Anatomy of Immune Responses

Micro 204: Molecular and Cellular Immunology

Lecturer: Jason Cyster

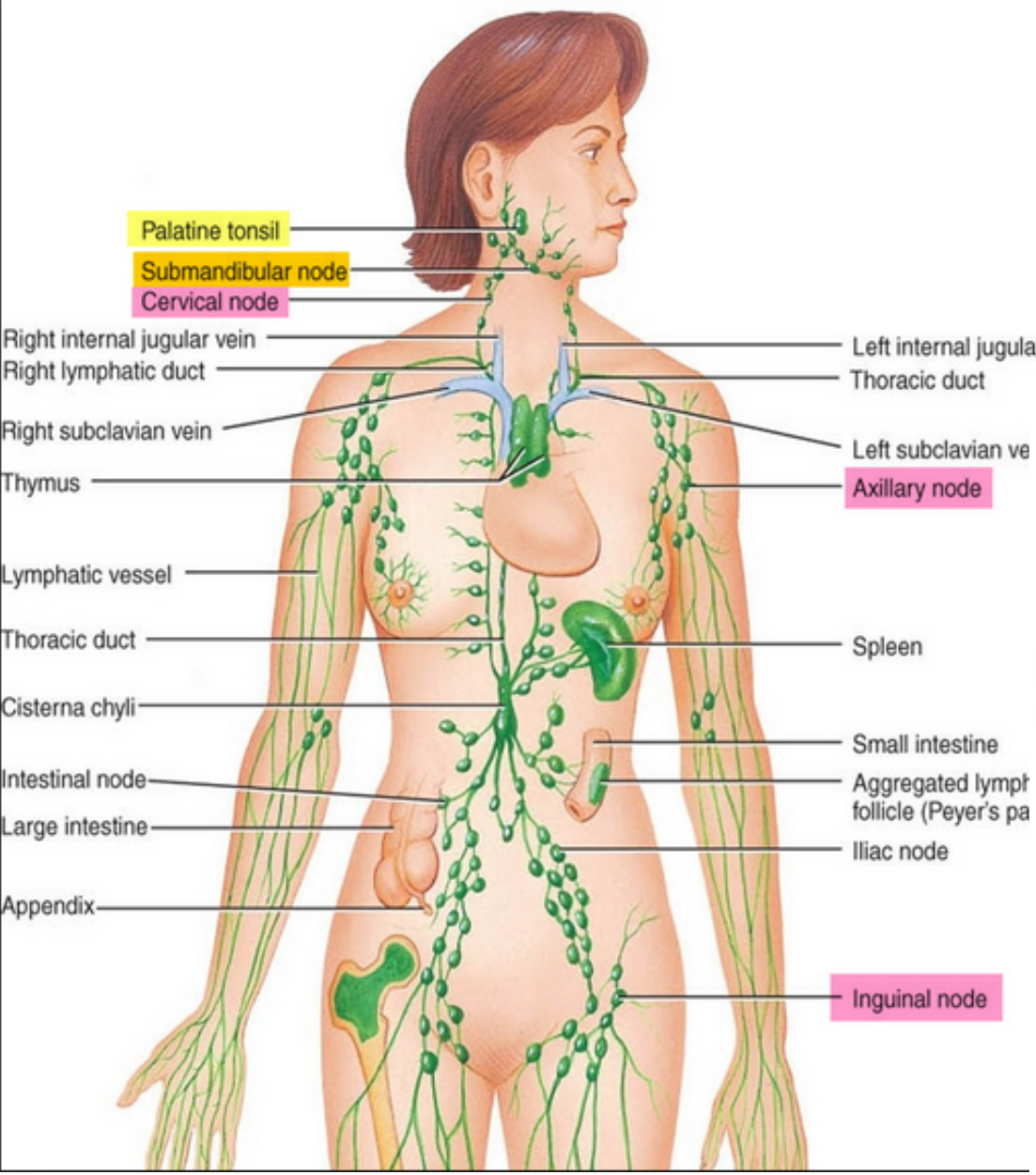
Lecture Outline

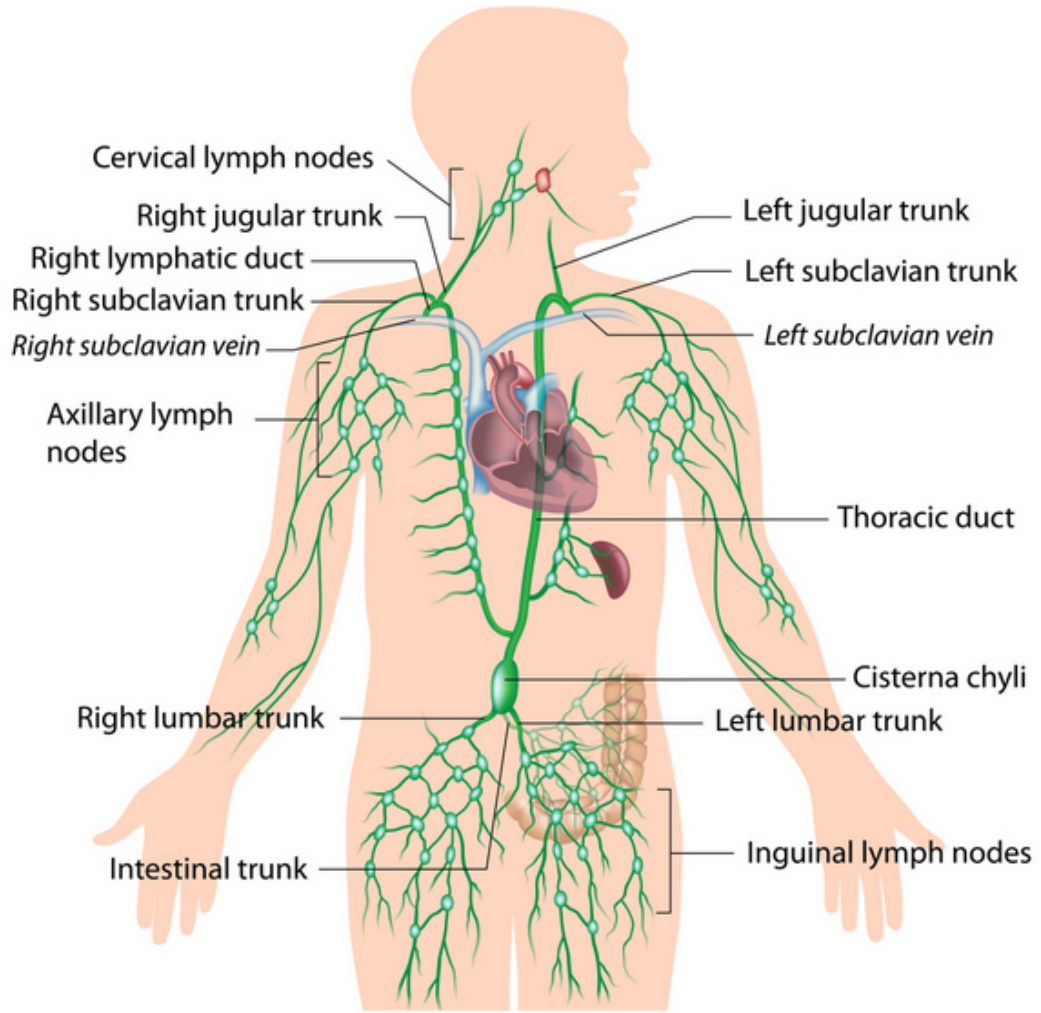
1. What are Secondary Lymphoid Organs and how do they function?

2. Why are Dendritic Cells so effective at initiating adaptive immune responses?

3. Where do B cells come in contact with antigen?

4. How do B cells find helper T cells specific for the same antigen?


Palatine tonsil
Submandibular node
Cervical node
Right internal jugular vein
Right lymphatic duct
Right subclavian vein
Thymus
Lymphatic vessel
Thoracic duct
Cisterna chyli
Intestinal node
Large intestine
Appendix
Left internal jugular vein
Thoracic duct
Left subclavian ve
Axillary node
Spleen
Small intestine
Aggregated lymph
follicle (Peyer's pa
Iliac node
Inguinal node

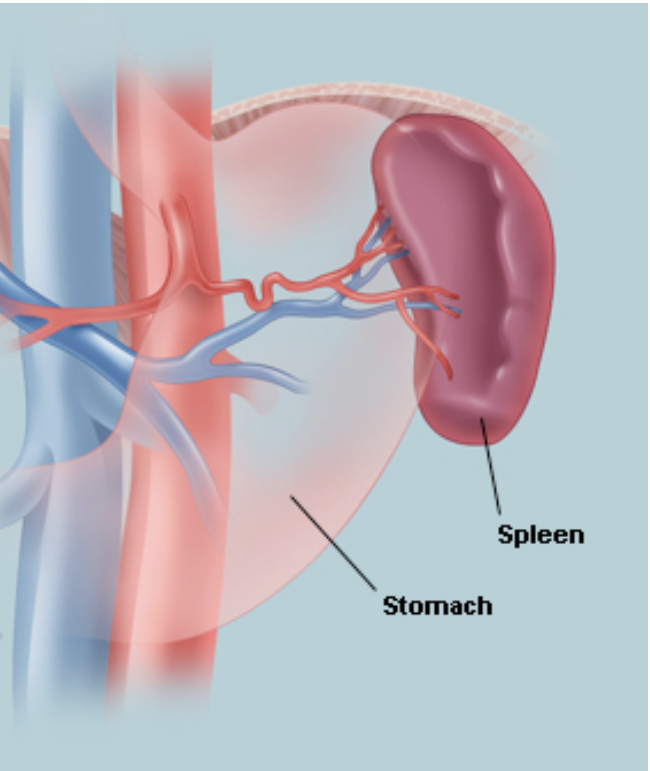

Cervical lymph nodes
Right jugular trunk
Right lymphatic duct
Right subclavian trunk
Right subclavian vein
Axillary lymph nodes
Left jugular trunk
Left subclavian trunk
Left subclavian vein
Thoracic duct
Cisterna chyli
Right lumbar trunk
Left lumbar trunk
Intestinal trunk
Inguinal lymph nodes

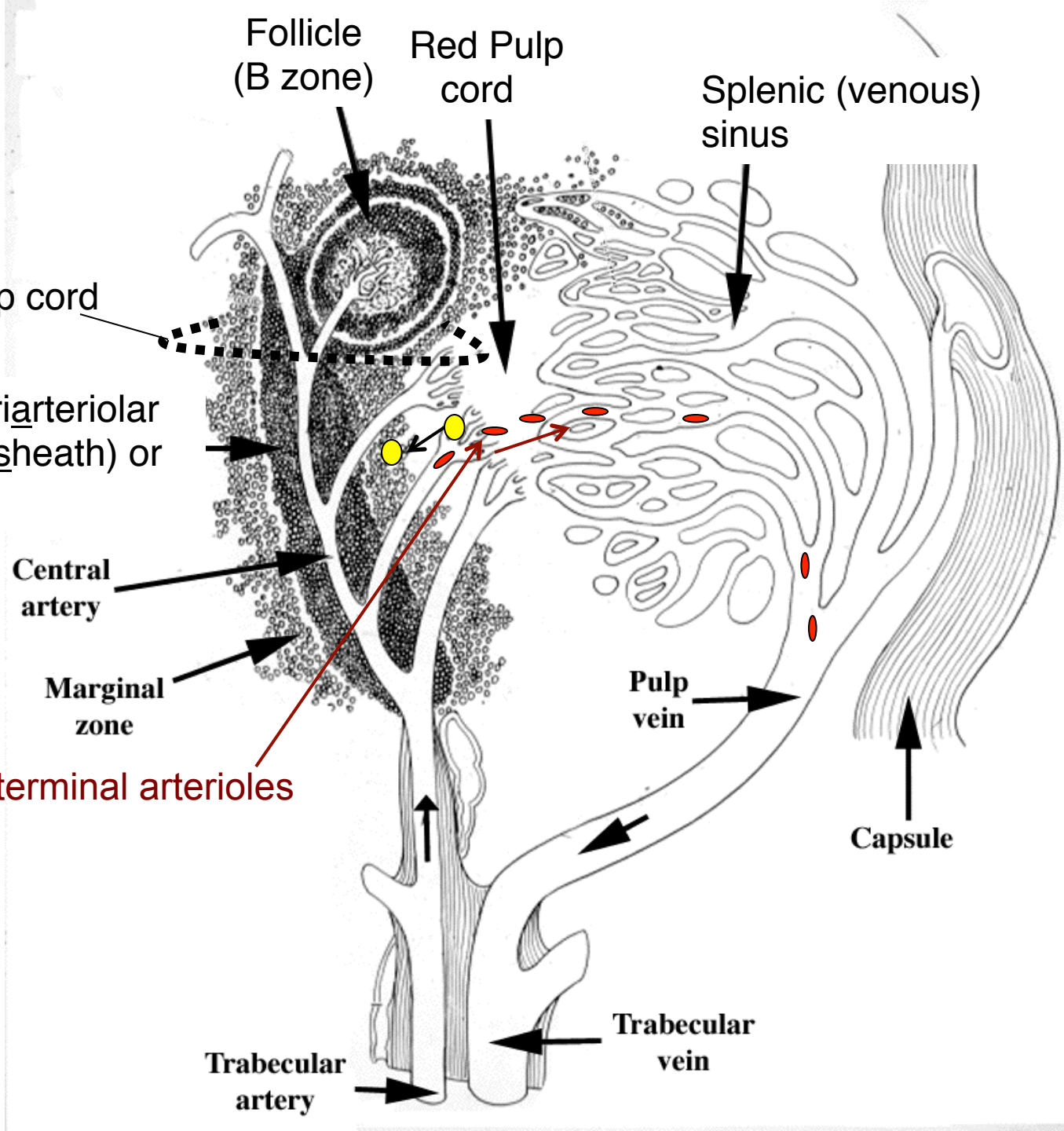
Spleen - A filter of the blood

- Two functions carried out in separate regions
  1) White-pulp is where immune responses against blood-borne antigens occur
  2) Red-pulp is responsible for monitoring and removing old or damaged RBCs

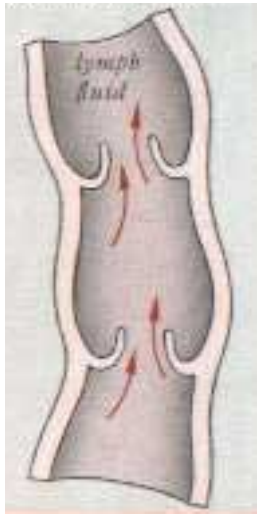
- Red-pulp consists of thin walled venous sinuses and dense collections of blood cells (including numerous macrophages) that form red-pulp cords (or cords of Billroth)
- Blood supply: branches of central arteries open directly into red-pulp cords, adjacent to the splenic sinuses (open circulation)
  - Released RBC must cross the sinus walls; interendothelial slits are a major mechanical barrier and only the most supple, mechanically resilient RBC survive; old and damaged cells are removed by macrophages

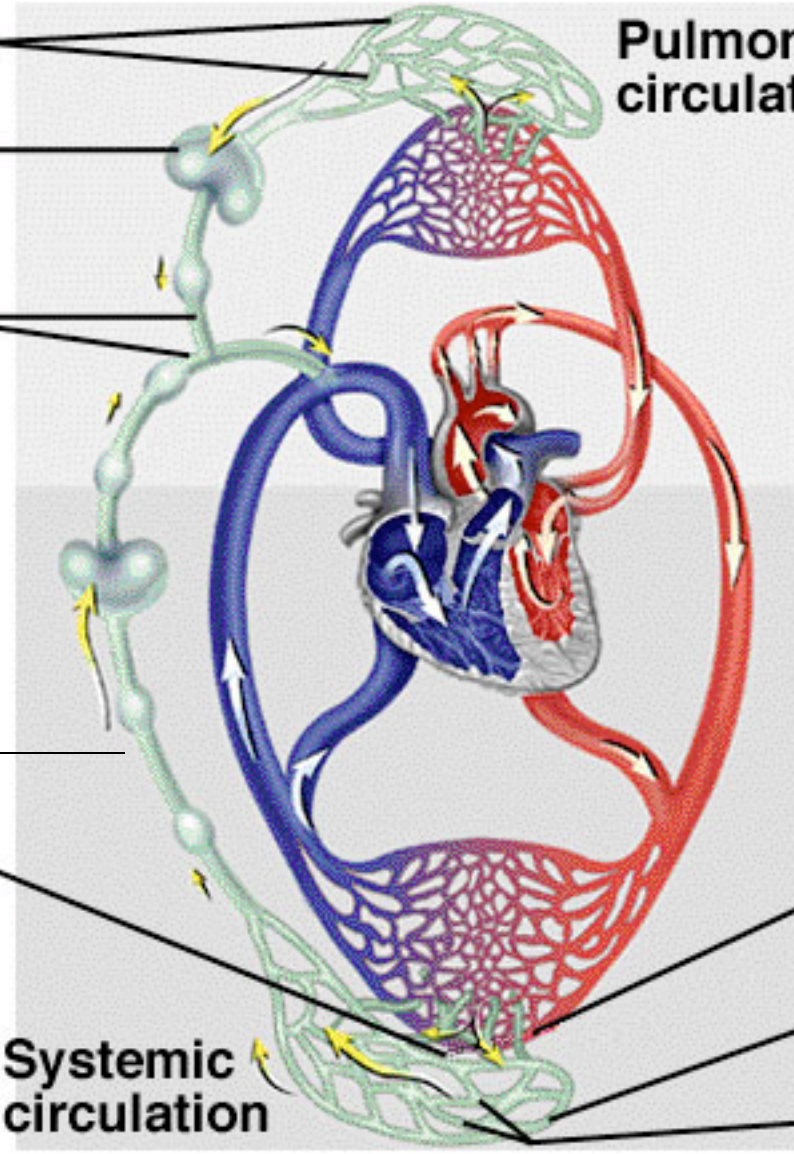
Anatomy of the Spleen





Lymphatics





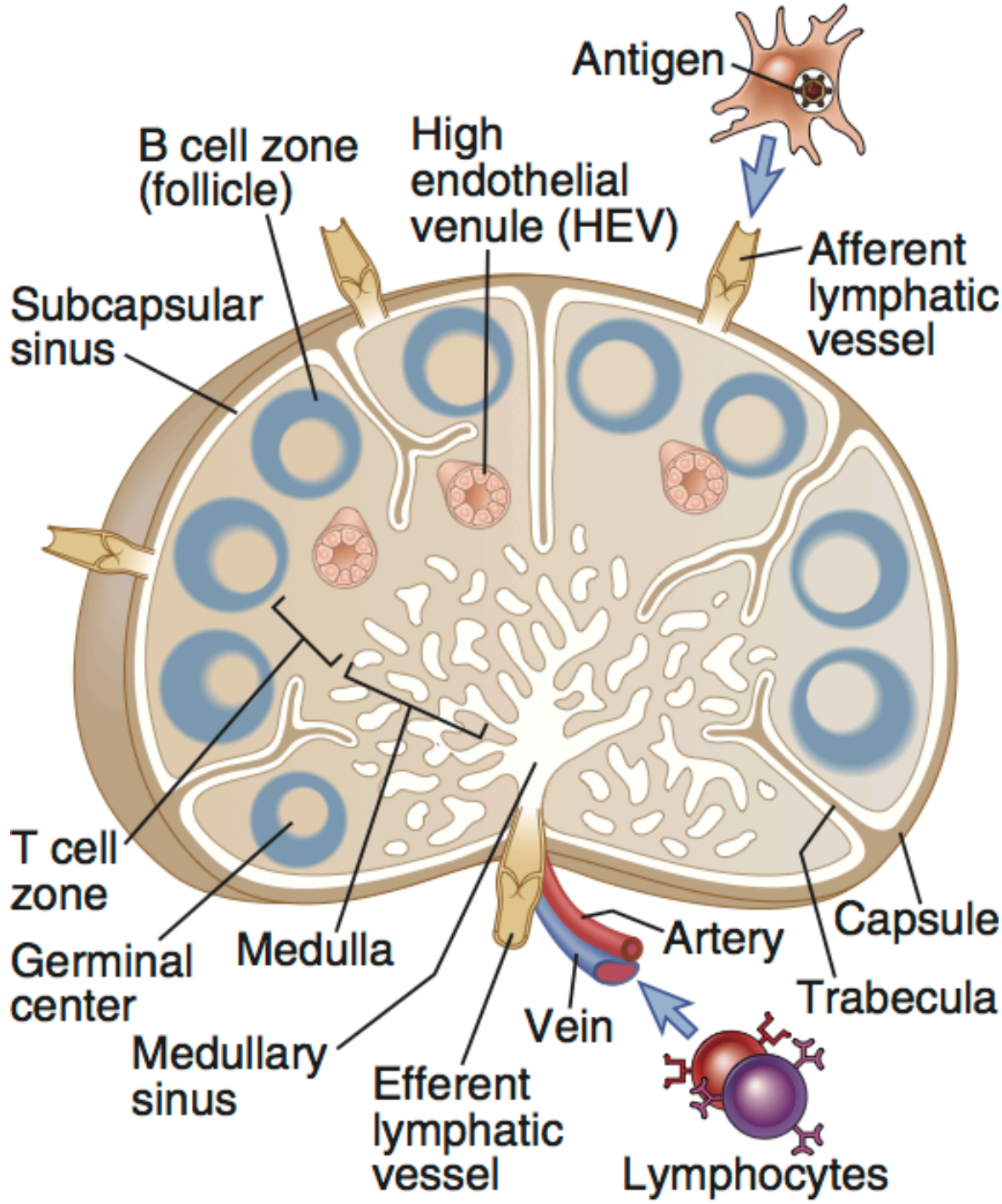
Lymph is filtered by Lymph Nodes before returning to circulation (liters per day)

Lymph contains T and B cells and dendritic cells

Anatomy of a Lymph Node

Filter antigens from the lymph

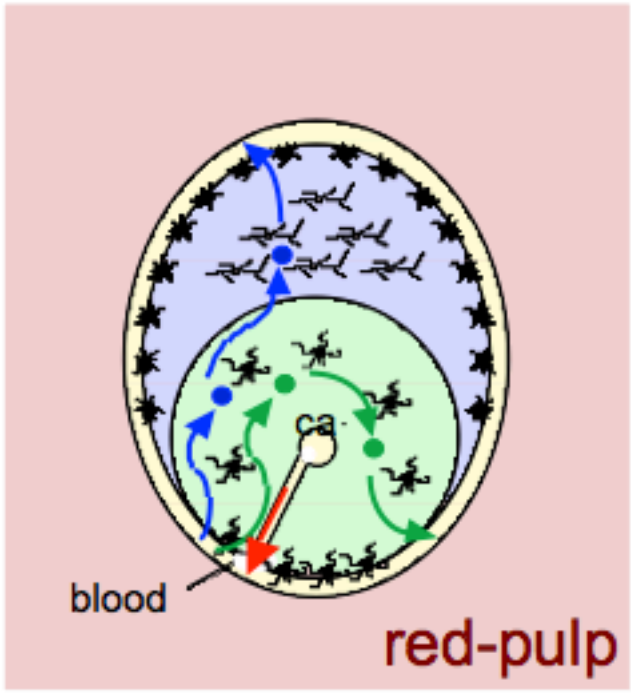
- for recognition by T and B cells
- for destruction by macrophages to prevent systemic spread



