC contacts
- Bound cognate cells accumulate TCR signal (-> S)
- Cognate T cell activation stage updated
- Cognate T cells divide (depends on S level)
- With permission, activated cells leave
- Cognate T cells may die (depends on S level)
- DC antigen decays
- DCs arrive, DCs die

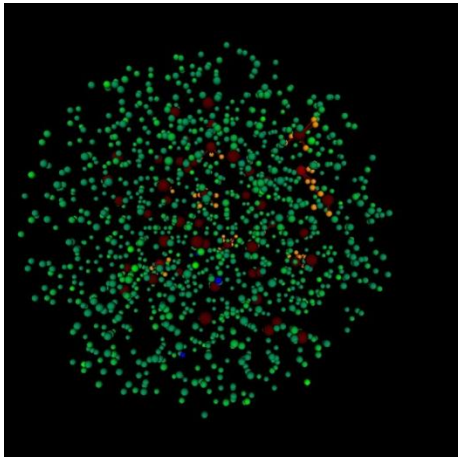
Typical simulated response progression



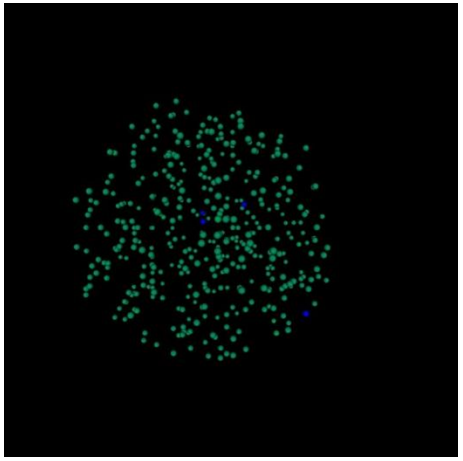
Day 0



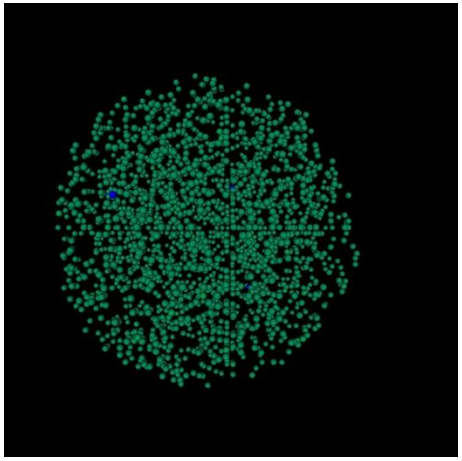
Day 2



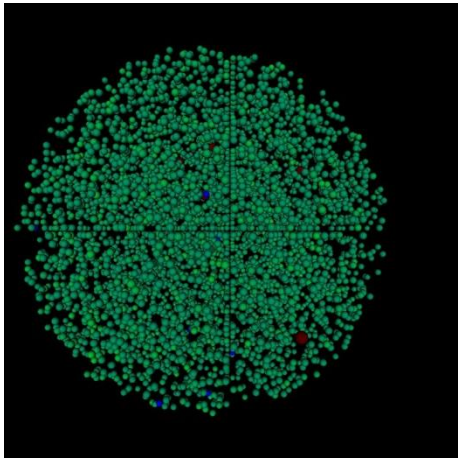
Day 4



Day 10



Day 8



Day 6

Adoptive transfer experiment

(Franca Ronchese, Evelyn Hyde)

Aim: To investigate early proliferative events in adoptive transfer model. How do Ag-specific CD8+ and CD4+ T cells proliferate in response to DC+OVA peptide in LN in a model where further lymphocyte entry is halted by αCD62-L treatment?