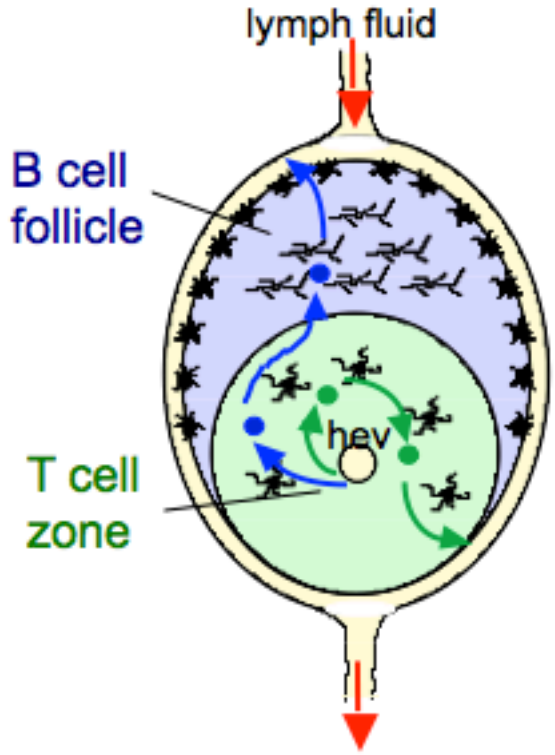
Abbas Fig. 1-12

SECONDARY LYMPHOID ORGANS

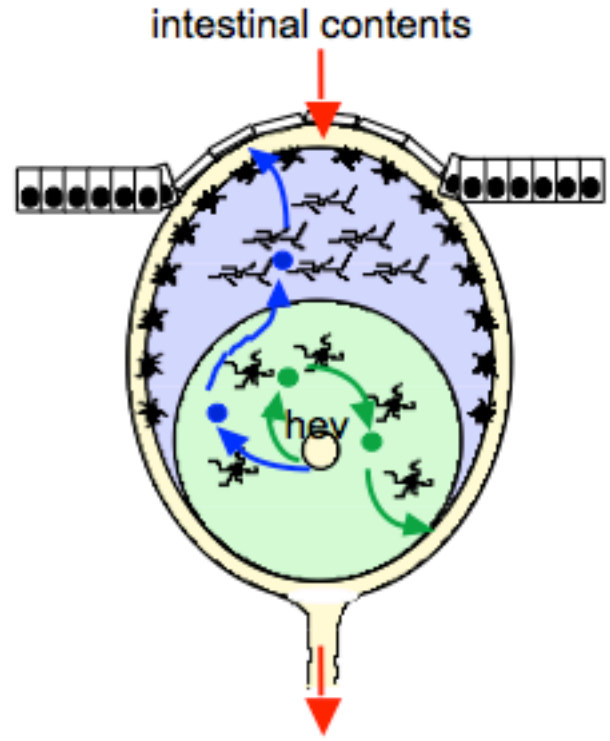
Splenic White-Pulp Cord



Lymph Node

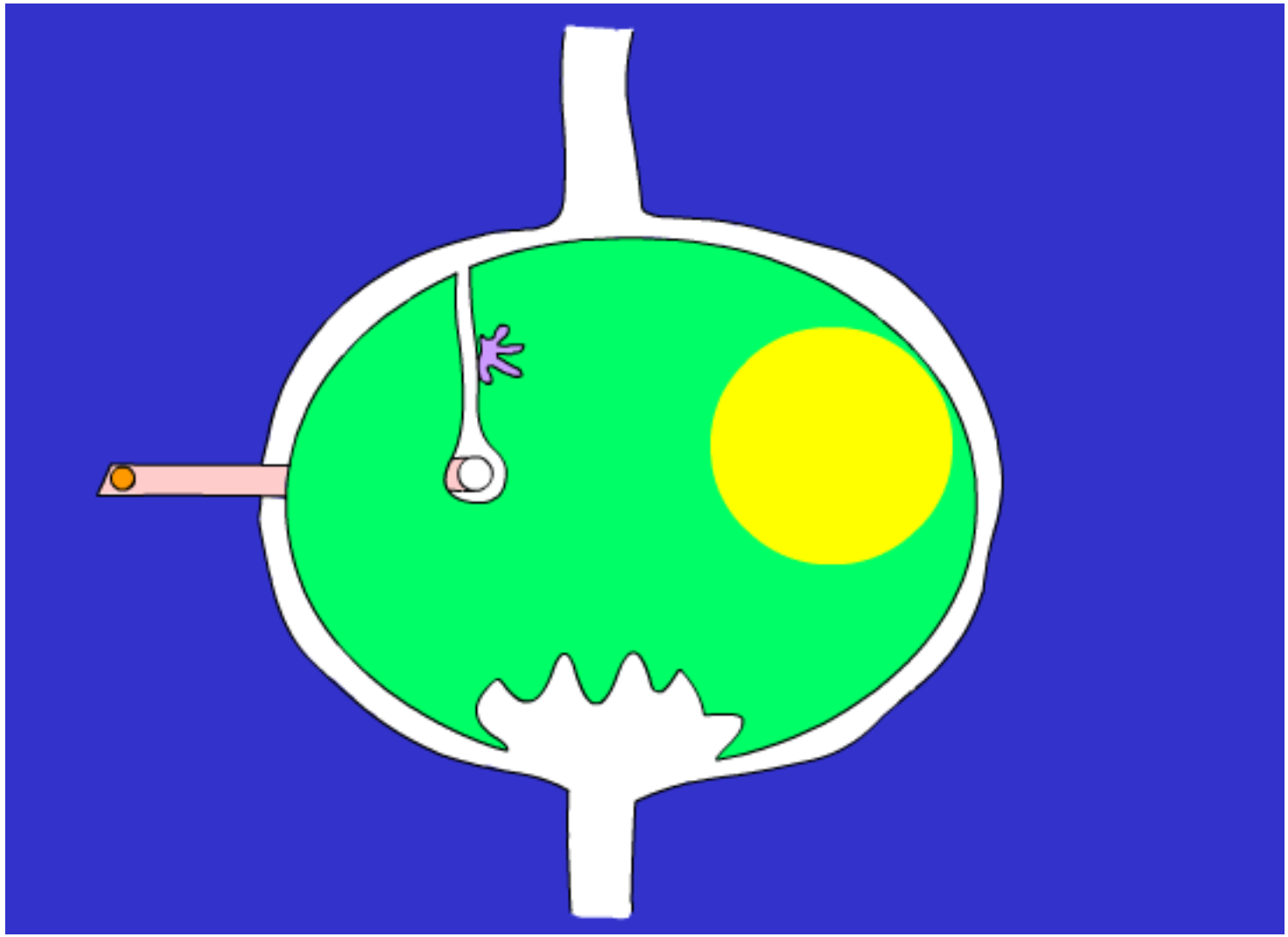


Mucosal lymphoid tissue
(e.g. Tonsil, Peyer's patch)



- filter antigens from body fluids
- bring together antigen presenting cells and lymphocytes
- help bring together antigen reactive (cognate) B and T cells

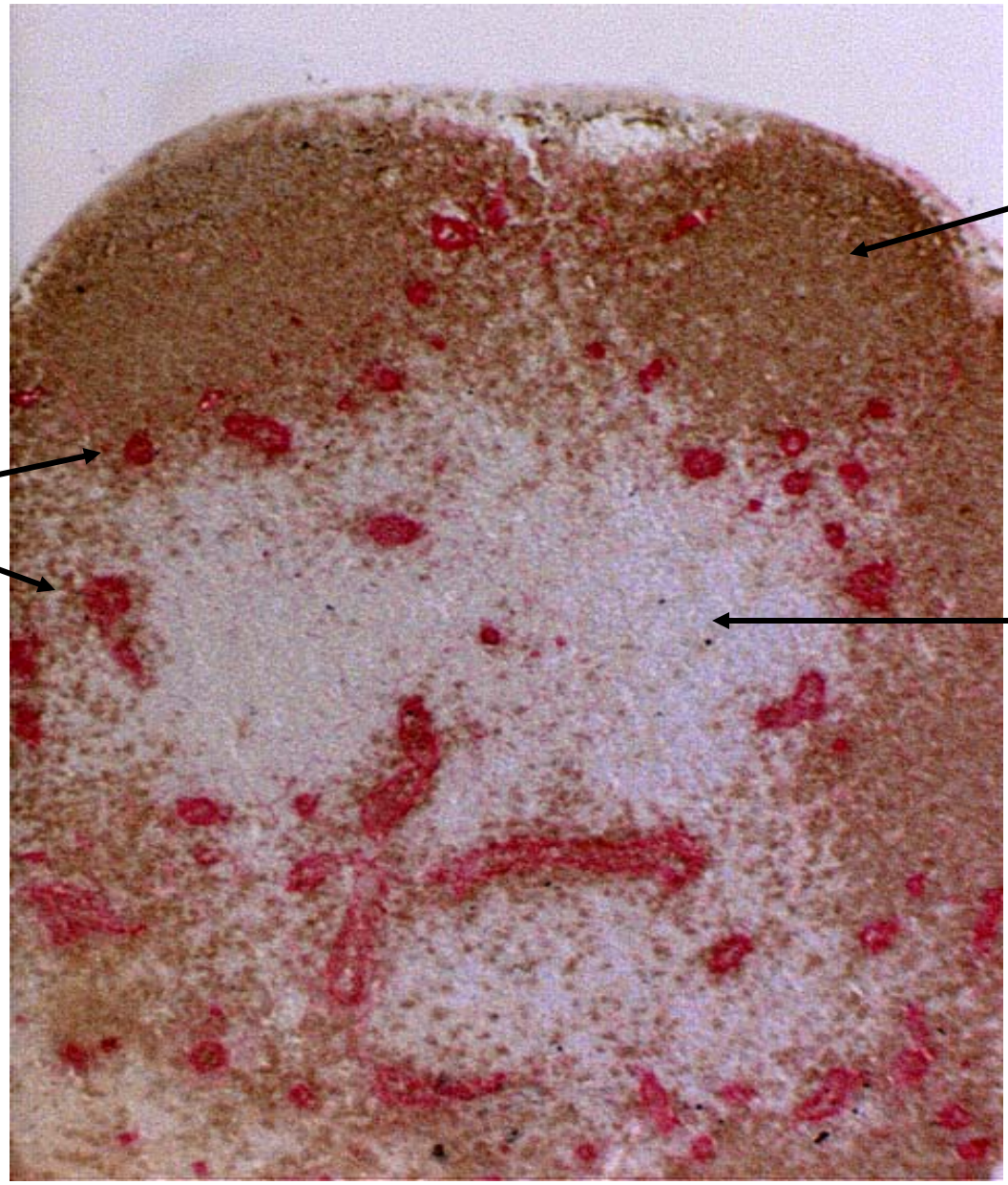
Primary immune response in a lymph node



See also Jenkins et al., (2001) Annu. Rev. Immunol. 19; 23

Provided by Marc Jenkins, Center for Immunology at the University of Minnesota

Lymphocytes traverse HEVs to enter lymph nodes and then compartmentalize in B cell follicles and T cell zones



HEV

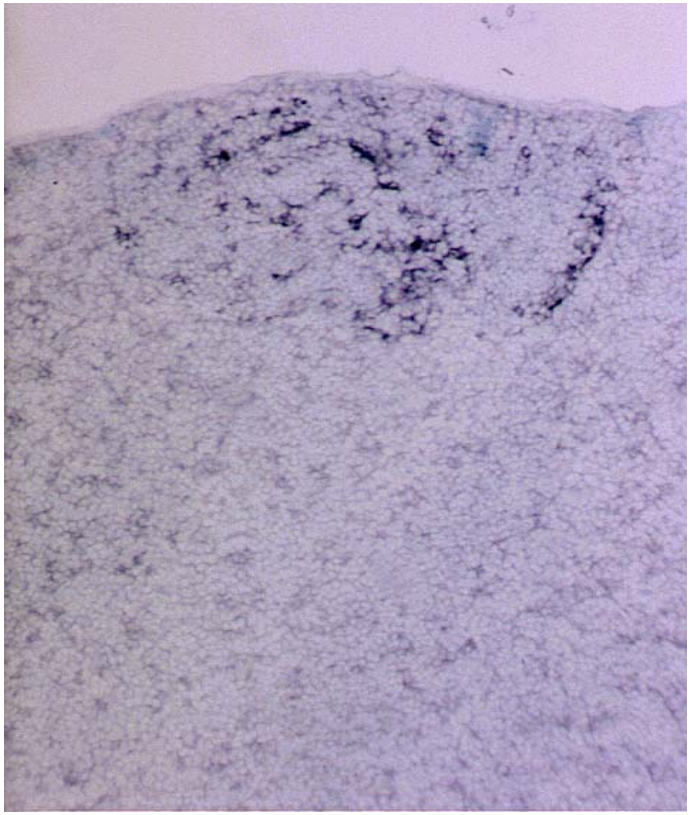
Follicle or B zone
- B cells
- FDCs

T cell area (paracortex)
- T cells
- DCs

LN section stained with:
B220
PNAd

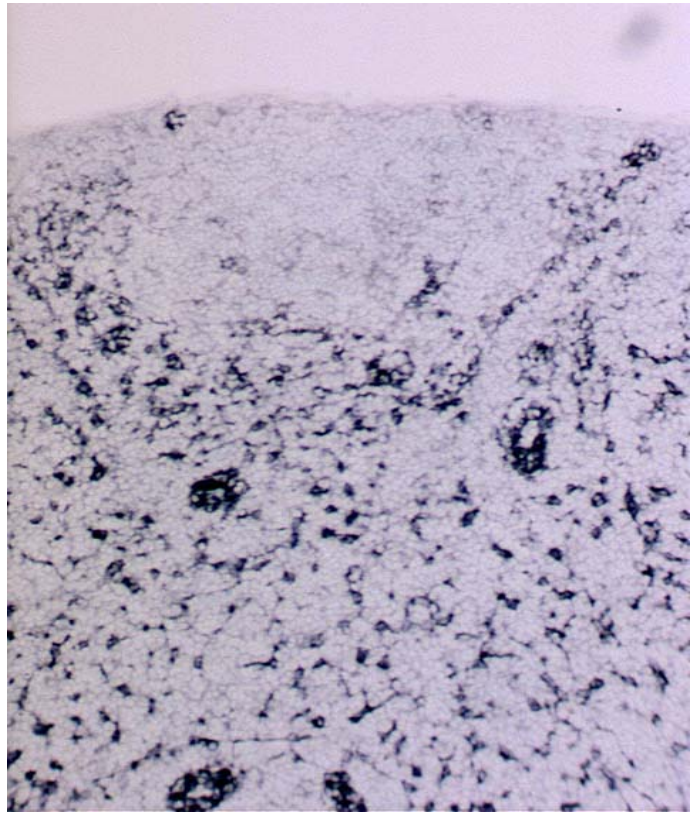
Lymphoid organ chemokine expression in lymph node

CXCL13 (BLC)



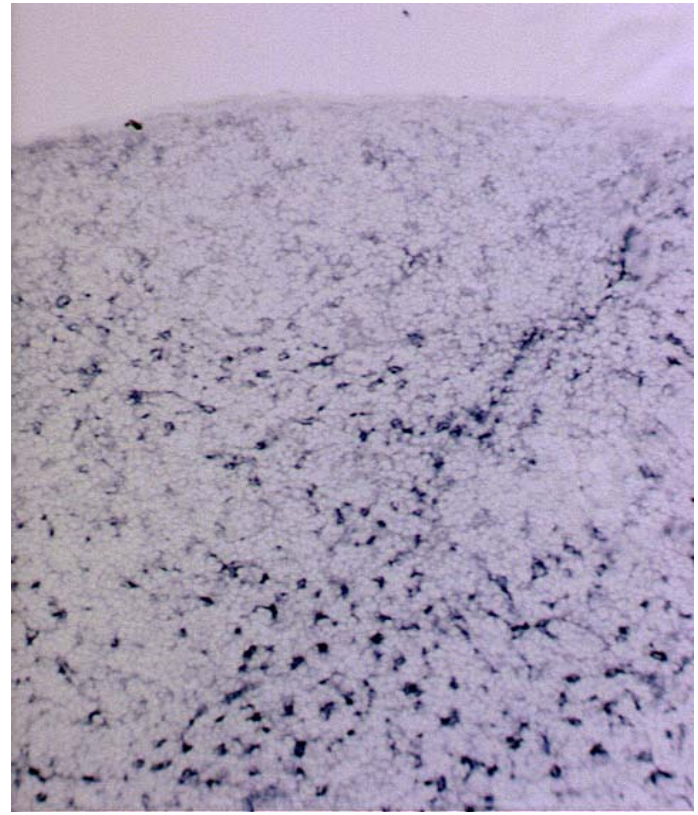
-> CXCR5

CCL21 (SLC)



-> CCR7

CCL19 (ELC)

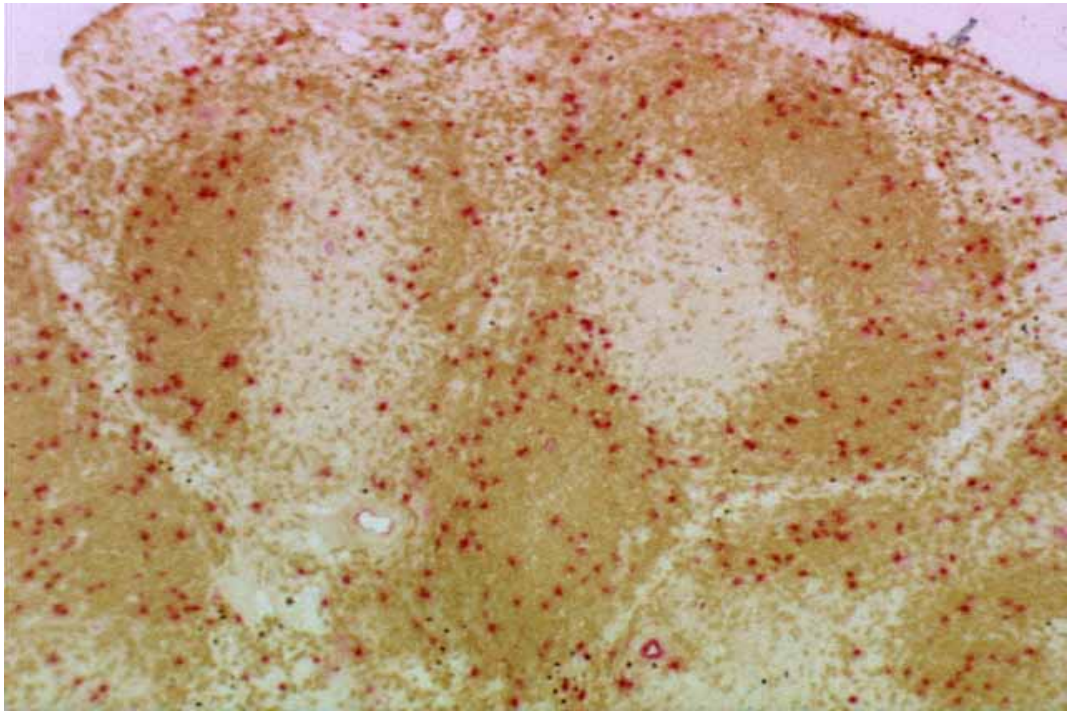


-> CCR7

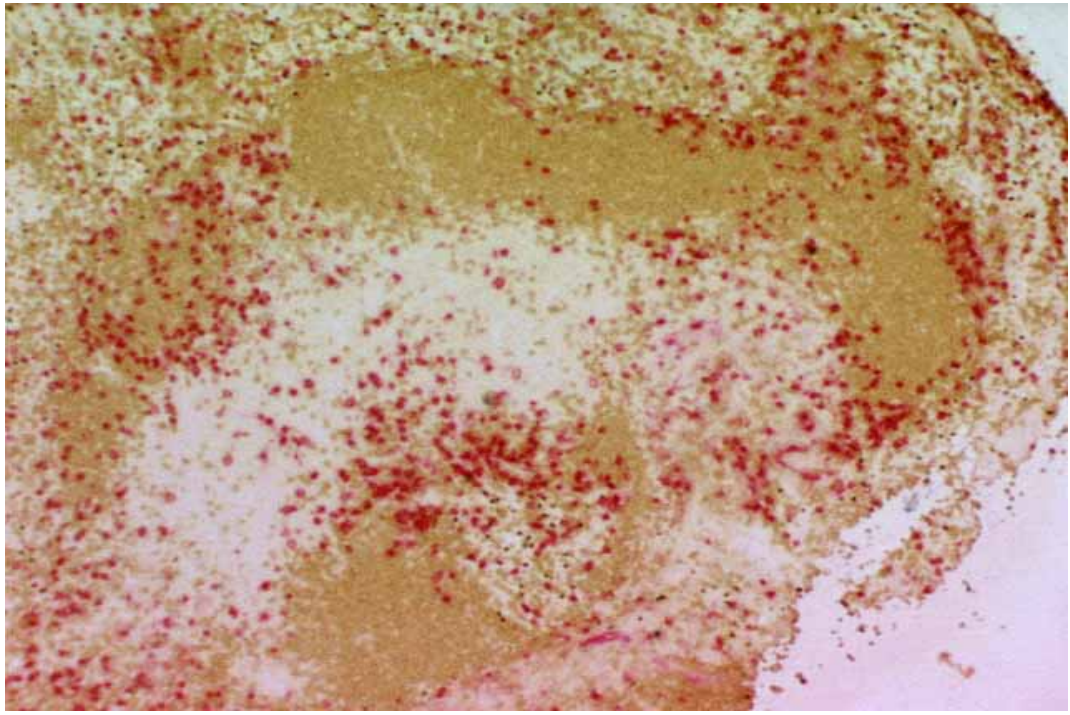
from Cyster, 1999
Science 286, 2098

CXCR5 is required for B cell migration into follicles

WT B cells -> WT



CXCR5-/- B cells -> WT

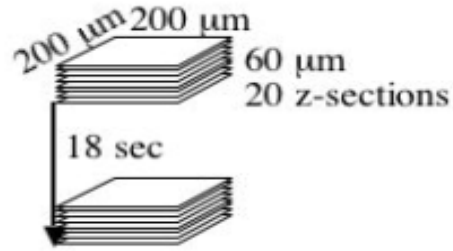


red = transferred B cells

brown = endogenous B cells

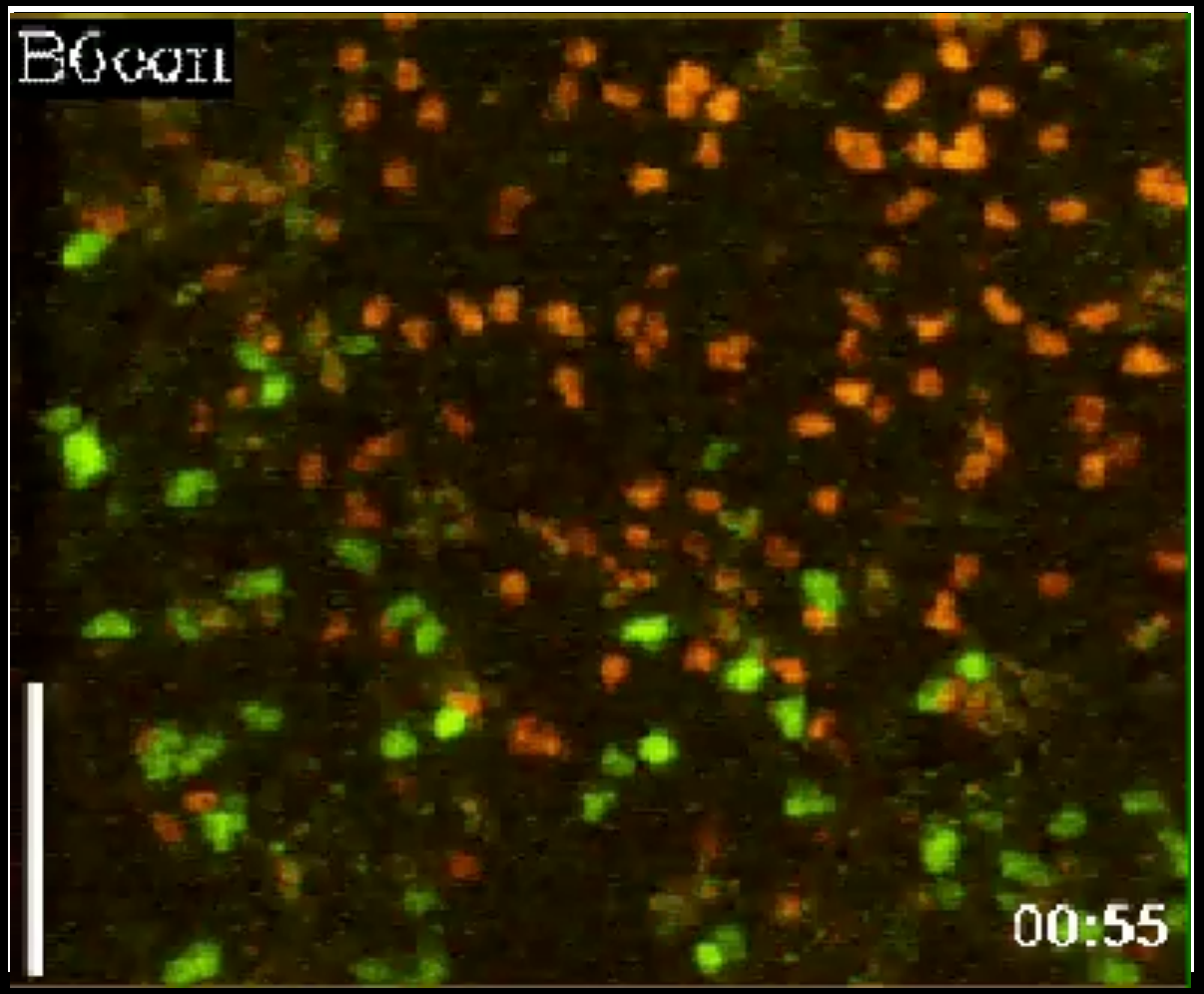
Lymphocyte migration within lymphoid tissue

- Two-photon microscopy of intact lymph node
  - High speed imaging at depths up to 500 μM



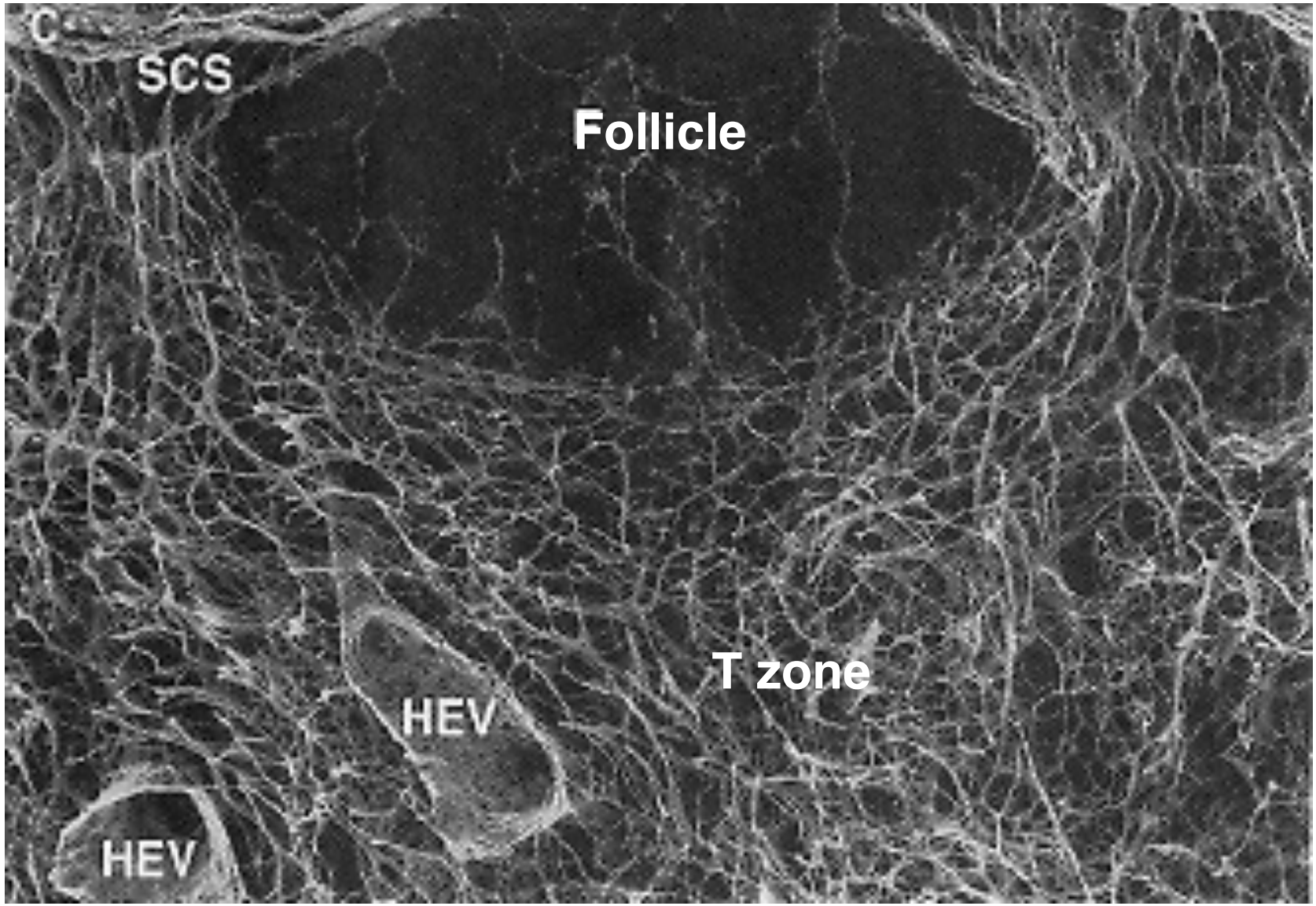
- Demonstrate that naïve B and T lymphocytes undergo extensive ‘random’ migration behavior
  - 5-6 μM / min for B cells
  - 10-12 μM / min for T cells

Movement of B and T cells within a lymph node in the absence of antigen



B cells (CMTMR labelled)
T cells (CSFE labelled)

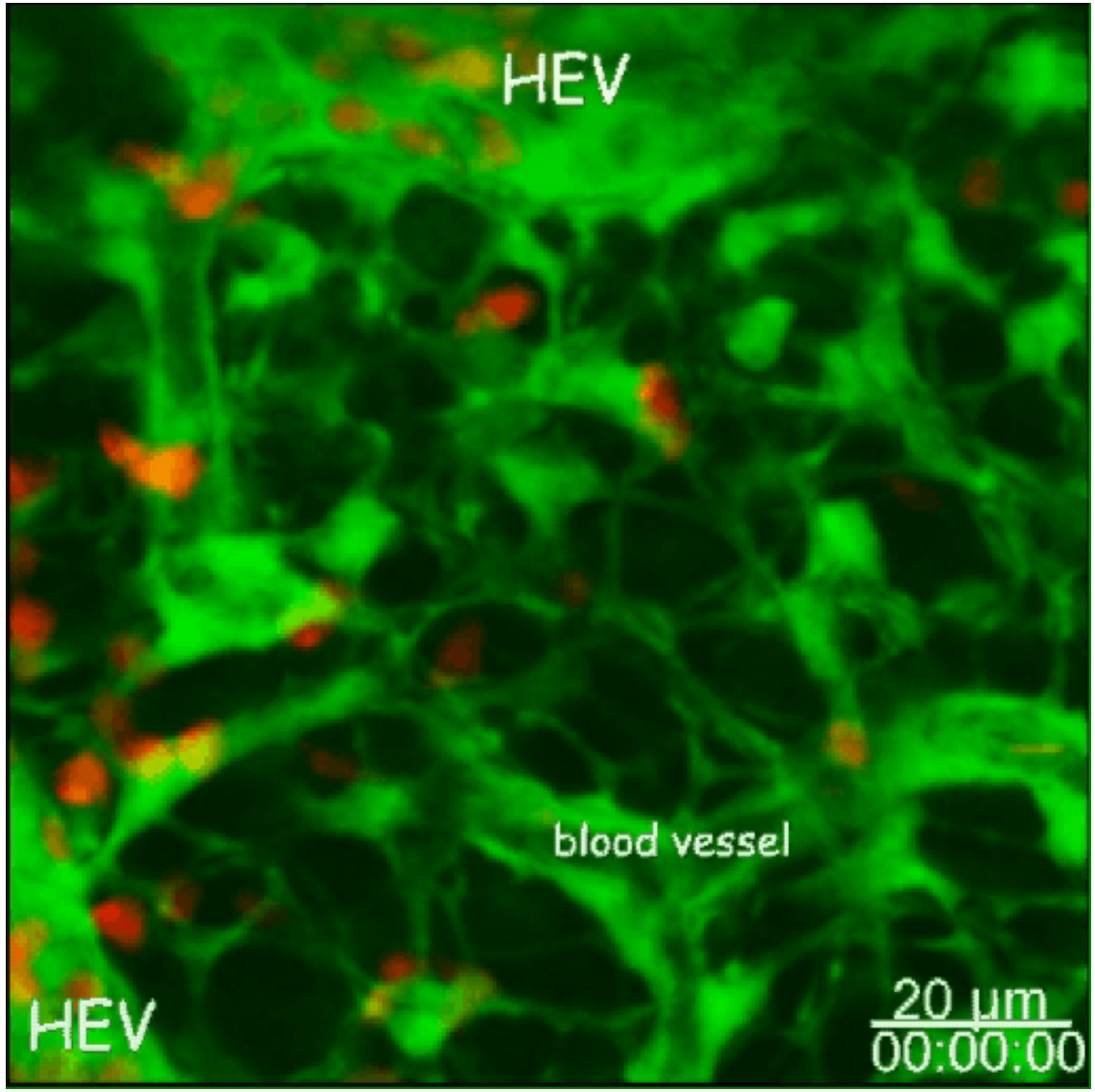
The ‘infrastructure’ of the lymph node

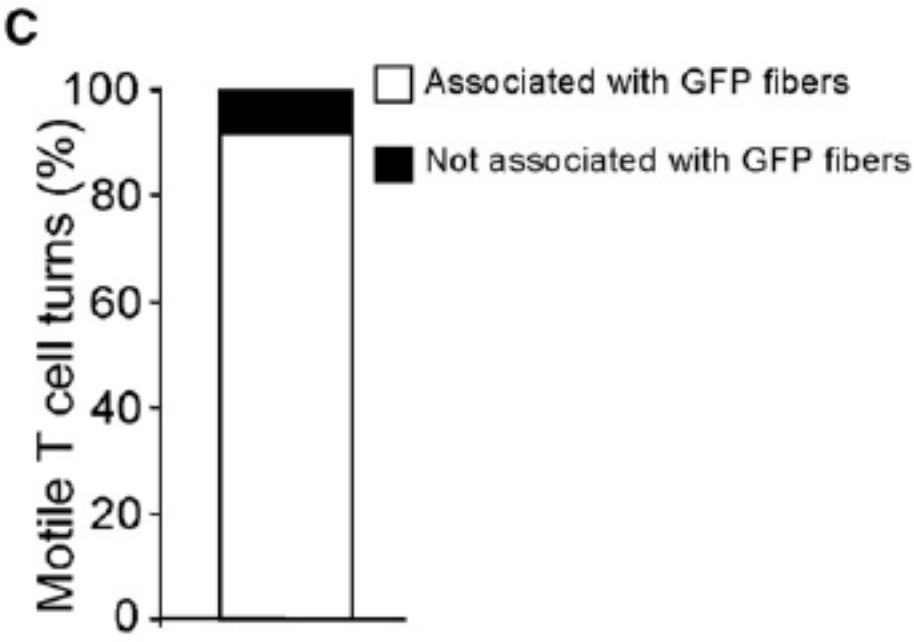


Scanning EM of collagen fiber network in rat LN after removal of cells

from Gretz et al., 1997, Imm. Rev. 156, 11

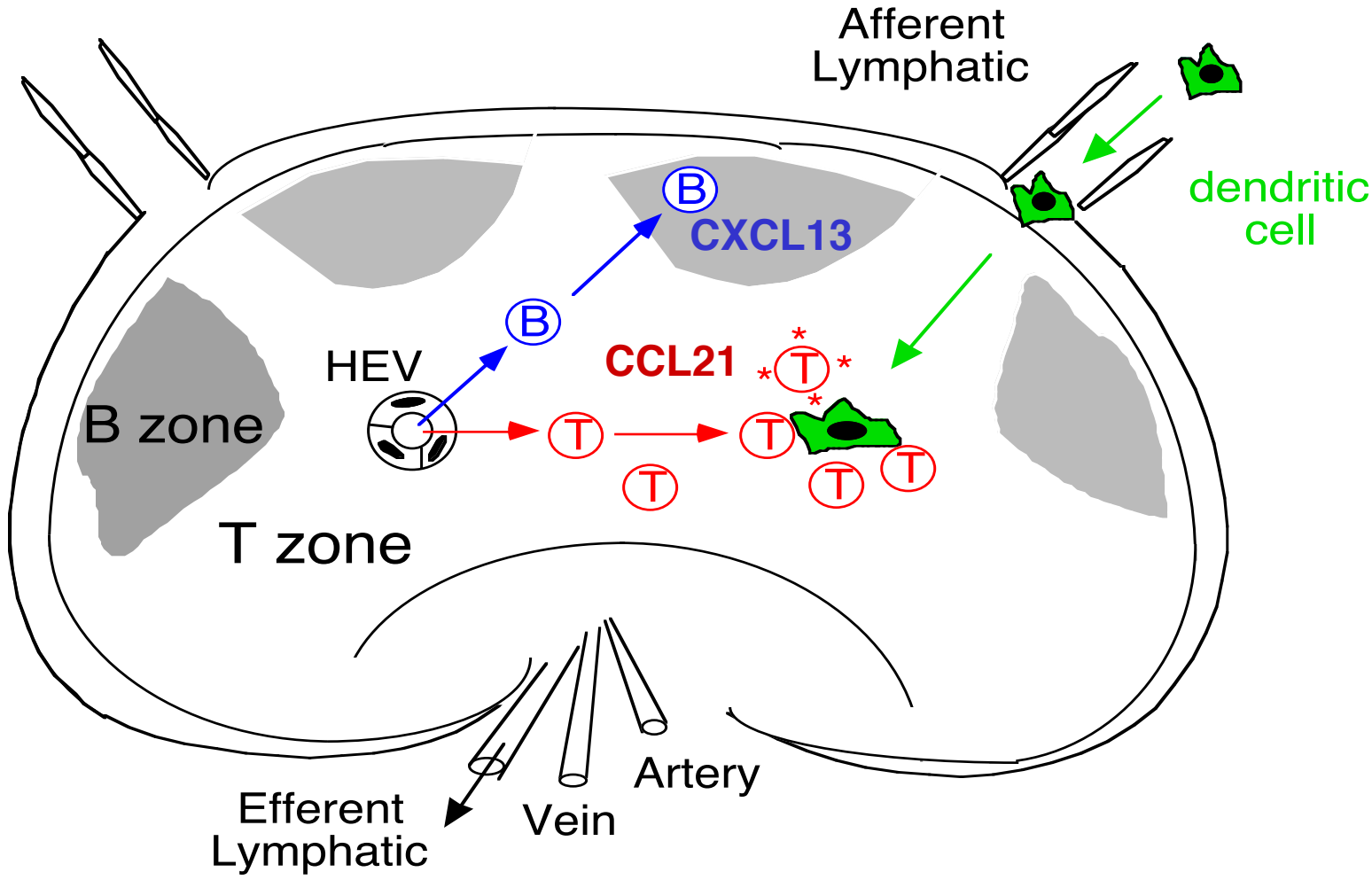
Lymphocytes migrate along stromal processes





Bajénoff et al., 2006
Immunity 25, 989

Schematic view of a lymph node



In mice lacking CXCL13 or CXCR5, B cells fail to home to B zones (follicles)
In mice lacking CCL21 and CCL19 or CCR7, T cells and DCs fail to home to T zones

Summary 1

Secondary lymphoid organs:

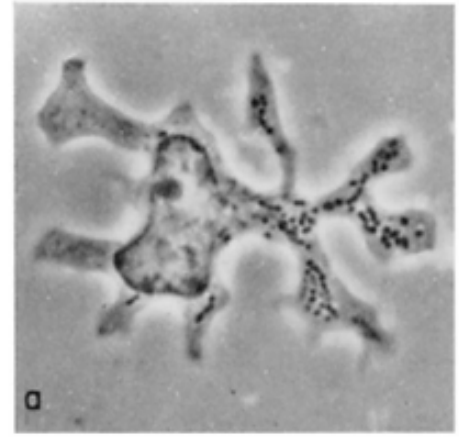
- lymph nodes, spleen, Peyer's patches
- function to filter antigen from body fluids
- bring together antigen, antigen-presenting cells and antigen-specific lymphocytes
- support lymphocyte activation and differentiation events

Question

- Are adhesion molecules needed for migration within the LN?
- Why do B and T cells home to separate zones?

2. Why are Dendritic Cells (DC) so effective at initiating adaptive immune responses?

- immature 'sentinel' DCs are present in most tissues, continually sampling their microenvironment for antigen
  - by pinocytosis, phagocytosis and engulfment of dying cells
- detection of pathogen-derived or damage associated signals (e.g. LPS, dsRNA, bacterial DNA, necrotic cells, TNF, IL-1, CD40L) causes the cells to mature
  - decrease adhesion to local tissue cells (e.g. keratinocytes)
  - increase expression of receptors (CCR7) for chemokines made by lymphatic endothelial cells and lymphoid organ T zones
  - process internalized Ag, upregulate MHC and costimulatory molecules
- migrate into lymphoid T zone
- present antigen to T cells
- some antigens also travel to T zone via lymph and are captured and presented by lymph node resident DCs

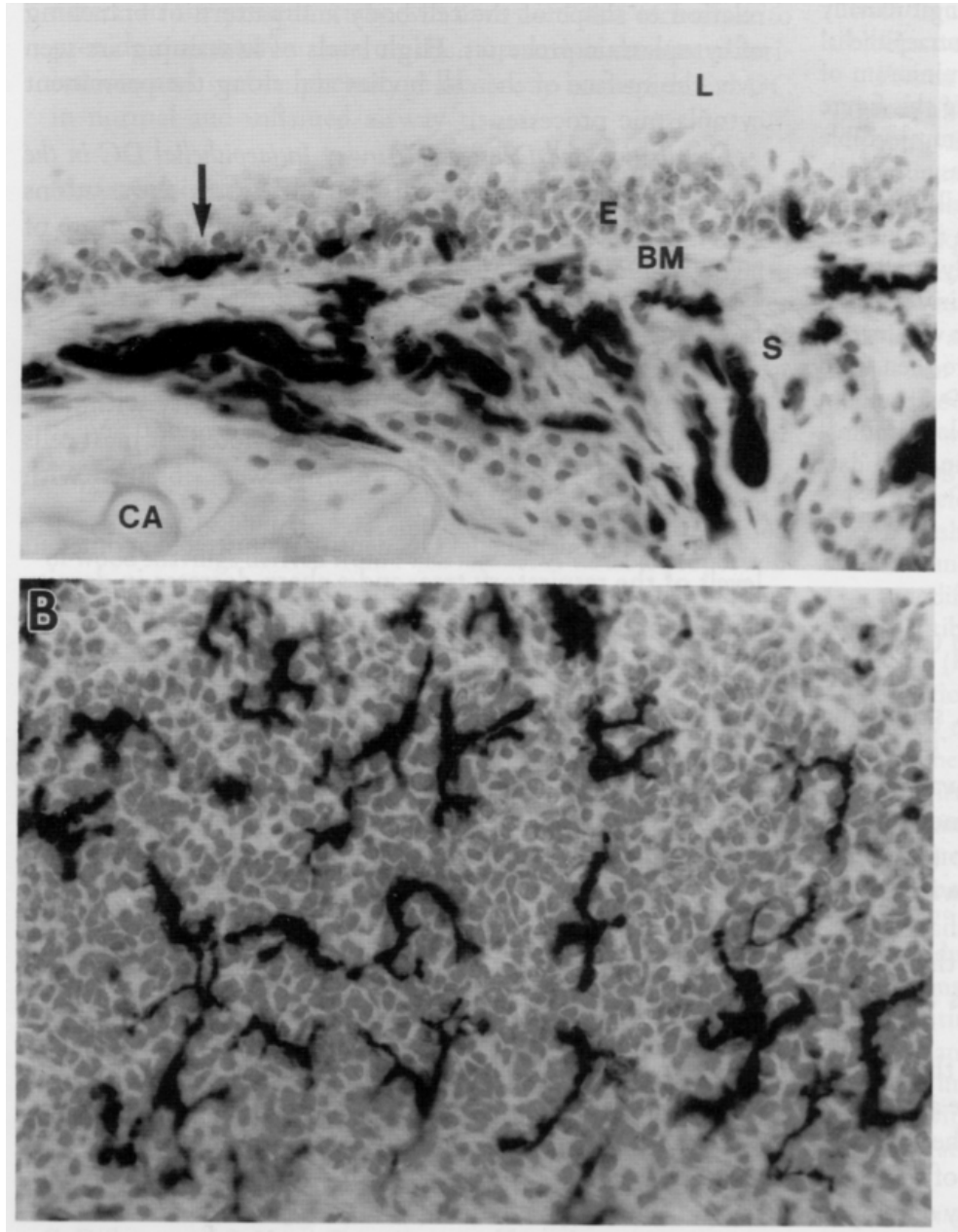


Steinman RM, Cohn ZA. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. *J Exp Med.* 137:1142

Steinman was awarded 2011 Nobel Prize in Physiology or Medicine "for his discovery of the dendritic cell and its role in adaptive immunity"