Protocol:

- Inject DCs pulsed with Class I and Class II peptides (labeled with Cell Tracker Orange) into mouse forearms.
- Next day inject i.v. 1.6 x 10^6 each of AutoMACS-enriched CD4+ or CD8+ CFSE-labeled T cells (OT-II, OT-I, respectively). 2 hours later treat mice with 100 μg αCD62L antibody (i.p.). Also treat mice remaining in experiment at 48 hours with αCD62L again (same dose/route)
- Remove draining lymph nodes from 4 mice at days 1, 2, 3 and 4 and analyse by flow cytometry for presence of dendritic cells and extent of CD4 and CD8 T cell proliferation (CFSE dilution). Also collect spleens at days 3 and 4 to check for T cell proliferation

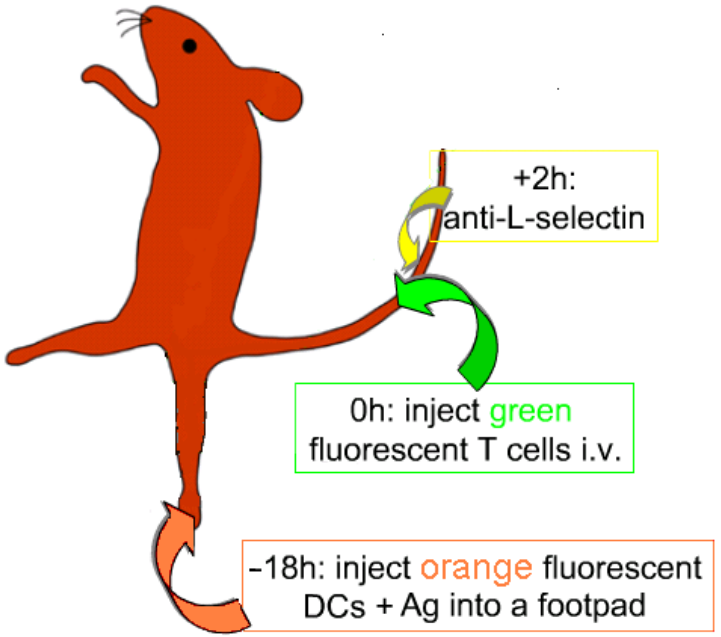
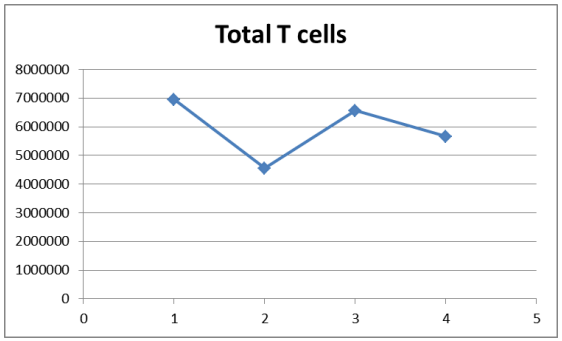
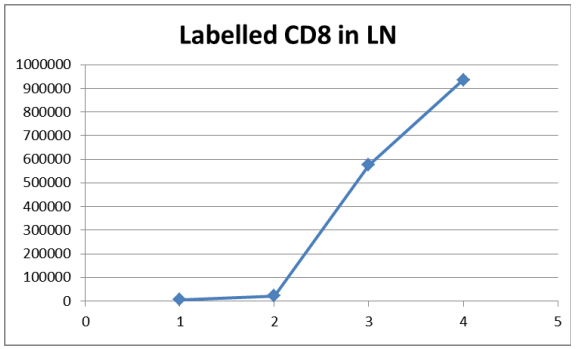


Figure adapted from: Henrickson et al. Science Signaling 1(12), 2008

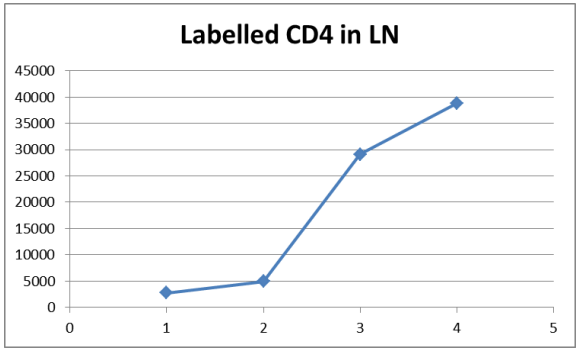
Experiment results



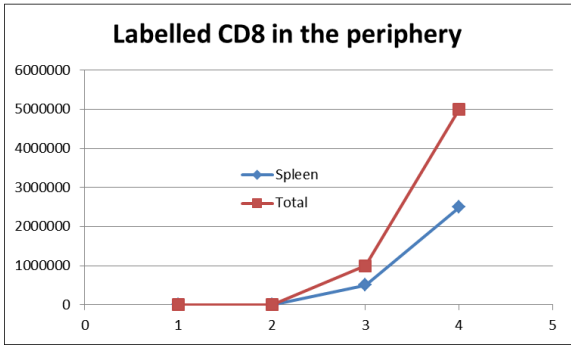
The total T cell population dropped somewhat but stabilized. (4 mice)