Immature (sentinel) DCs in peripheral tissue

rat tracheal epithelium



longitudinal section

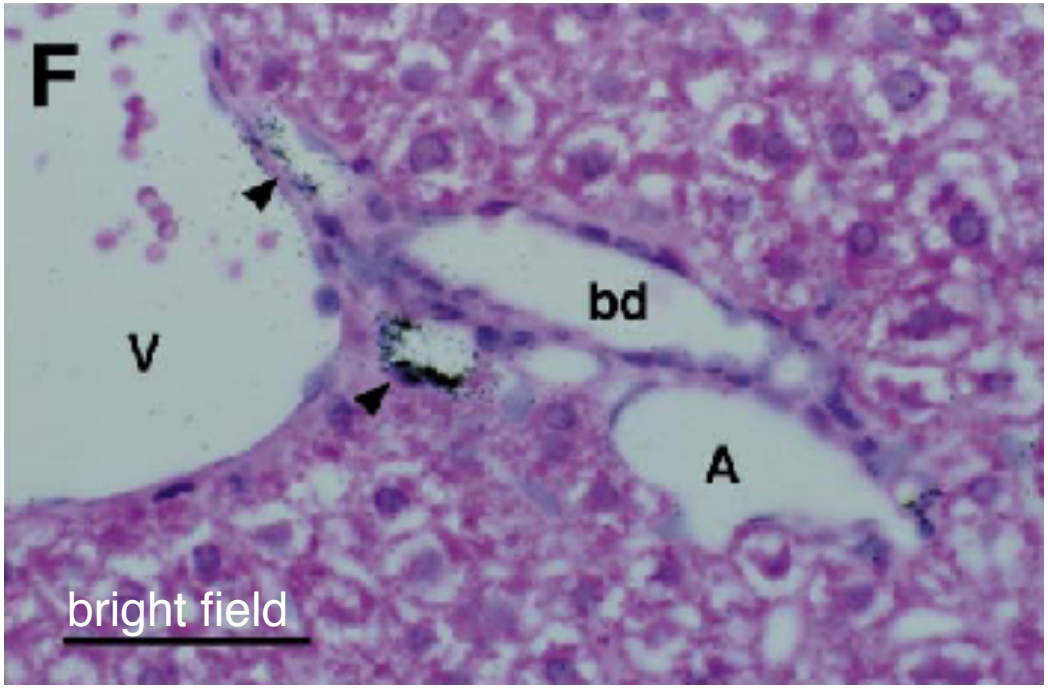
tangential section

Schon-Hegrad et al., (1991) J. Exp. Med. 173, 1345

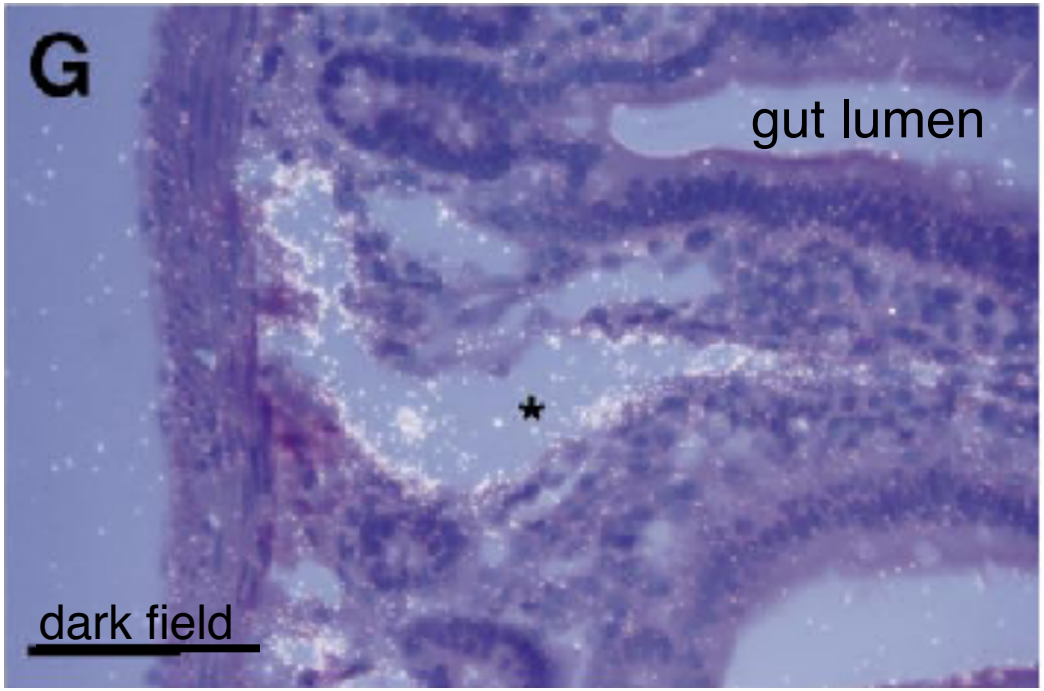
Chemokine CCL21 (SLC) expression by lymphatic endothelium

in situ hybridization to detect CCL21 mRNA expression

Liver



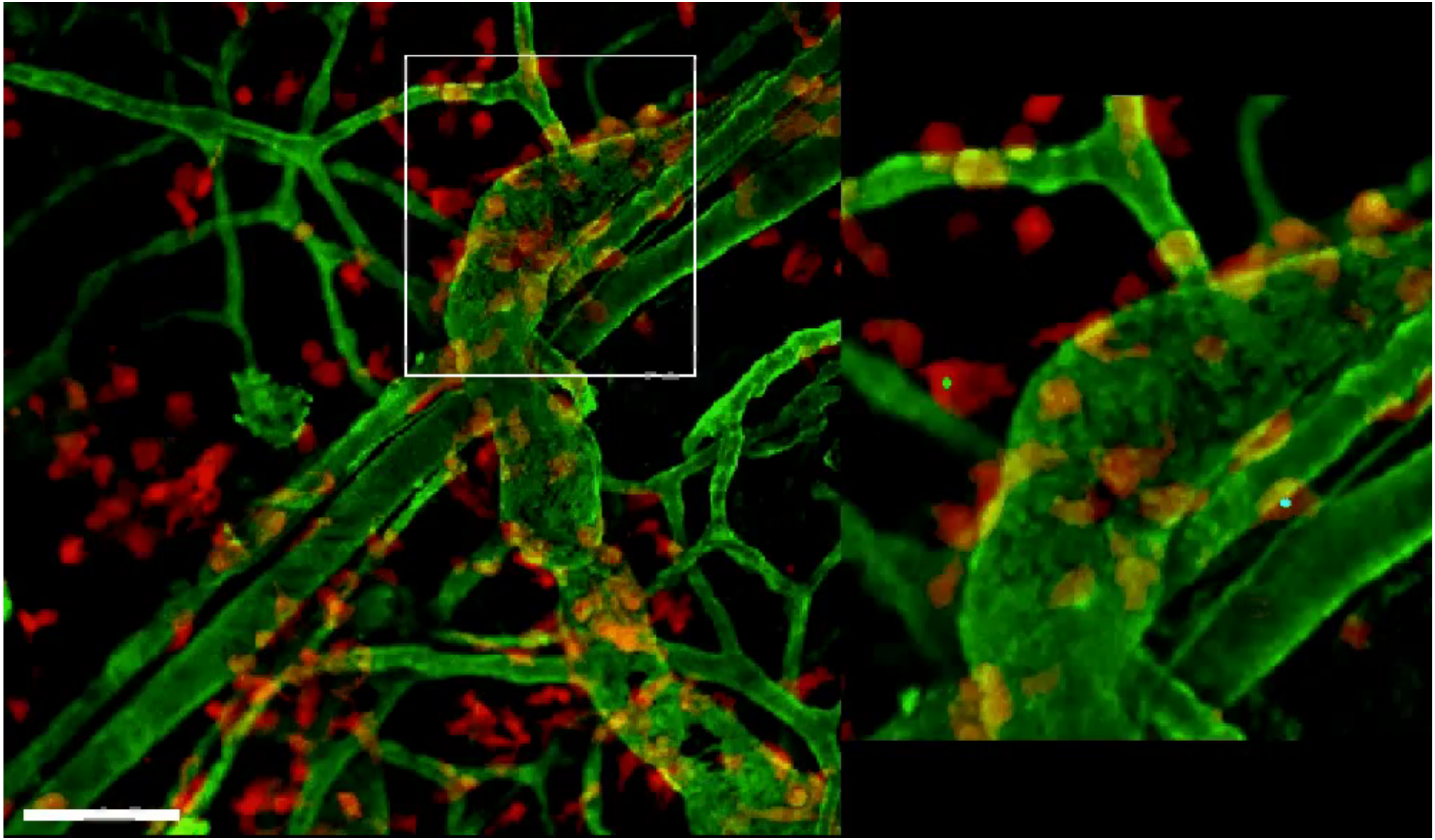
Small Intestine



under bright field illumination, deposited silver grains appear black;
under dark field (Nomarski) optics they appear silver

from Gunn et al.,
1999, PNAS 95, 258

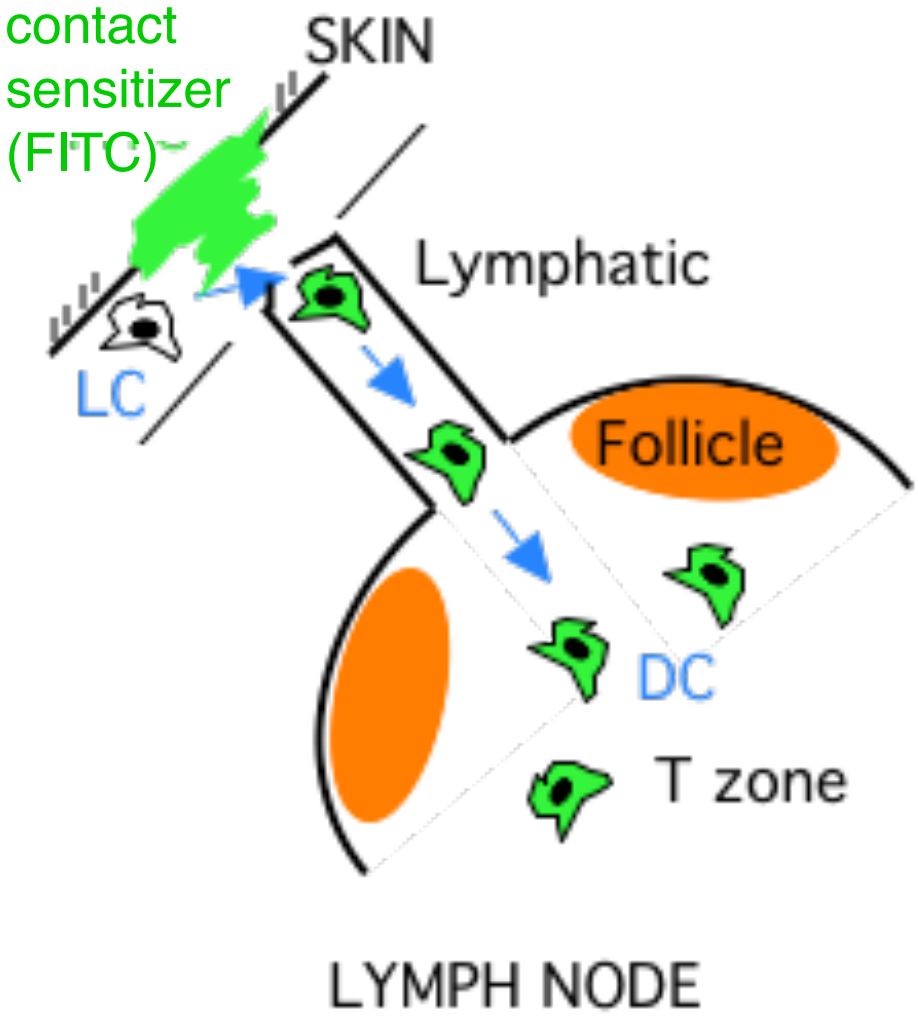
DC migration into skin lymphatics



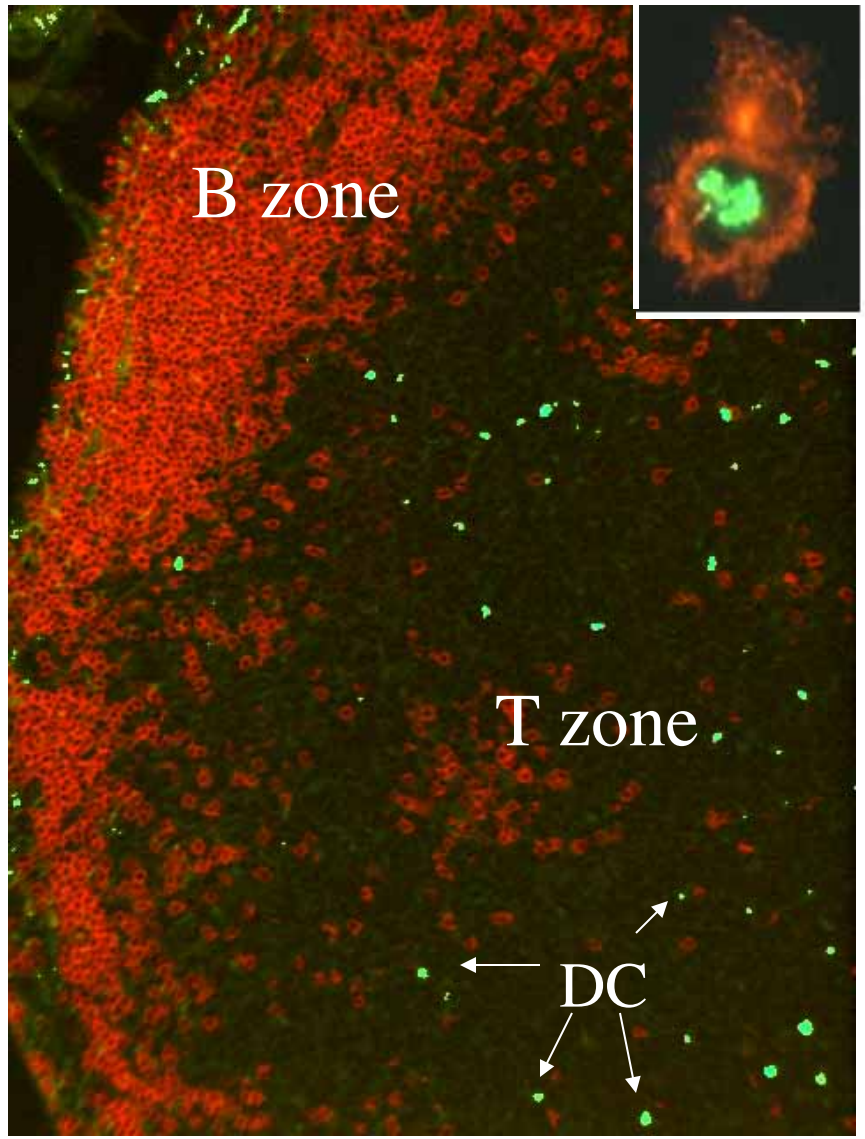
DCs (red), vessel basement membrane (laminin, green)

Pflicke& Sixt, JExpMed 2009

DCs migrate from periphery to lymphoid organ T zone bearing antigen

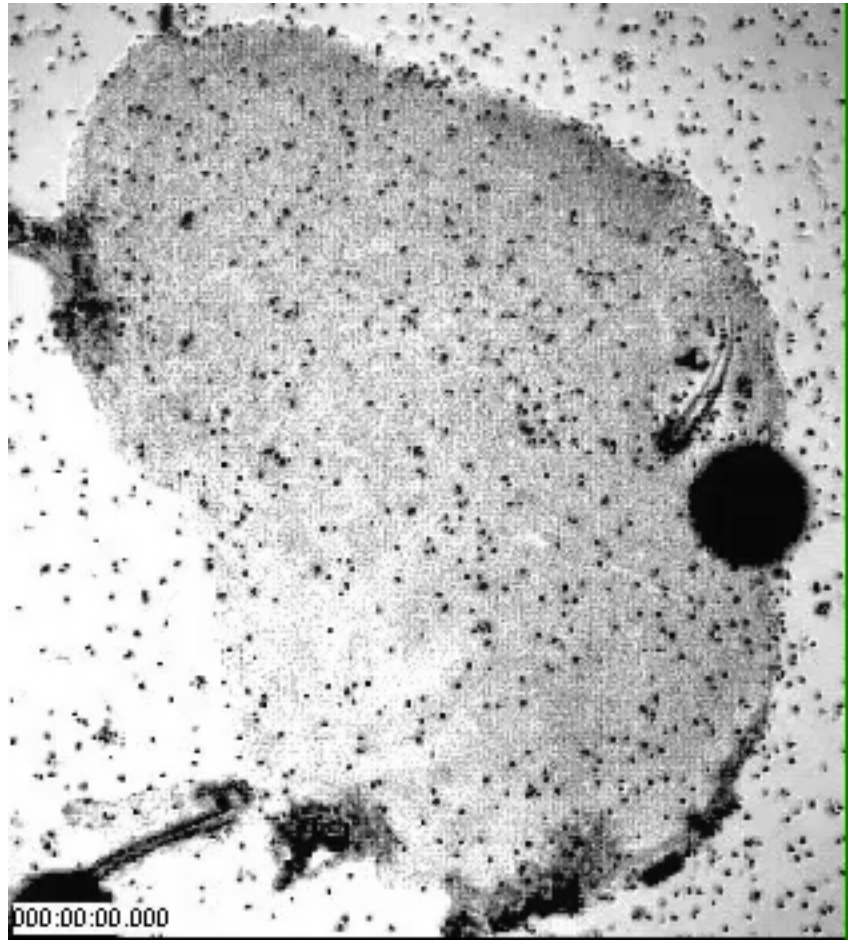


Skin draining Lymph Node (day 1)

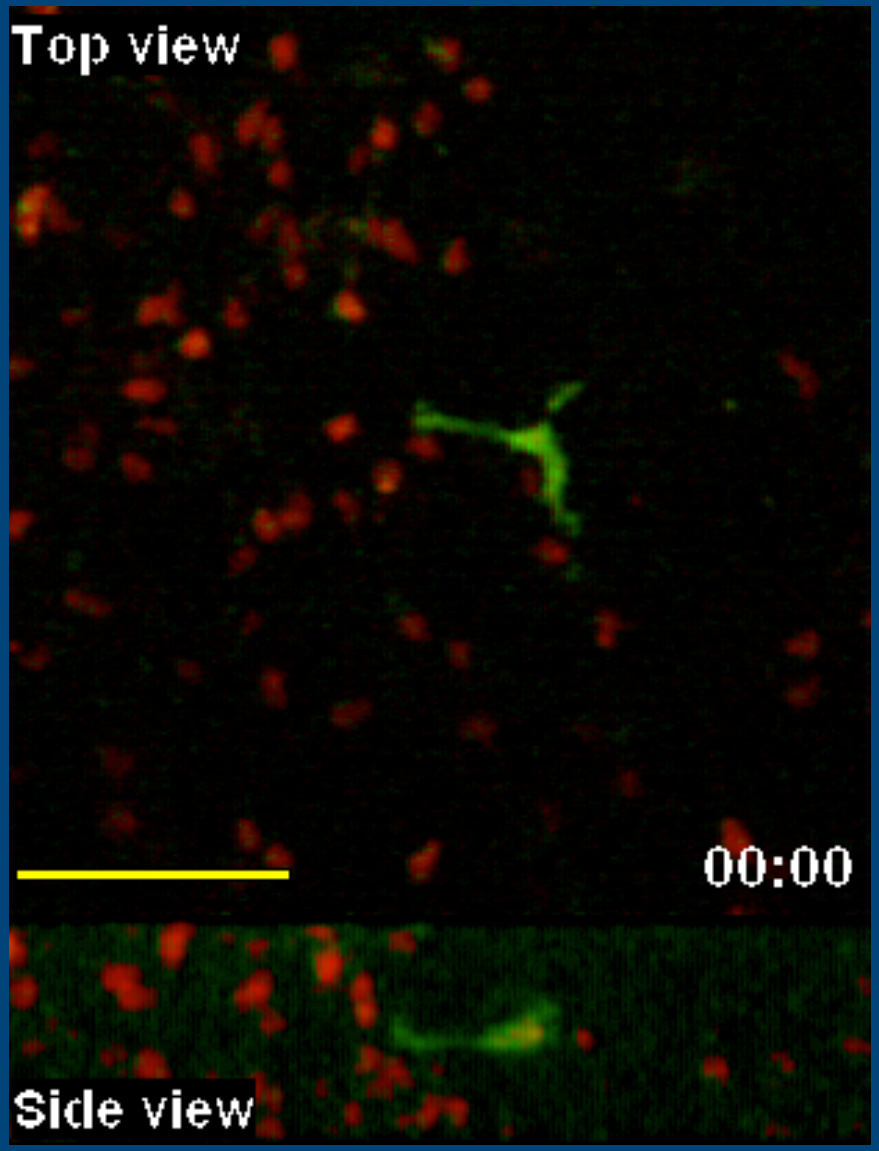


Note: immature DC of skin are known as Langerhan's Cells

DCs follow chemokine (CCR7 ligand) gradients into LN T zone



Naïve T cells survey a dendritic cell in the lymph node T zone



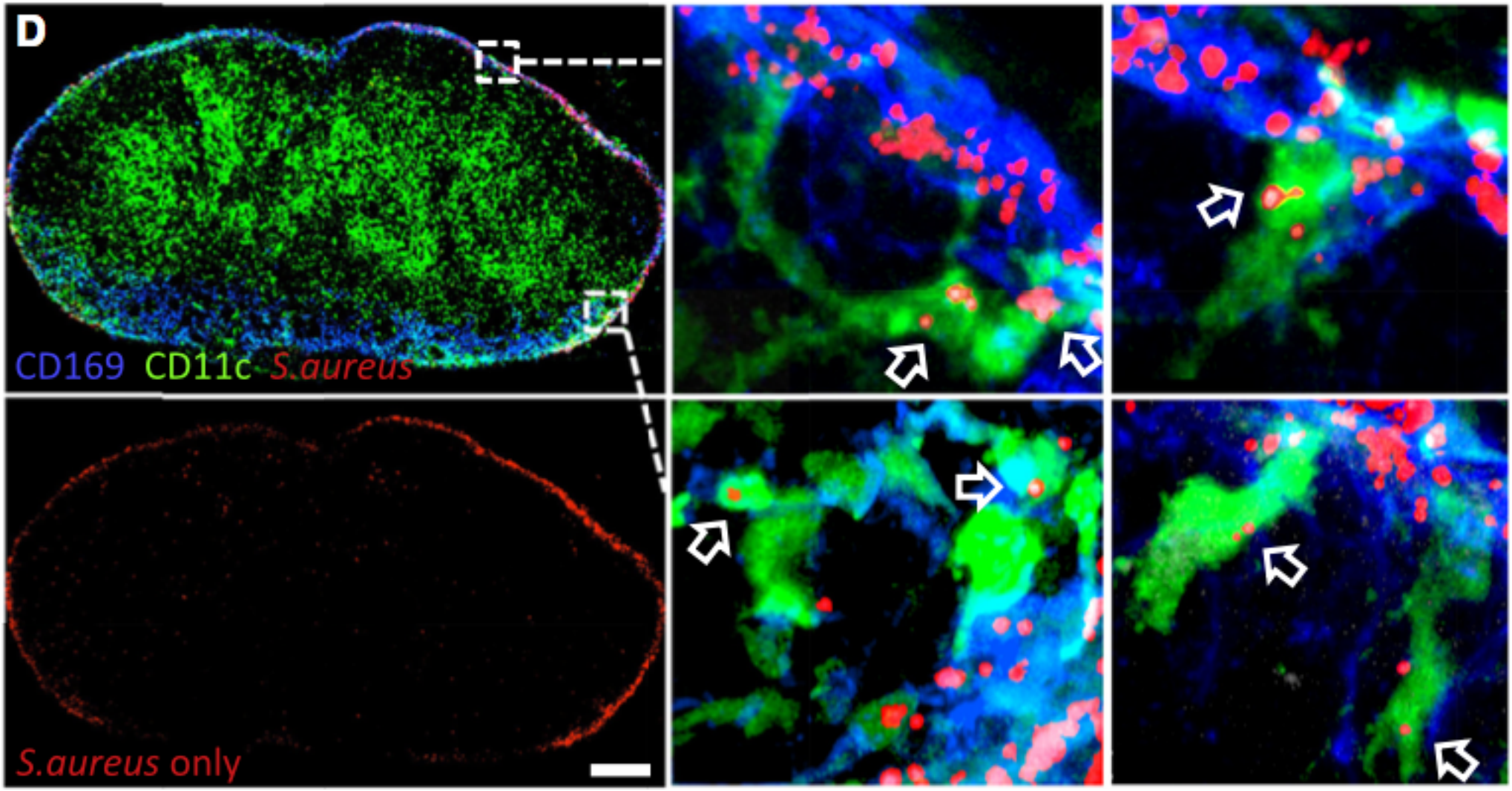
Summary 2

DC are effective at initiating immune responses because:

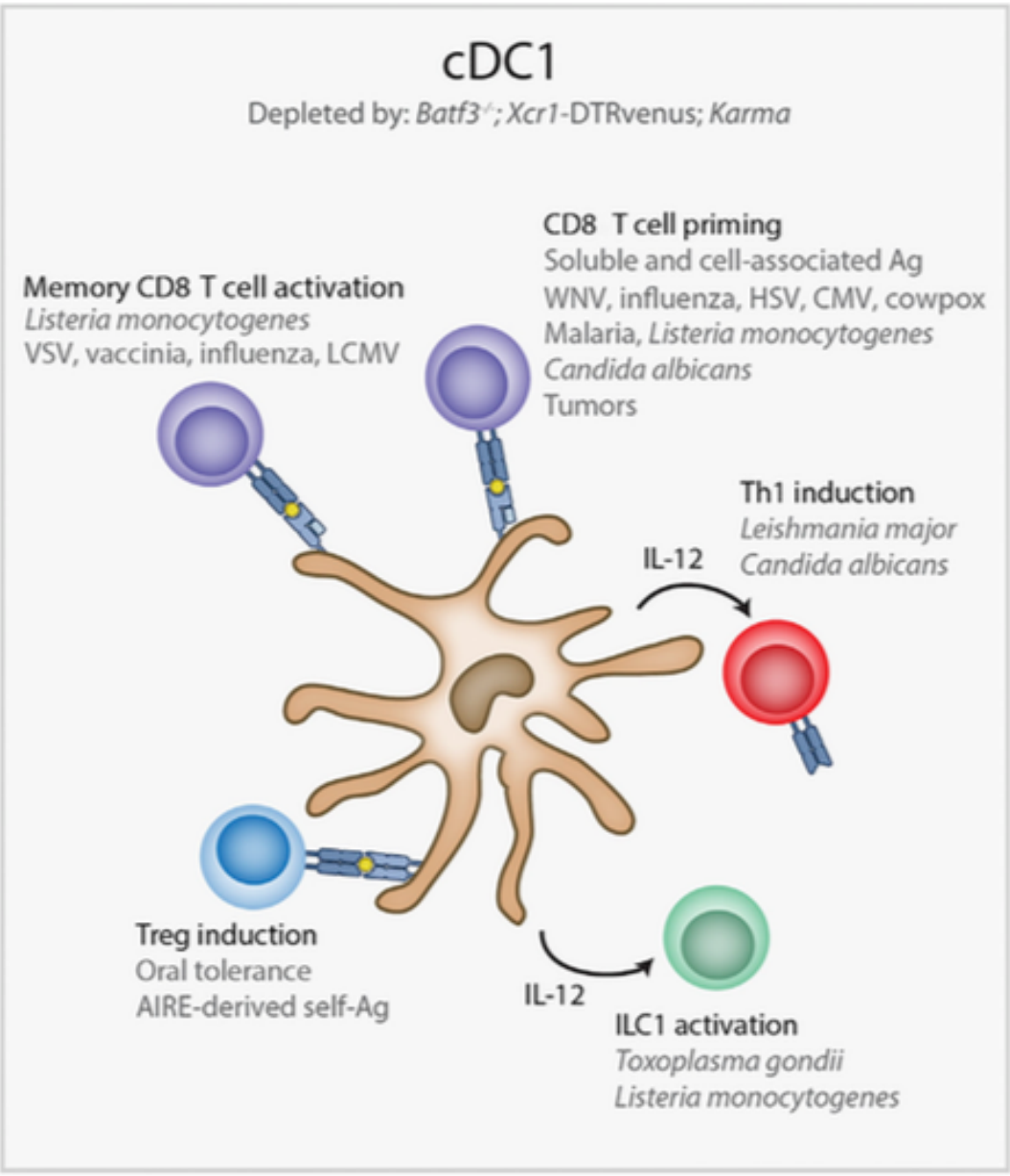
- The immature cells are located in sentinel positions
- They are highly efficient at processing and presenting antigen
- They migrate rapidly to lymphoid T zones
- They express high levels of costimulatory molecules for provoking activation of T cells
- DC influence the differentiation pathway of the T cell in terms of cytokine induction and homing receptor profile

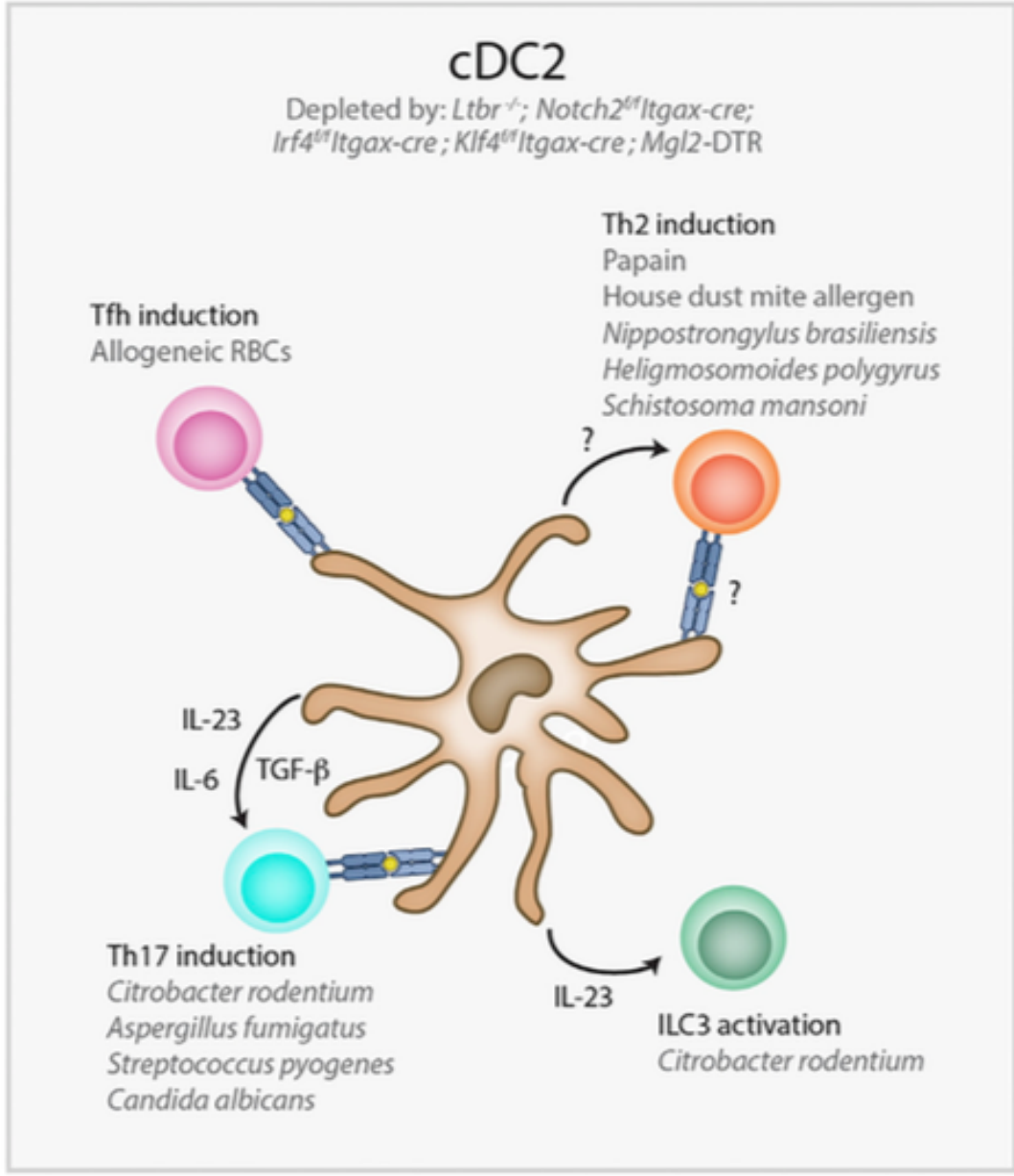
Question

- Can DC in the LN capture antigens directly?
- There is more than one type of DC. Any thoughts on why? What properties may differ between different DC types?



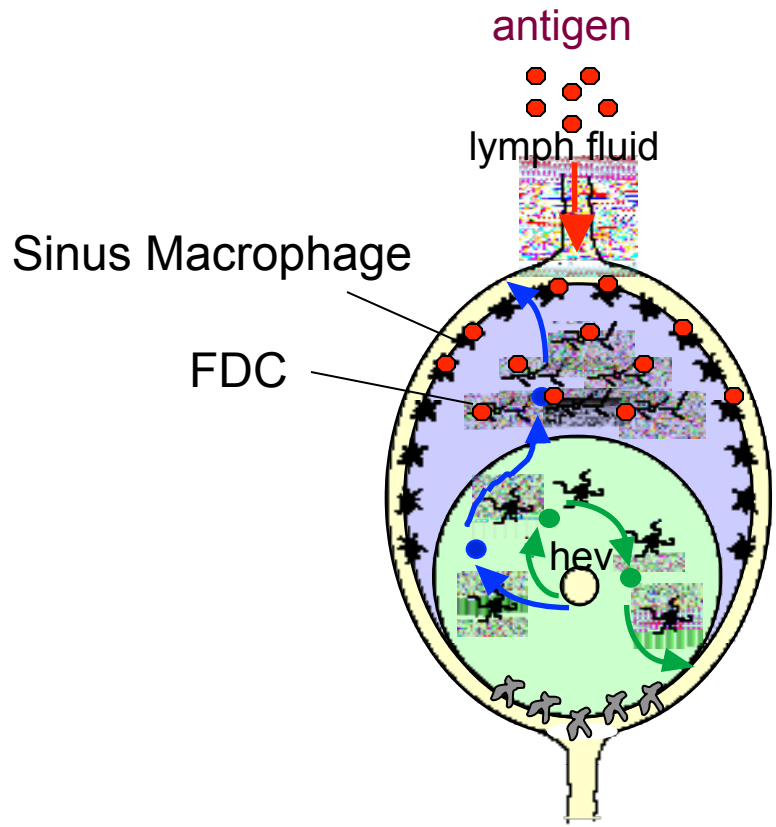
Gerner et al., *Immunity* 2015





Durai & Murphy 2016 *Immunity*

3. Where do B cells come in contact with antigen?

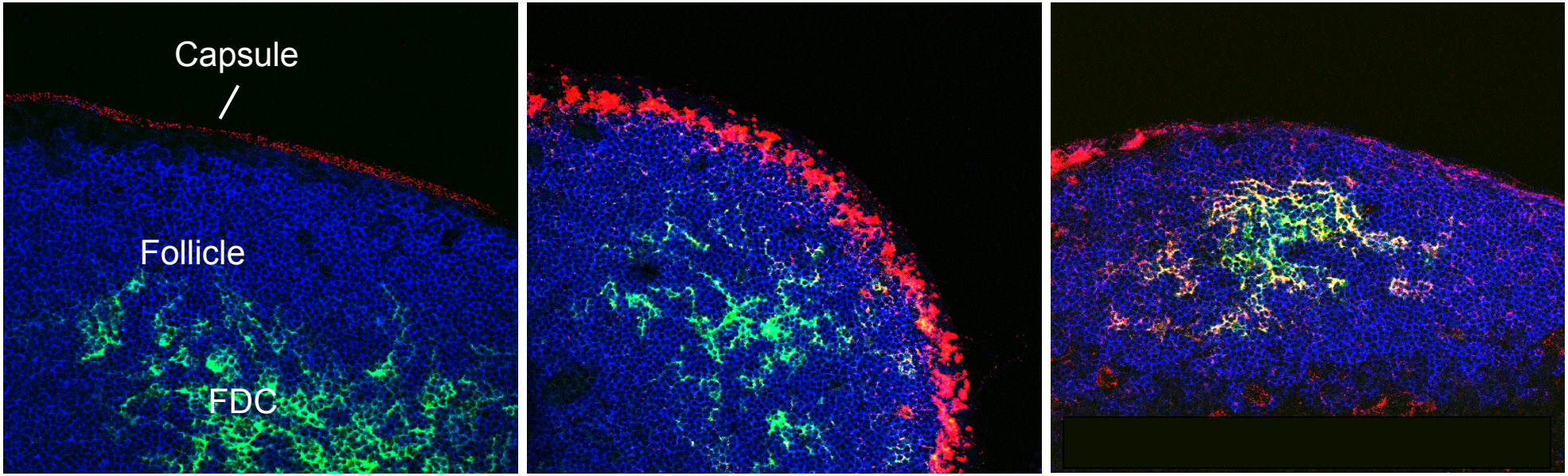


- **B cells bind intact antigen through their surface Ig / B cell receptor (BCR)**
- **Antigen that enters via blood or lymph reaches the follicle and can be captured directly by B cells**
- **Follicular dendritic cells (FDC) can display antigen on their surface in an intact form for long periods**

Deposition of Immune Complexes occurs in distinct phases

What is an immune complex?

15 min | 2 h | 8 h

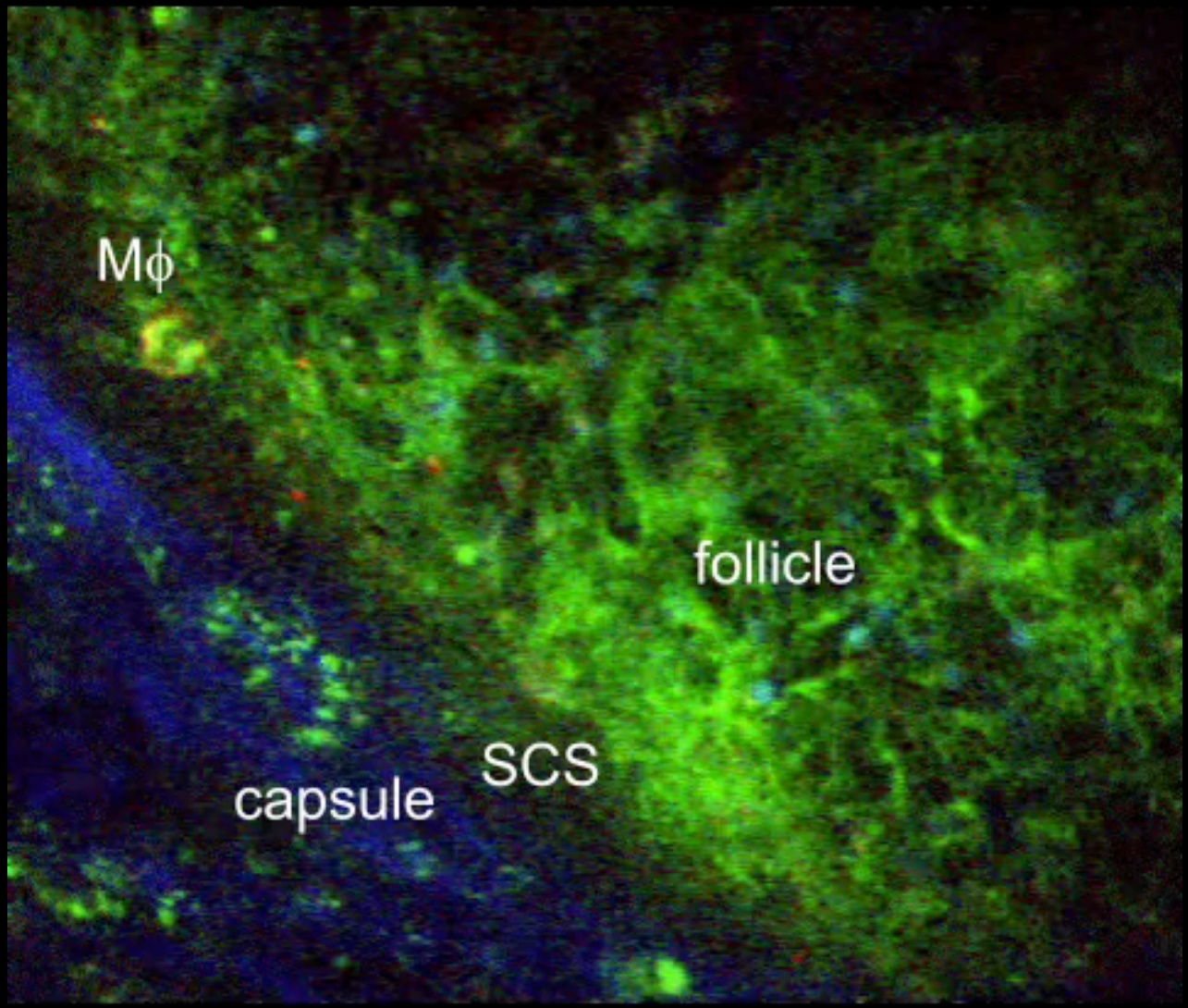


PE Immune Complex
Complement Receptor-1 (CD35)
B cells (B220)

Immune Complexes are made up of Antigen, Antibody (IgM or IgG) and (typically) Complement (C3b). They are a form of opsonized antigen. They will usually be multivalent (contain multiple units of the antigen). Antigens coated by C3b alone are also termed opsonized and are handled in a similar way

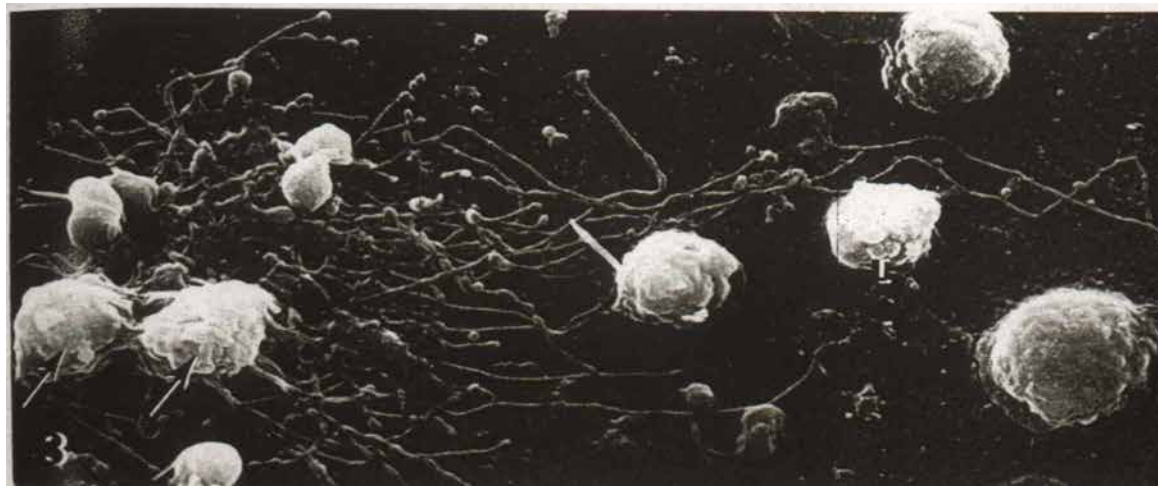
Macrophage capture of PE ICs

Collagen
(capsule)



Follicular Dendritic Cells (FDCs)

- Resident in lymphoid follicles
  - highly extended processes, can contact many migrating B cells
  - produce CXCL13
  - not of hematopoietic origin and thus not related to DCs of T zone (instead they are of mesenchymal 'fibroblastic' origin)
- Express receptors that bind antigen coated in complement C3d (CRs) and antibody (FcRs)
- Play a role in the Germinal Center reaction



Scanning EM of isolated FDC

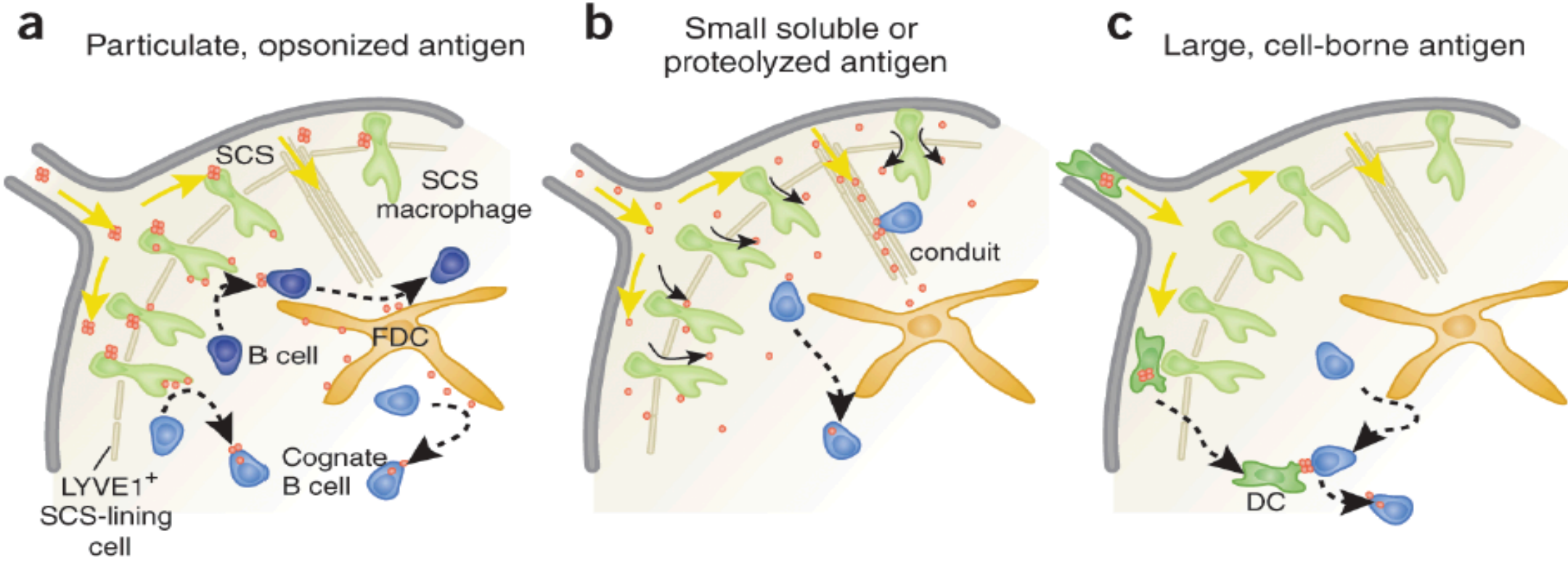
Skazal et al. 1985 JI 134, 1349

Antigen capture by cognate B cell from FDC

Antigen (red)
Specific B cell
(green)

Cognate (MD4) B cell Non-cognate (CFP) B cell HEL-PE

Multiple modes of follicular B cell encounter with antigen

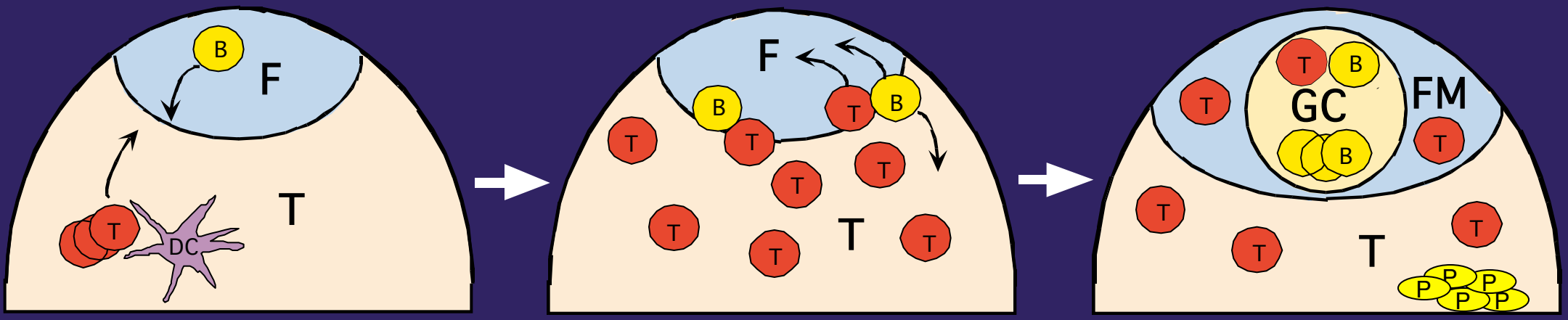


Questions

- How does complement coat an antigen?
- If FDC only bind opsonized antigens, can they be involved in presenting antigen during a primary immune response?