CD8 cells proliferated very strongly in day 3 (from an initial count of 5180). Apparent proliferation in day 4 is much reduced, because cells are leaving the LN.



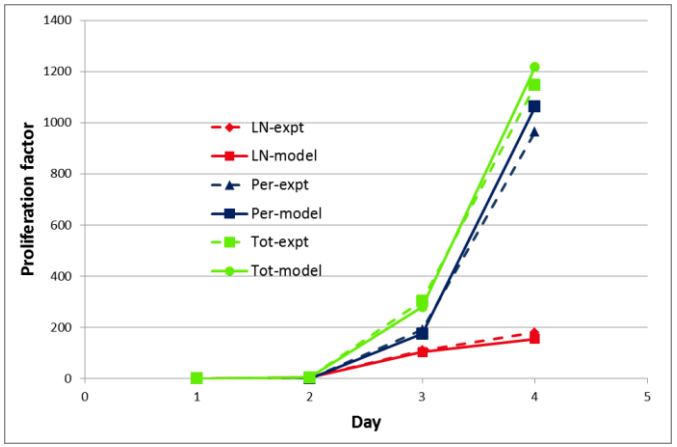
A low level of CD4 proliferation (from an initial count of 2770) compared with CD8. (Presumably reflects much lower TCR avidity for OT-II peptide.)

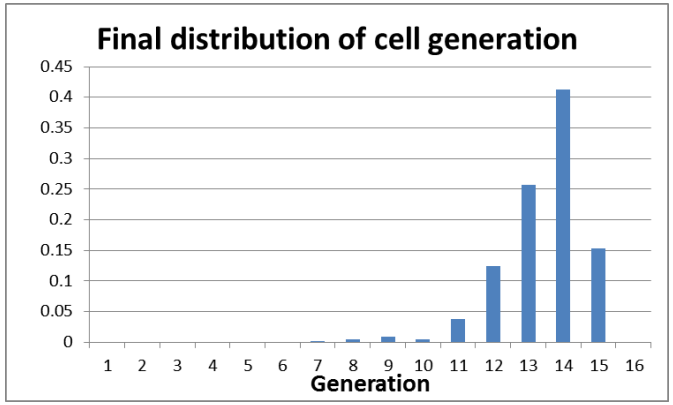


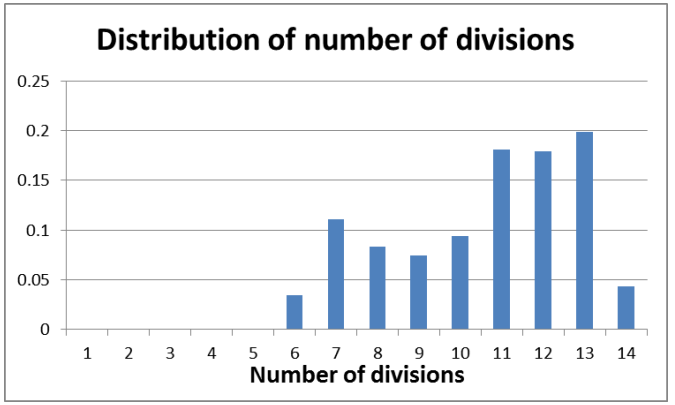
Labelled cells start to appear in the spleen in day 3, and clearly proliferation continues. The total count for the periphery is crudely estimated to be twice the spleen count (2 mice).

Typical simulation results

- First cell divides at about 40h
- Subsequent divisions take about 3.5h
- Division effectively ends before end of day 4
- Activated cell egress suppressed until generation 6
- Big burst of egress in day 3, continuing in day 4







Probabilistic chemotaxis on a lattice

For each $i = 1,.., N$, let $\boldsymbol{u}(i)$ be the vector representing a jump in the ith direction, then the angle between the chemokine gradient vector $\boldsymbol{v}$ and $\boldsymbol{u}(i)$ is θ_i, where

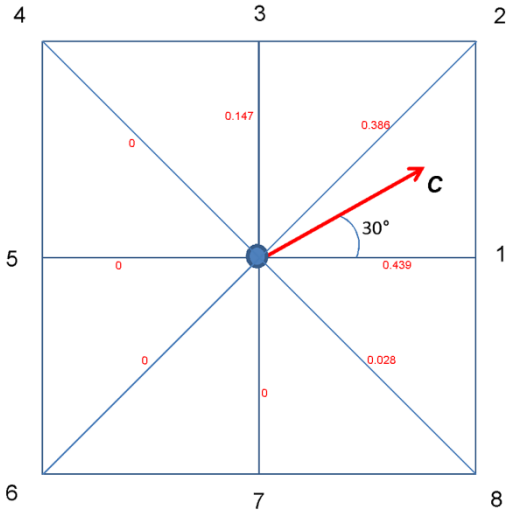
$$\cos(\theta_i) = \frac{v \cdot u(i)}{|u(i)|}$$

The relative amount of chemotactic influence in the direction $\boldsymbol{u}(i)$ is given by $w(i)$, where

$$w(i) = 0 \quad \text{if } \cos(\theta_i) < 0, \text{ else } \quad w(i) = \frac{\cos^2(\theta_i)}{|u(i)|}$$

For each $i = 1,..,N$, the pure chemotaxis jump probability $p_c(i)$ is proportional to $w(i)$:

$$p_c(i) = \frac{w(i)}{\sum_{k=1}^{N} w(k)}$$



(2D for clarity)

If the jump probability in the absence of chemotaxis is $p(i)$, then the net probability $p^*(i)$ is

$$p^*(i) = (1-\alpha)\,p(i) + \alpha\, p_c(i)$$

where the weighting α is determined by the magnitude of the chemokine gradient

DC-T cell chemotaxis

How would DC scanning rates be affected if T cells were attracted to them?

Which T cells?

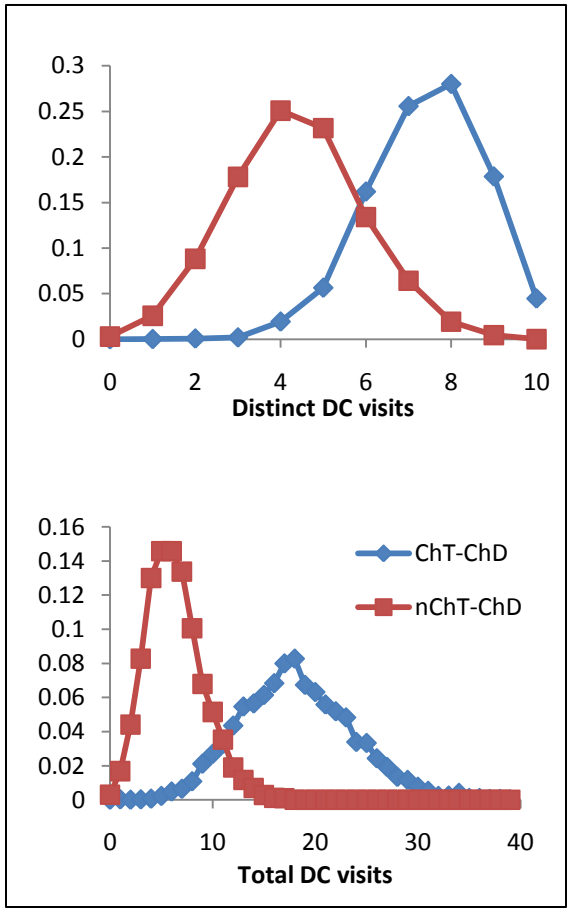
Influence on CD4 help delivery?