4. How do B cells find helper T cells specific for the same antigen?

Changes in lymphocyte homing during T-dependent antibody responses



Antigen encounter

T/B collaboration near the follicle/T zone boundary

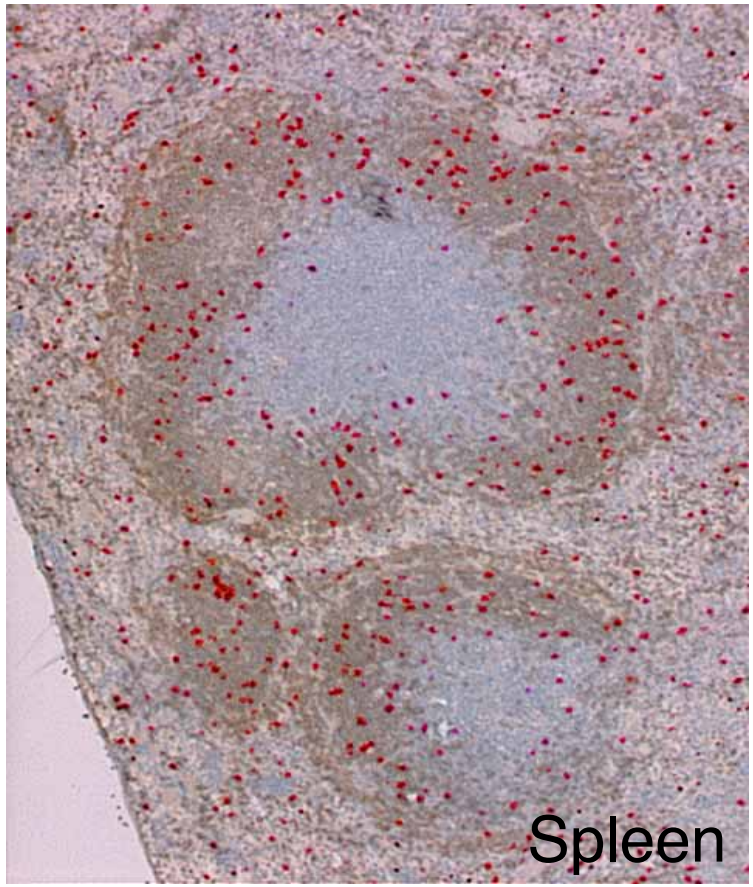
Plasma Cell and Germinal Center formation

B Antigen-specific B cell

P Antigen-specific Plasma cell

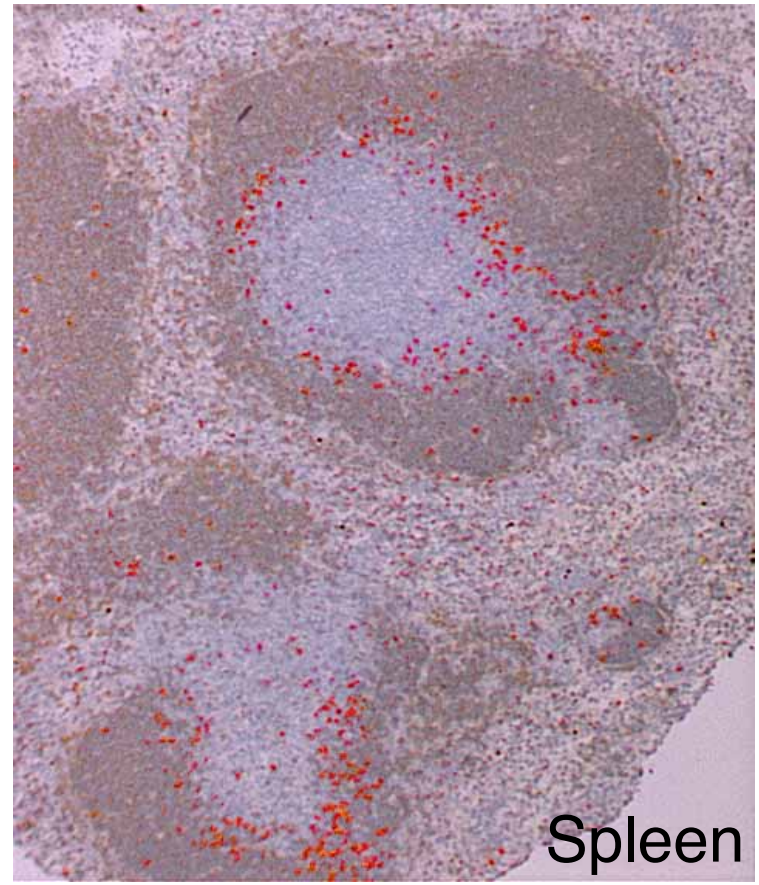
T Antigen-specific T cell

B cell antigen receptor engagement induces B cell movement to outer T zone



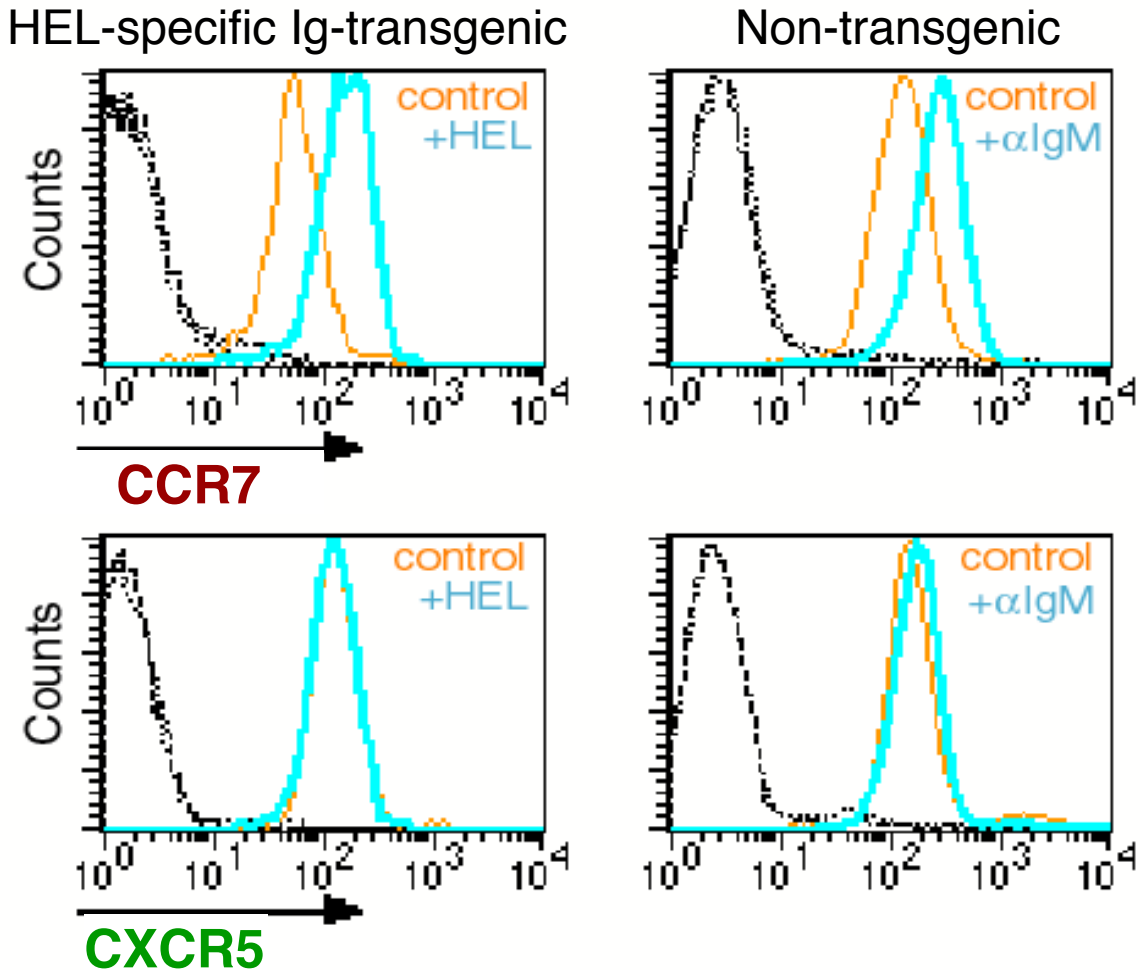
i.v. antigen

6-8 hr



brown = all endogenous B cells
red = antigen specific B cells

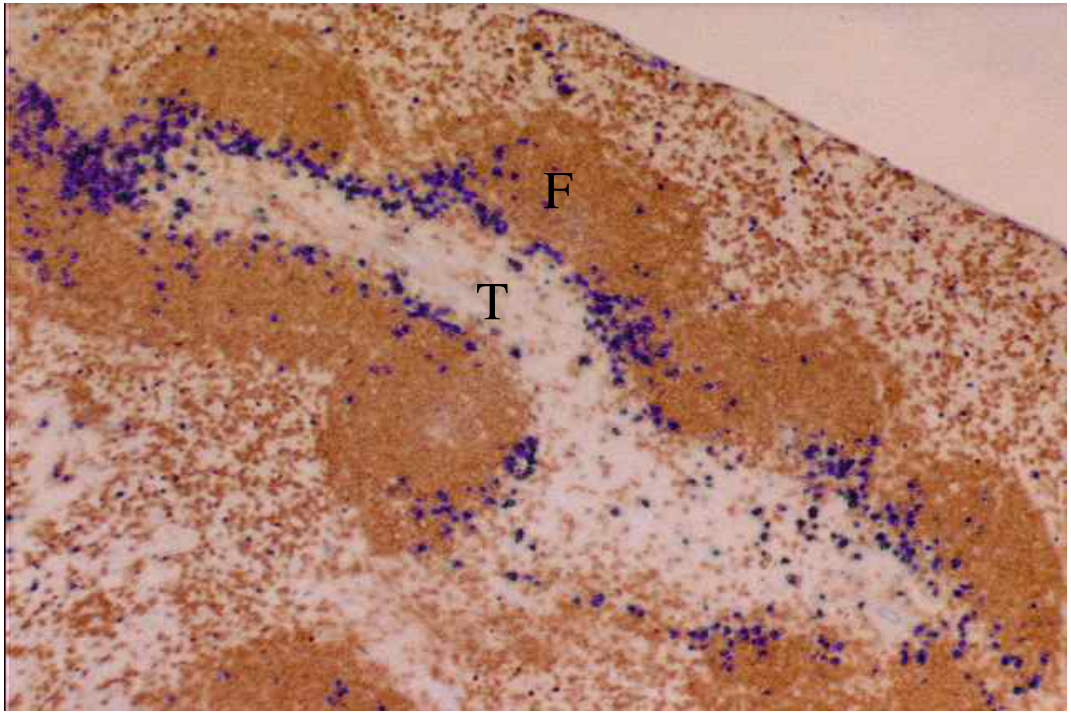
BCR engagement increases CCR7 surface levels



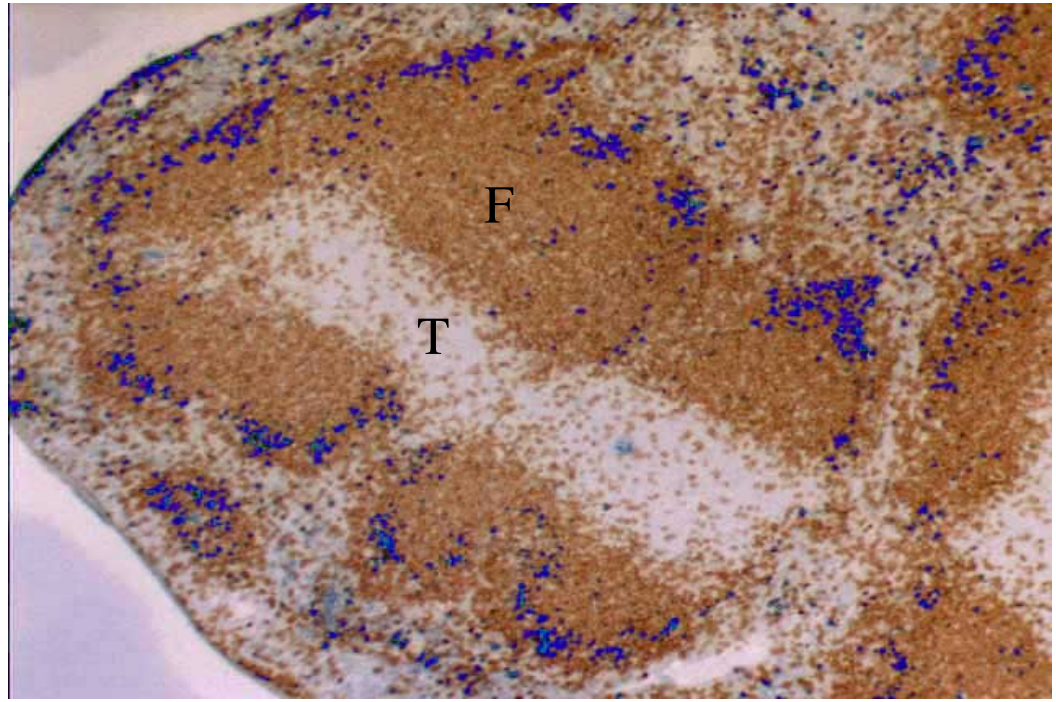
HEL – hen egg lysozyme
(model antigen)

B cells deficient in T zone chemokine receptor fail to migrate to follicle / T zone boundary

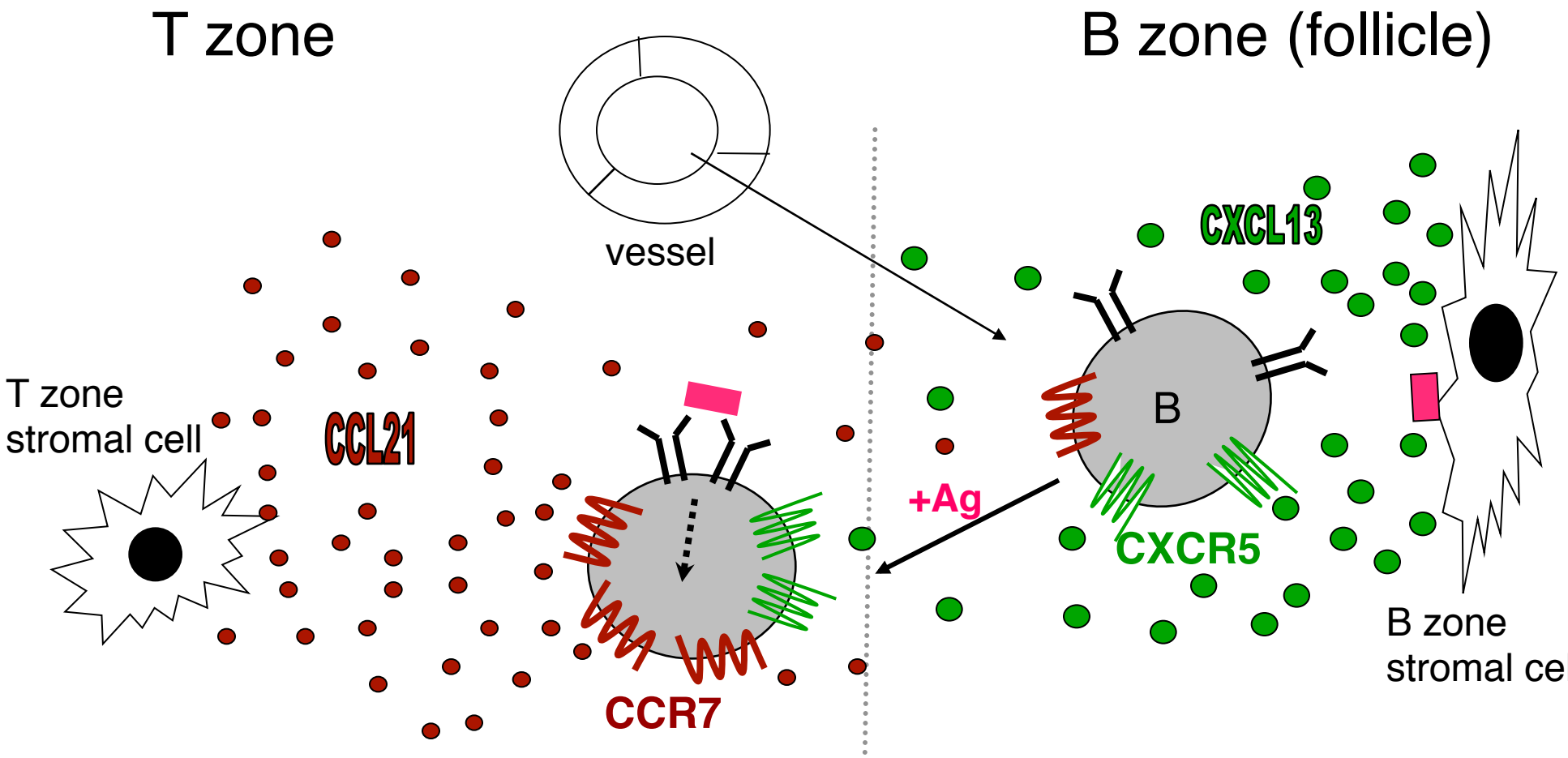
Wildtype Ig-tg B cells



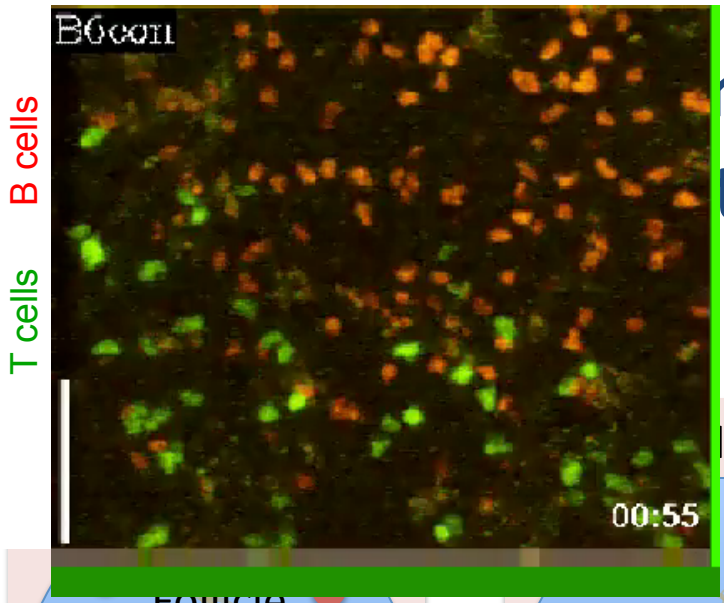
CCR7 deficient Ig-tg B cells

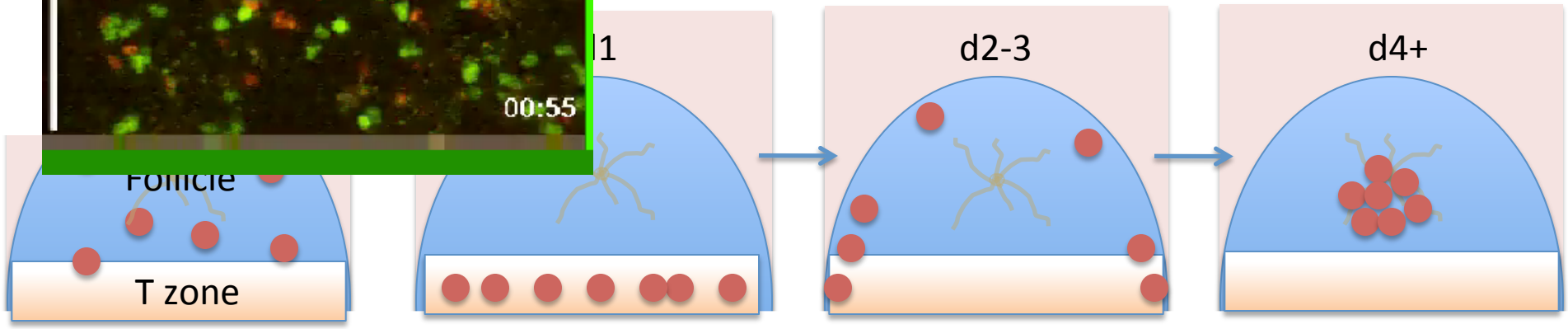


Activated B-cell localization in outer T zone determined by balanced responsiveness to T and B zone chemokines



ng during the T-cell dependent tibody response





Chemokine CXCL13

Chemokine CCL21

CXCR5hi CCR7lo

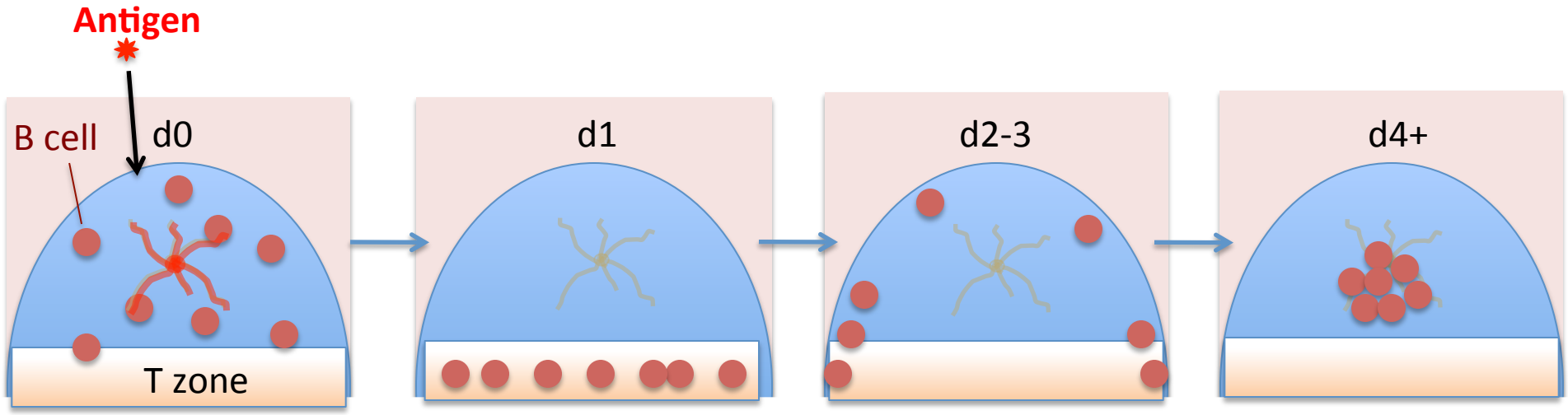
CCR7

Chemokine -> Gαi coupled GPCR

CXCL13->CXCR5 (Gαi)