Preliminary exploratory simulations:

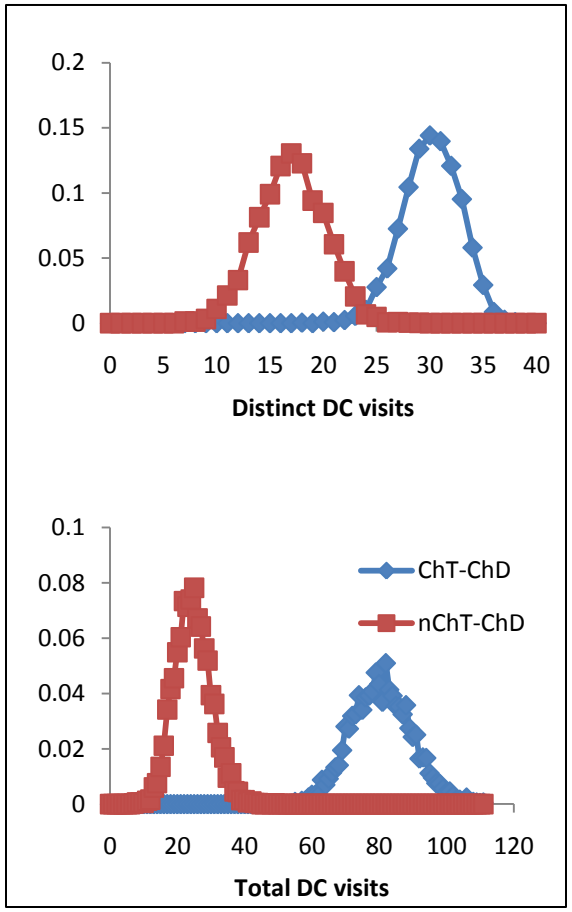
- Fixed population of 50k, no trafficking
- Simulations of cell movement over 48h
- A fraction (20%) of cells are tagged and their DC contacts counted
- Only brief contacts (mean 3 min), no cognate TCR-pMHC
- Variable fractions of DCs and T cells are secreting chemokine and chemotactically receptive, respectively.
- Count total DC encounters, and distinct encounters
- Record first contact time
- Variable number of DCs

DC visit distributions

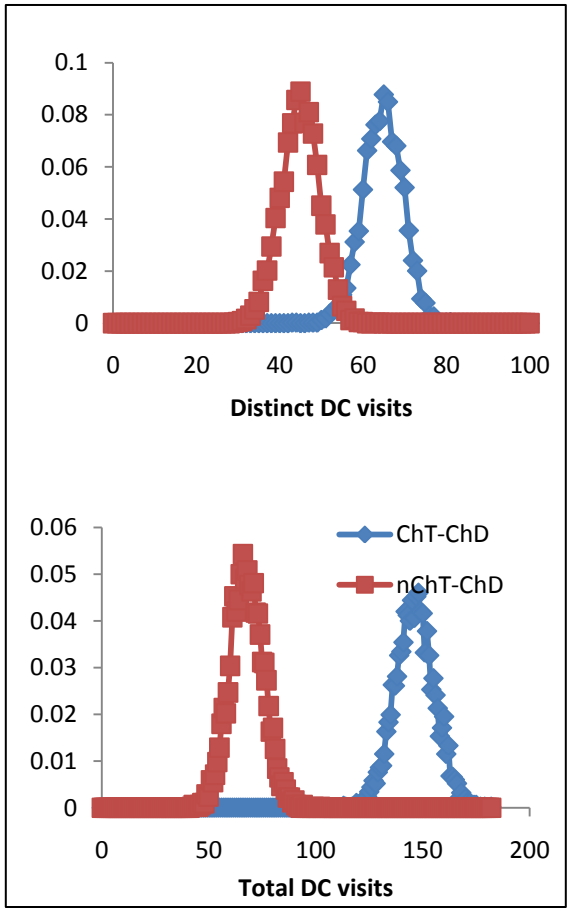
ChT = chemotactic T cell
nChT = non-chemotactic T cell
ChD = chemokine secreting DC



10 DCs
5000 Tcells/DC

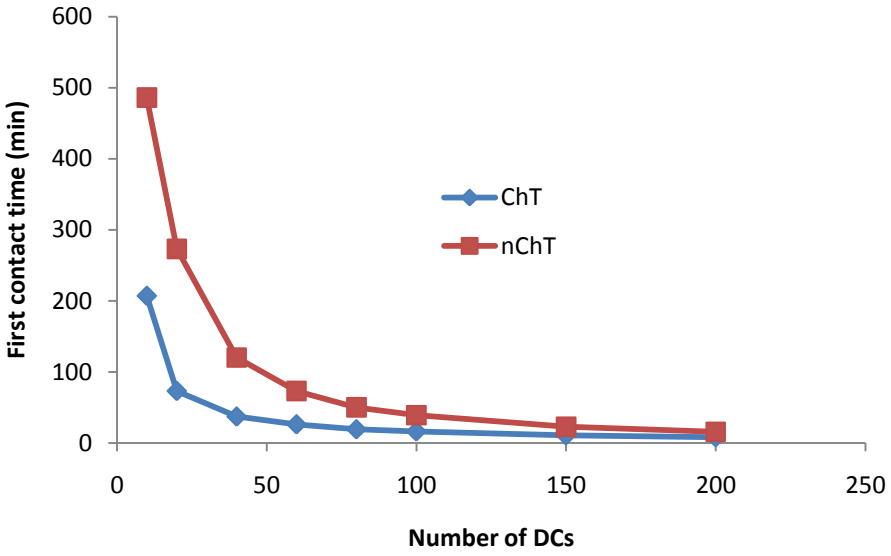


40 DCs
1250 Tcells/DC



100 DCs
500 Tcells/DC

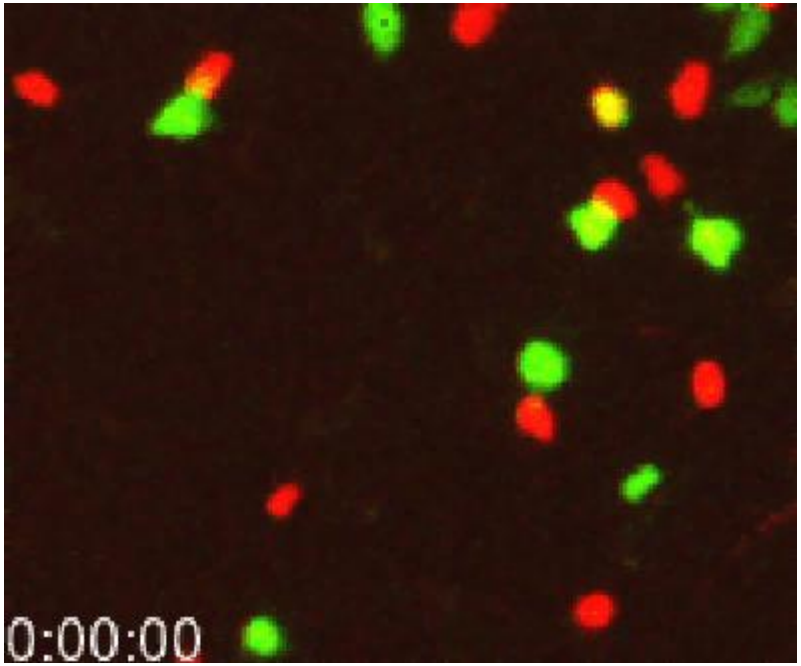
Average first contact time



B cell model

A collaboration with Takaharu Okada
RIKEN Research Centre for Allergy and Immunology, Yokohama.