CCL21->CCR7 (Gαi)

B cell repositioning during the T-cell dependent antibody response



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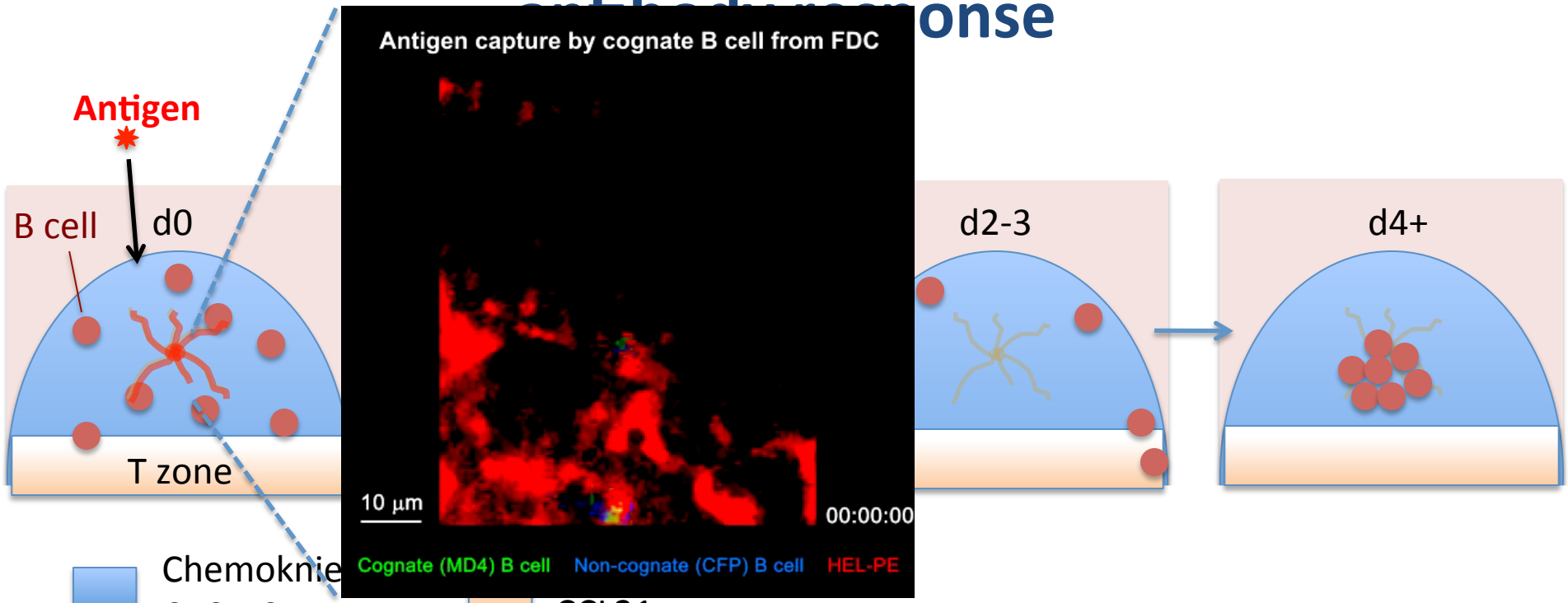

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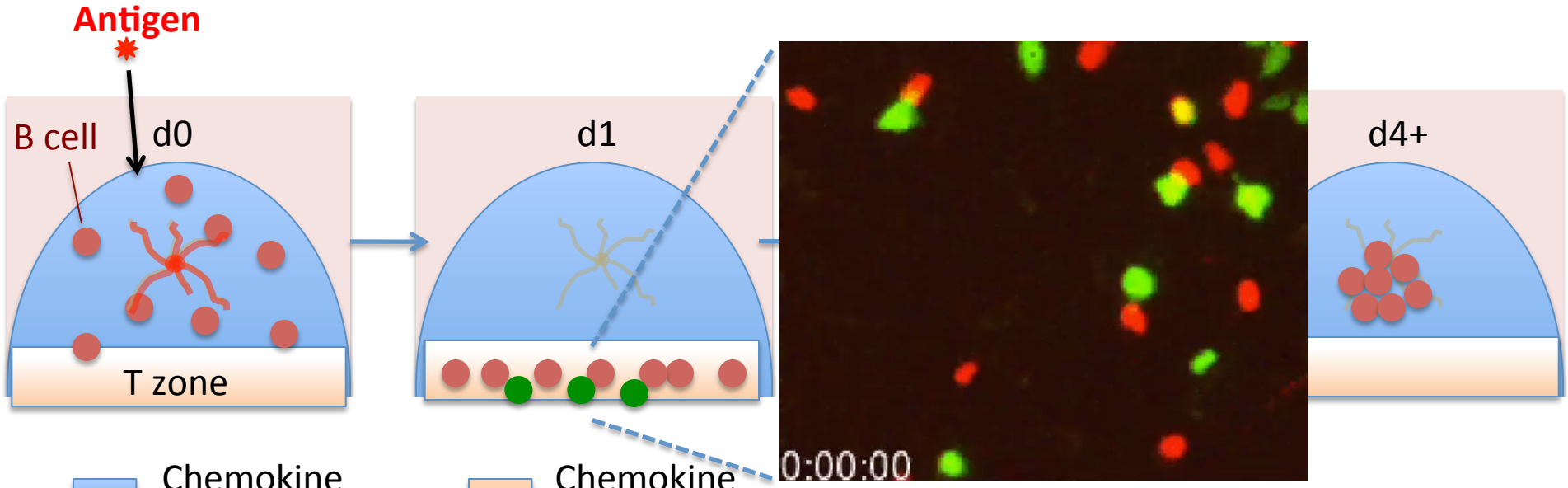


Chemokine
CXCL13

Chemokine
CCL21

CXCR5hi
CCR7lo

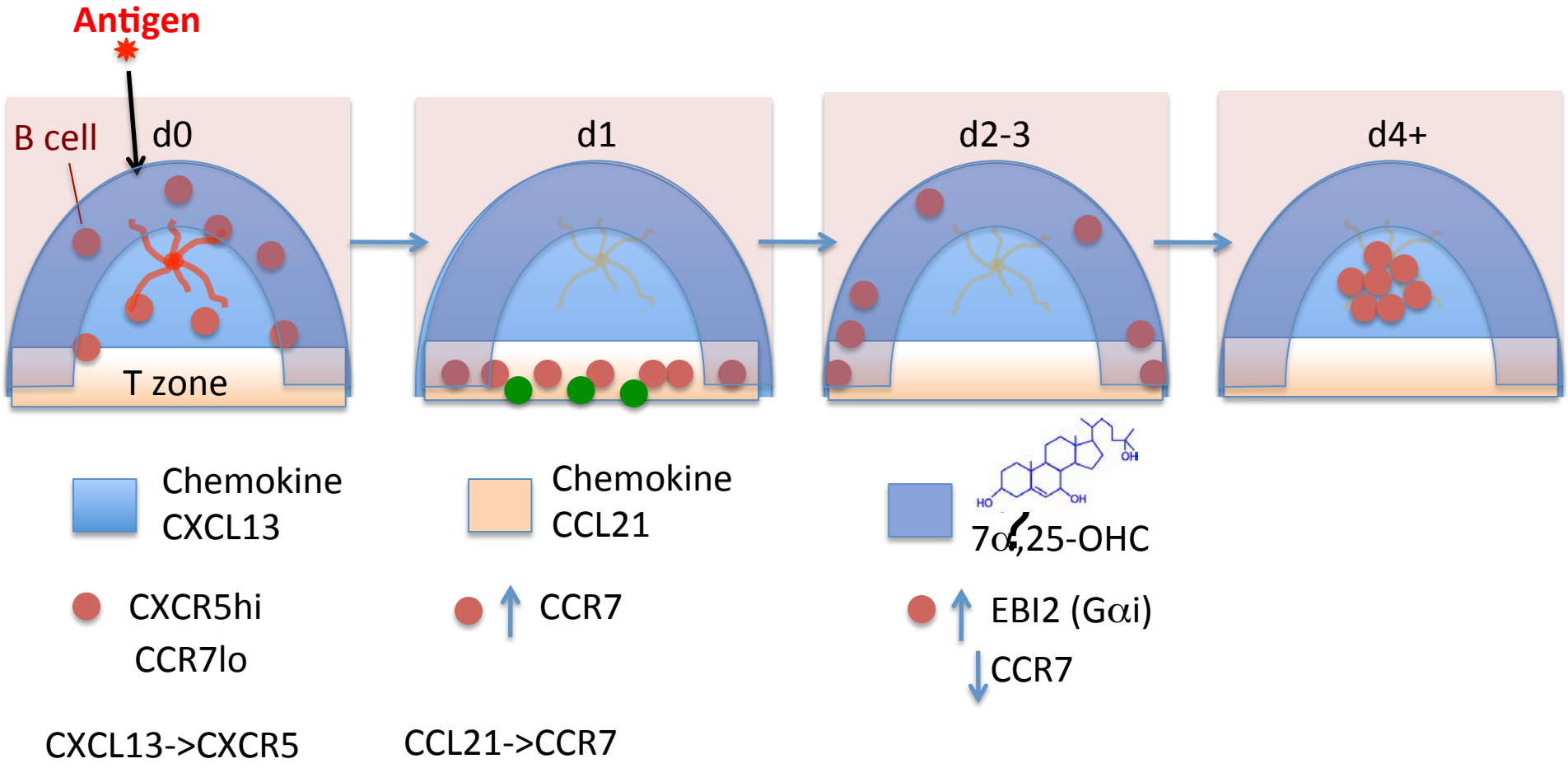
CCR7

Chemokine -> Gαi coupled GPCR

CXCL13->CXCR5
(Gαi)

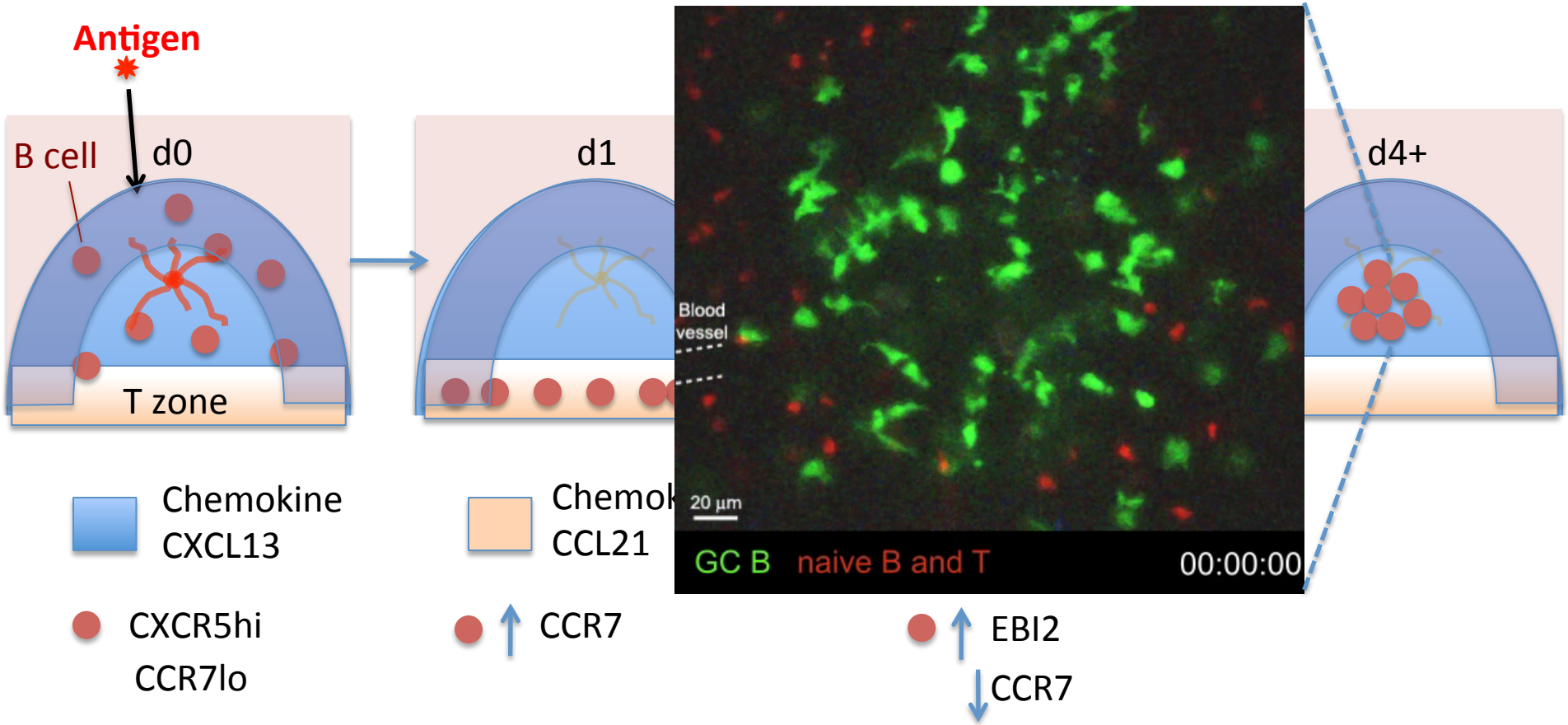
CCL21->CCR7
(Gαi)

B cell repositioning during the T-cell dependent antibody response



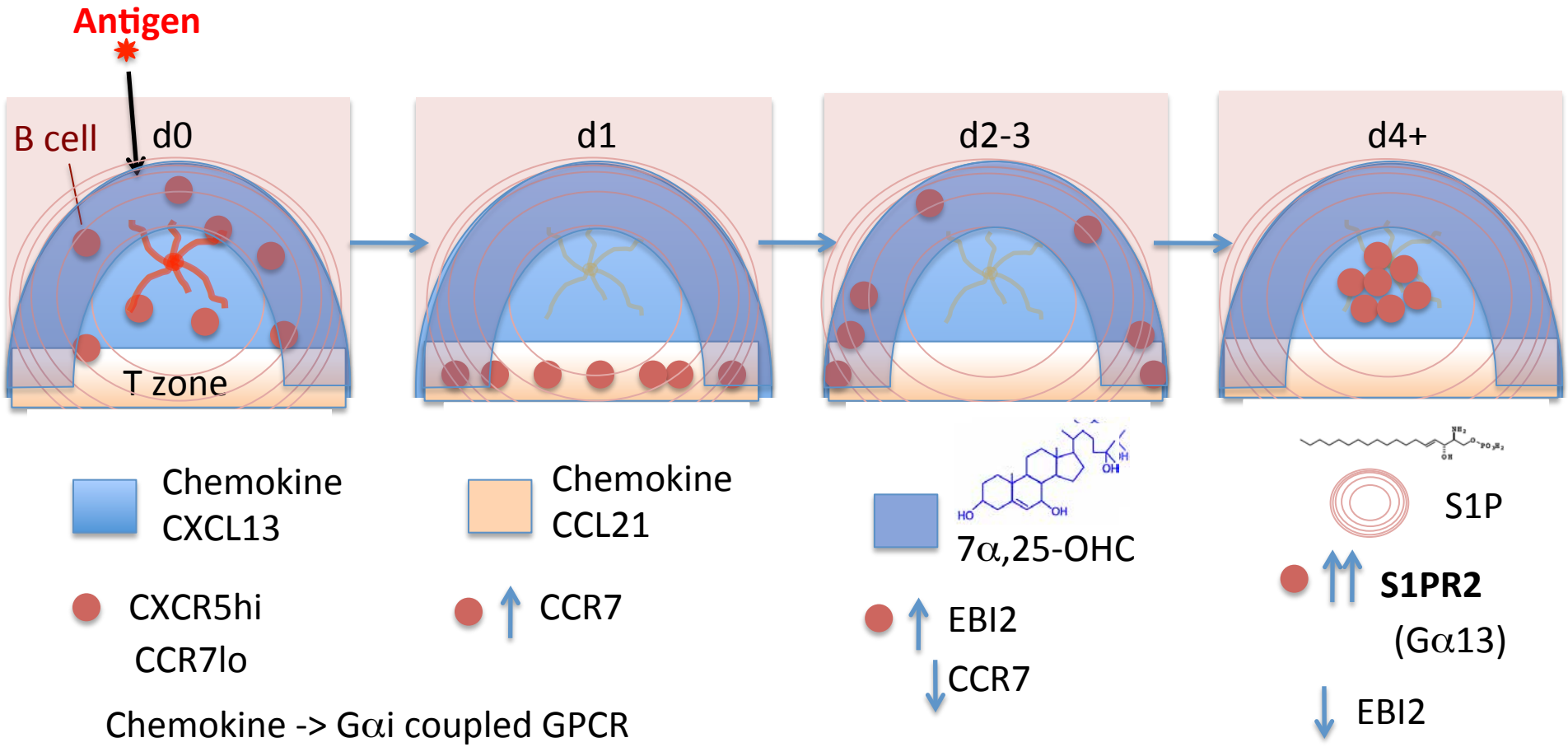
- EBI2 in B cells and DCs and 7α,25-dihydroxycholesterol gradients in lymphoid tissue are required for mounting normal T cell-dependent antibody responses

B cell repositioning during the T-cell dependent antibody response

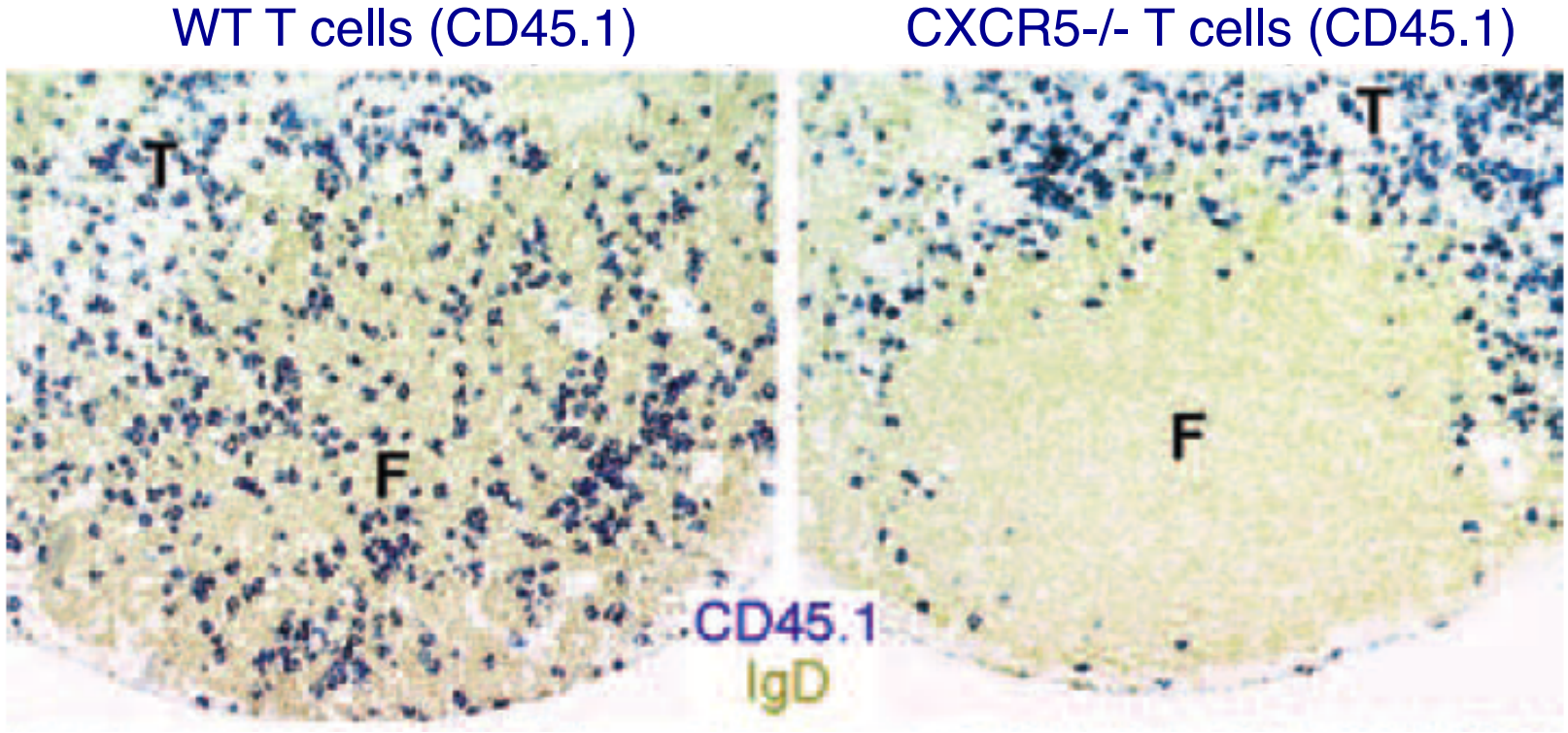


- EBI2 (GPR183) in B cells and DCs and 7α,25-OHC gradients in lymphoid tissue are required for mounting normal T cell-dependent antibody responses