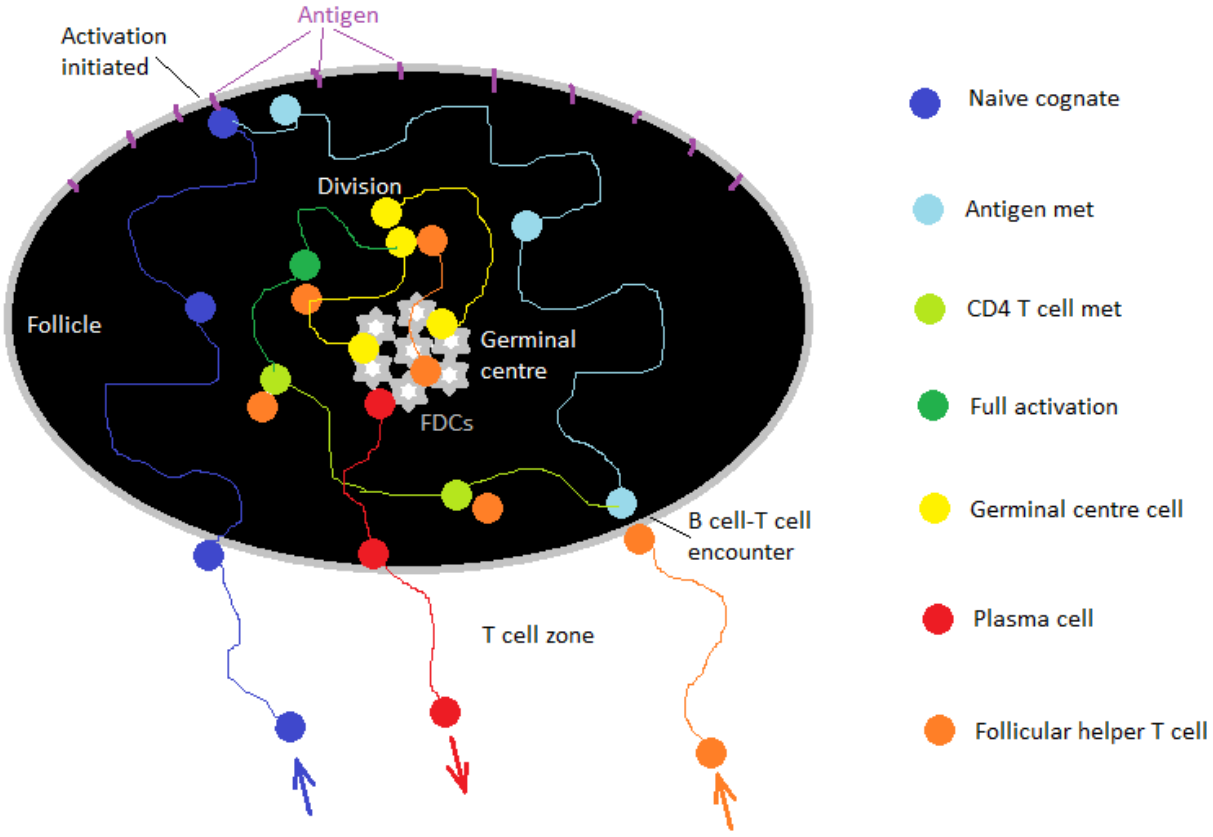
Taka's lab specializes in using two-photon intravital microscopy to observe lymphocyte interactions in the mouse lymph node.



B cell
T cell

Germinal centre formation

- B cell behaviour in the LN is strongly influenced by chemotactic cues
- As cognate cells pass through stages of activation, receptor expression changes and different chemokines act
- At least four chemokine/receptor pairs are known to be important in choreographing B cell motion and activation:
  - S1P S1PR1, S1PR2
  - CCL21 CCR7
  - Oxysterols EBI2
  - CXCL13 CXCR5
- The initial aim of the modelling is to explore hypotheses about the action of chemokines and chemotaxis in forming and maintaining a germinal centre



Stages of B cell activation

0 enters follicle, roams and randomly approaches the top surface (EBI2, S1PR1?)
1 binds antigen, activation initiated, CCR7 upregulated, roams with bias to T zone interface as a result of CCL21/19 secreted by stromal cells in the T cell zone.
2 encounters T cell, achieves full activation, downregulates CCR7, upregulates EBI2
3 begins to divide, cycle time about 10 h, roam with bias to top surface (oxysterols)
4 for some fraction, BCL6 upregulated, commitment to GC differentiation. EBI2 and S1PR1 expression abolished, S1PR2 is expressed for the first time.
5 continuing cell division, and some rate of cell death.

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You for listening!