B cell repositioning during the T-cell dependent antibody response



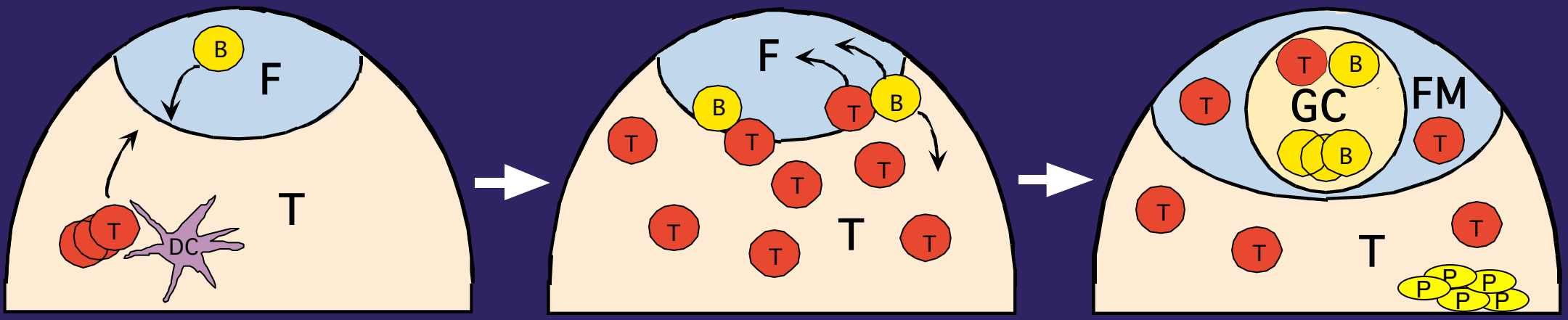
Migration of activated T cells in to B cell follicles is CXCR5 dependent



day 3 after activation of blue (cognate) CD4 T cells

- some activated CD4 T cells become 'T Follicular Helper cells (T_{FH})'
- upregulate CXCR5
- depend on transcription factor Bcl6
- upregulate costimulatory molecules (e.g. ICOS) and cytokines (e.g. IL21) that facilitate B cell responses
- undergo prolonged (SAP-dependent) adhesive interactions with B cells

Changes in lymphocyte homing during T-dependent antibody responses



Antigen encounter

T/B collaboration near the follicle/T zone boundary

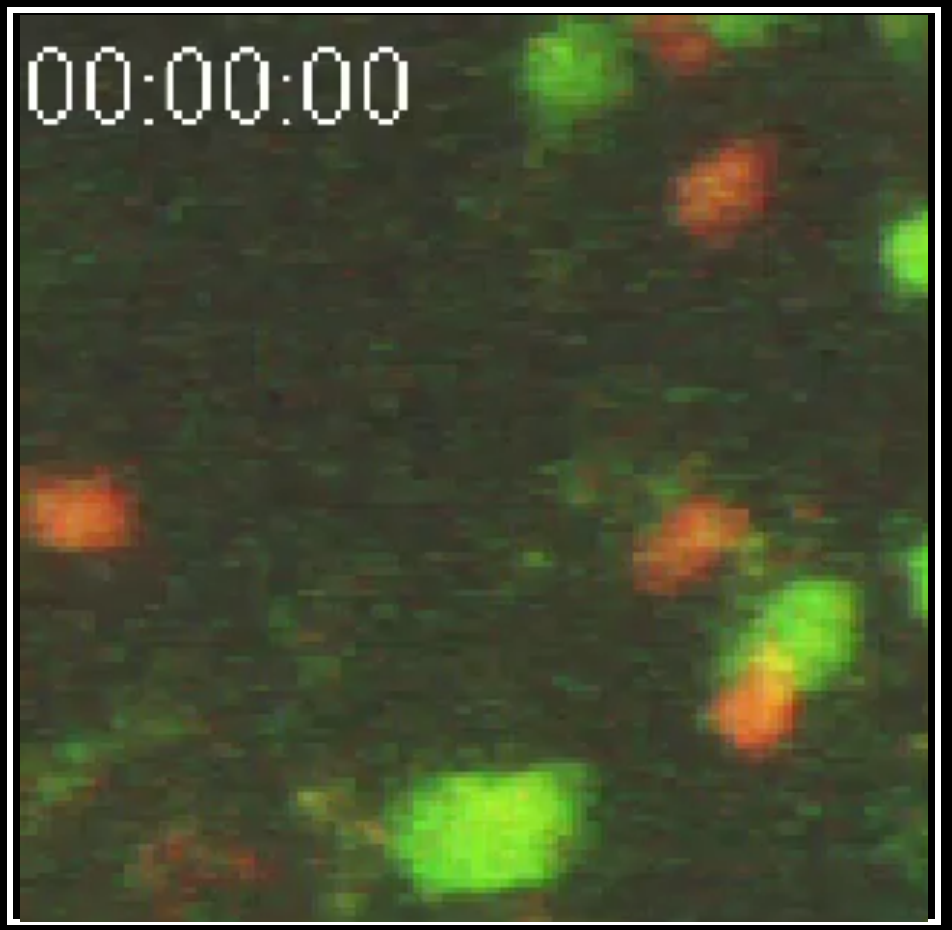
Plasma Cell and Germinal Center formation

B Antigen-specific B cell

P Antigen-specific Plasma cell

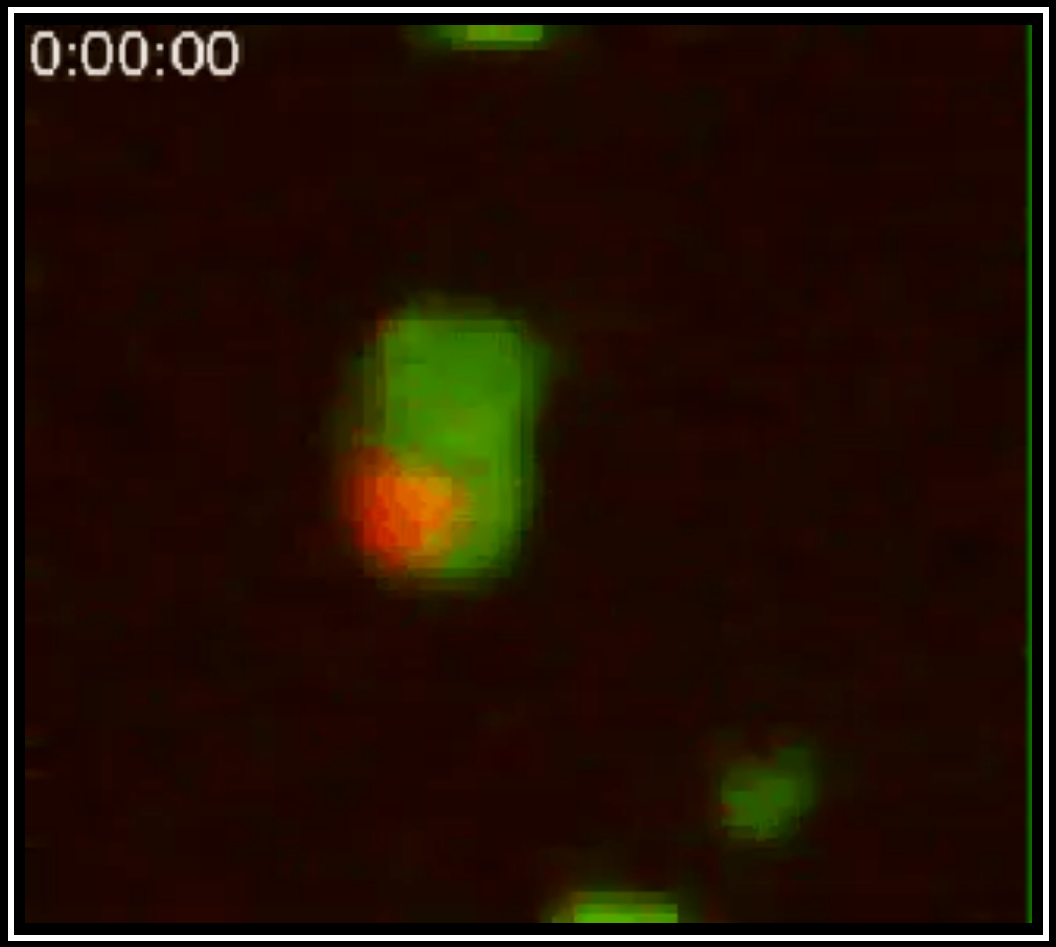
T Antigen-specific T cell

Onset of B-T interaction



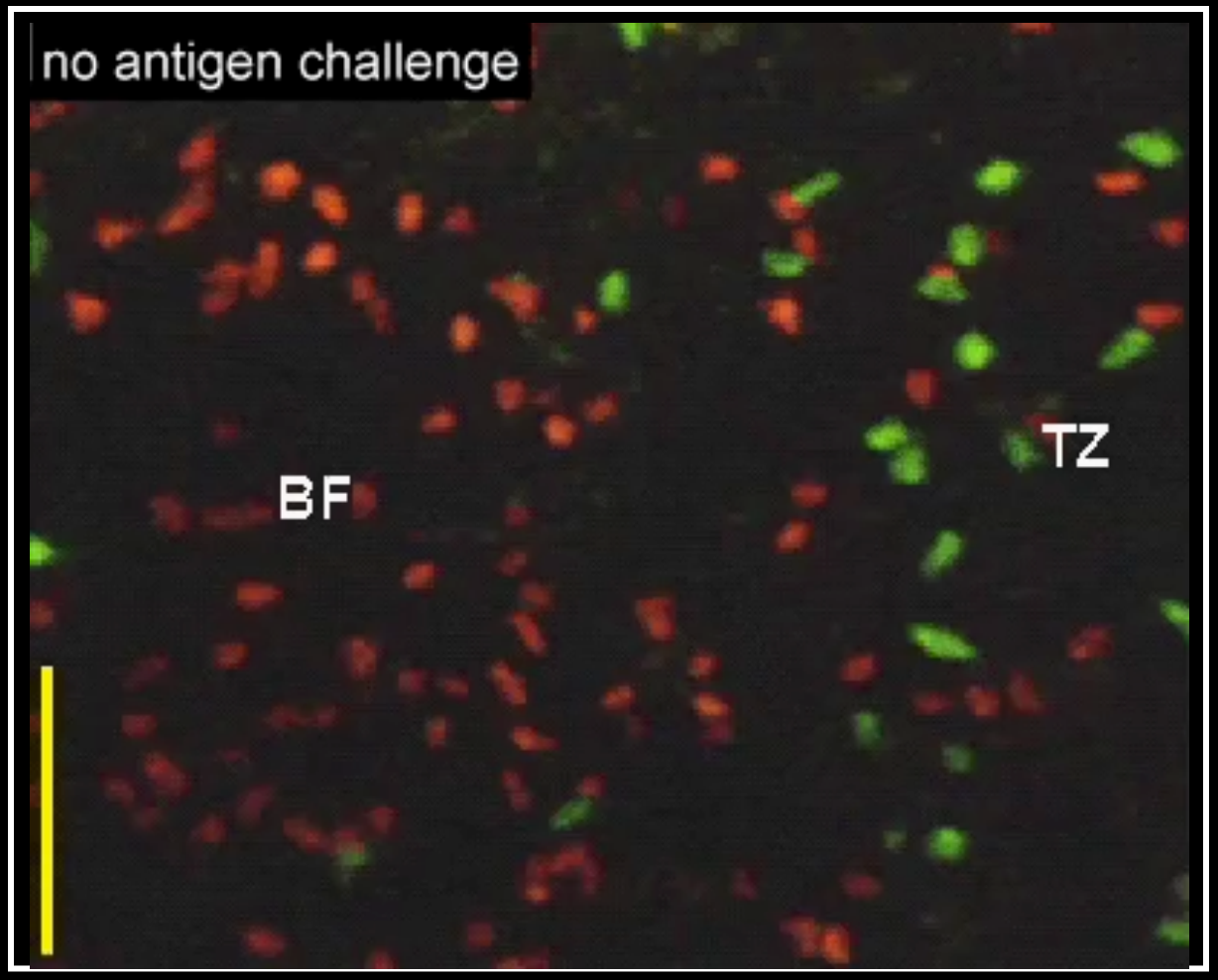
HEL-specific B cells
HEL-specific T cells

B cells can interact with multiple T cells



HEL-specific B cells
HEL-specific T cells

Time series showing the dynamics of B-T interaction during the early phase of the antibody response



HEL-specific B cells
HEL-specific T cells

Summary 4

Antigen specific B cell - CD4 T encounter:

- Cells move to a common location in lymphoid tissue
- B-T conjugate pairs are highly motile
- Antigen specific conjugates persist for >10 min, some for more than 1 hr
- Antigen non-specific conjugates persist <10 min

Questions

- How much contact time is needed for a T cell to ‘help’ a B cell?
- Do B cells integrate the help they receive over time?

Effector T cell Trafficking

- **Activated T cells exit lymphoid tissue -> circulation**
  - ability to re-enter lymphoid tissue is reduced (decrease in CCR7, L-selectin)

- **Increased ability to enter inflammed tissue due to increased expression of:**
  - ligands for E- and P- selectins
  - receptors for inflammatory chemokines (e.g. CXCR3)
  - adhesion molecules (e.g. integrin a4b7)

Question

- What receptor do activated T cells upregulate so they can exit the lymphoid tissue?

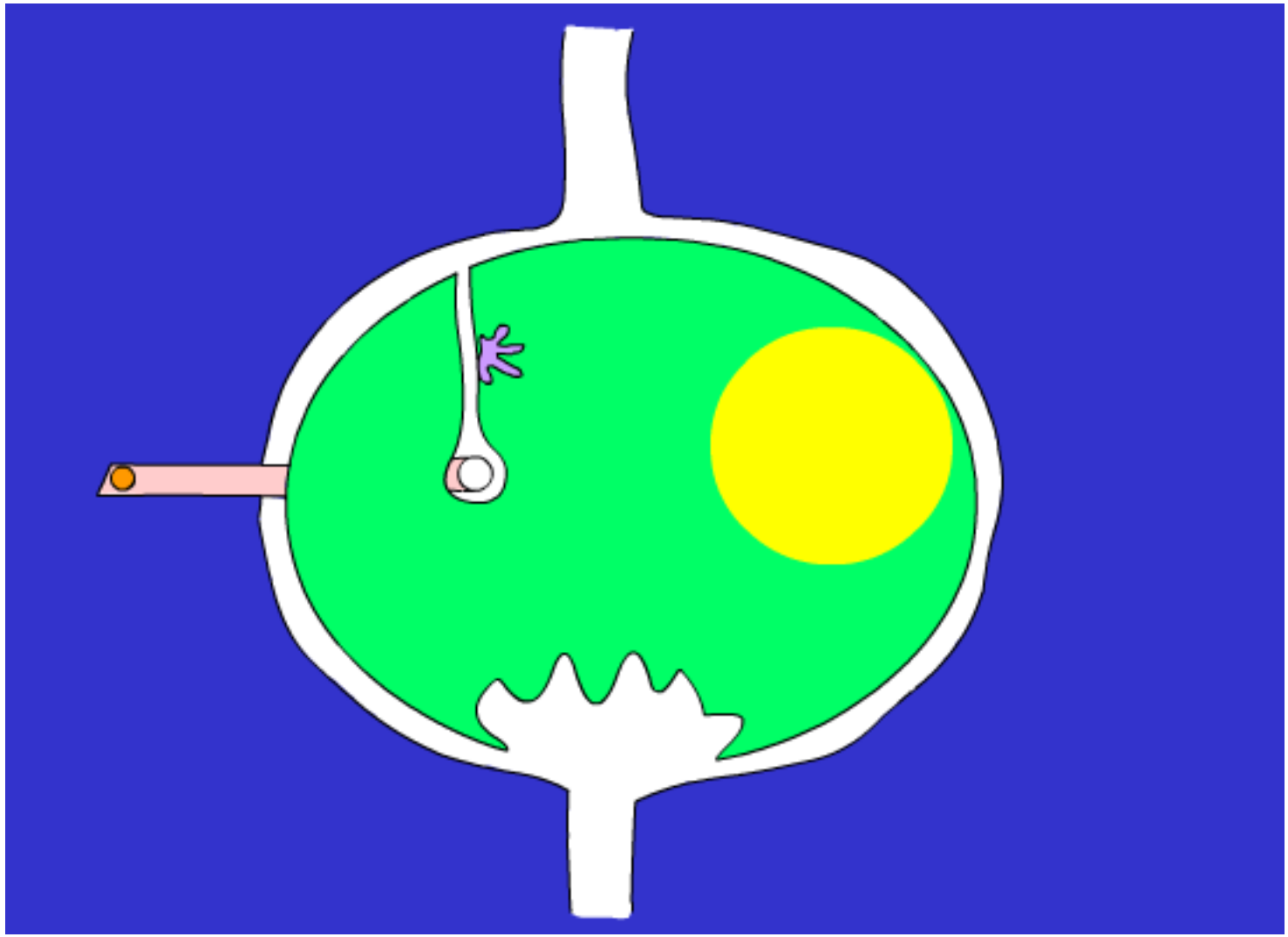
Effector T cells in non-lymphoid tissue

- Effector T cells attracted to site in response to chemokines
  - produced by tissue cells exposed to microbial products (e.g. epithelial cells, keratinocytes, mast cells, macrophages)
  - some memory CD8 T cells take up long-term residence in the tissue and are termed T resident memory (Trm) cells
- Macrophages and DCs in tissue present Ag to CD4 T cells
  - CD4 T cells release cytokines that activate macrophages to promote killing of ingested organisms
- All cells (except RBC) express MHC class I and can be recognized (and killed) by effector CD8 T cells

Question

- What are some of the requirements for effector T cells to access inflammed tissues?

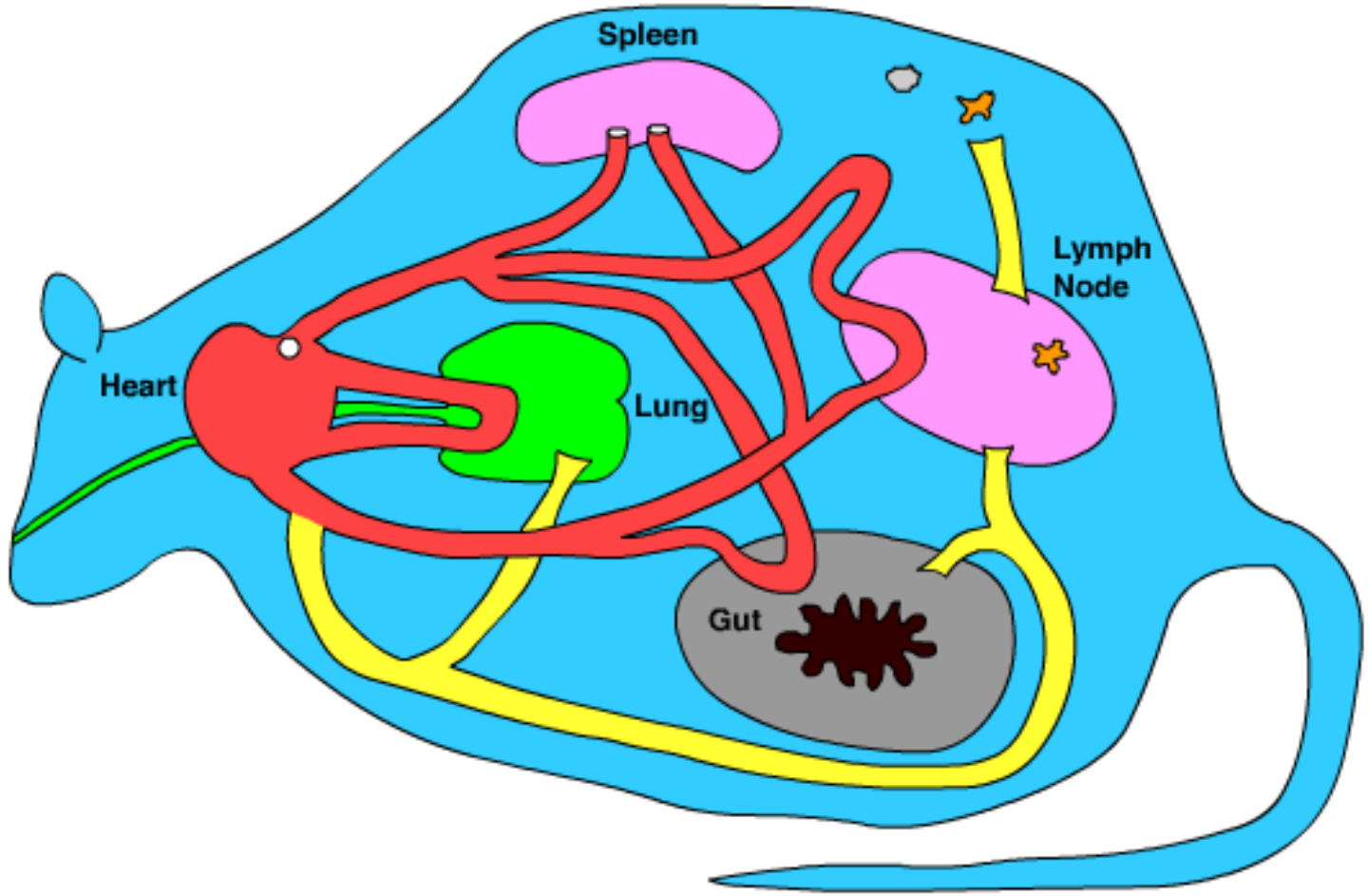
Primary immune response in a lymph node



See also Jenkins et al., (2001) Annu. Rev. Immunol. 19; 23

Provided by Marc Jenkins, Center for Immunology at the University of Minnesota

Adaptive immune response to s.c. infection



Animation provided by Marc Jenkins, Center for Immunology at the University of Minnesota

Translational impact of understanding anatomy of immune responses

- Augmenting effects of pre-existing antibody on antibody response (vaccination)
- Use of DCs as anti-cancer adjuvants
- Use of chemokines to agument vaccine response
  - Shin & Iwasaki 2012 Nature
- Using engineered cells to attack tumors – requires correct homing properties
- Efforts ongoing to build ‘artificial LNs’

Recommended Reading

Schulz, O … Forster, R (2016) Chemokines and Chemokine Receptors in Lymphoid Tissue Dynamics. Annu. Rev. Immunol. 34, 203-42

Qi H, Kastenmuller W, Germain RN (2014) Spatiotemporal Basis of Innate and Adaptive Immunity in Secondary Lymphoid Tissue. Annu. Rev. Cell Dev. Biol. 30, 141-167

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Cyster JG and Schwab SR (2012) Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. Annu. Rev. Immunol. 30:69-94

Cyster, JG (2010) B cell follicles and antigen encounters of the third kind. Nat. Immunol. 11, 989

Lammermann T and Sixt M (2008) The microanatomy of T cell responses. Imm. Rev. 221, 26-43

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Mebius RE and Kraal G (2005) Structure and Function of the Spleen. Nat. Rev. Immunol. 5, 606-616

Itano AA, Jenkins MK (2003) Antigen presentation to naive CD4 T cells in the lymph node. Nat Immunol. 4:733-9