Institute for Research in Biomedicine

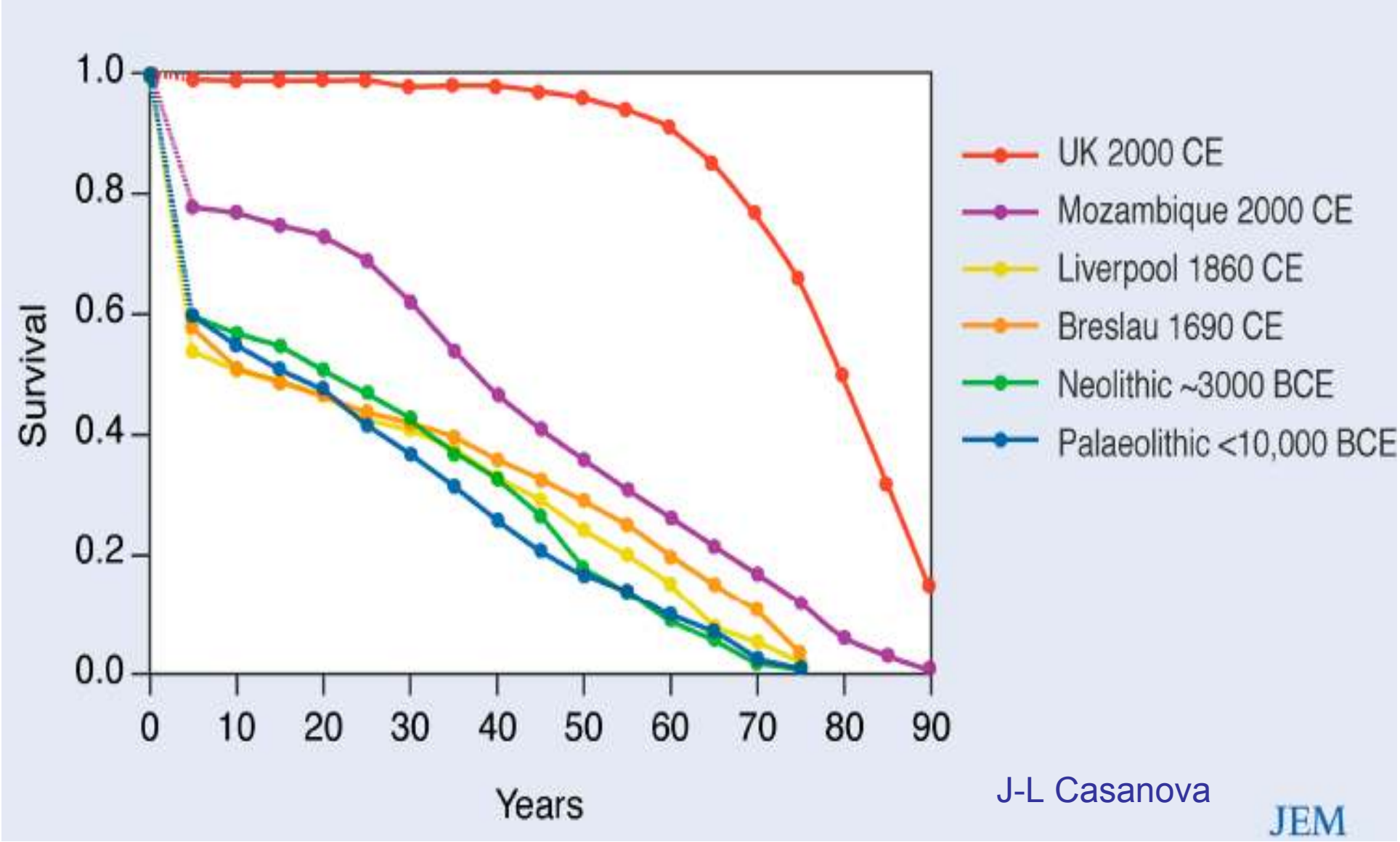
T cell differentiation, migration and immune regulation

Antonio Lanzavecchia

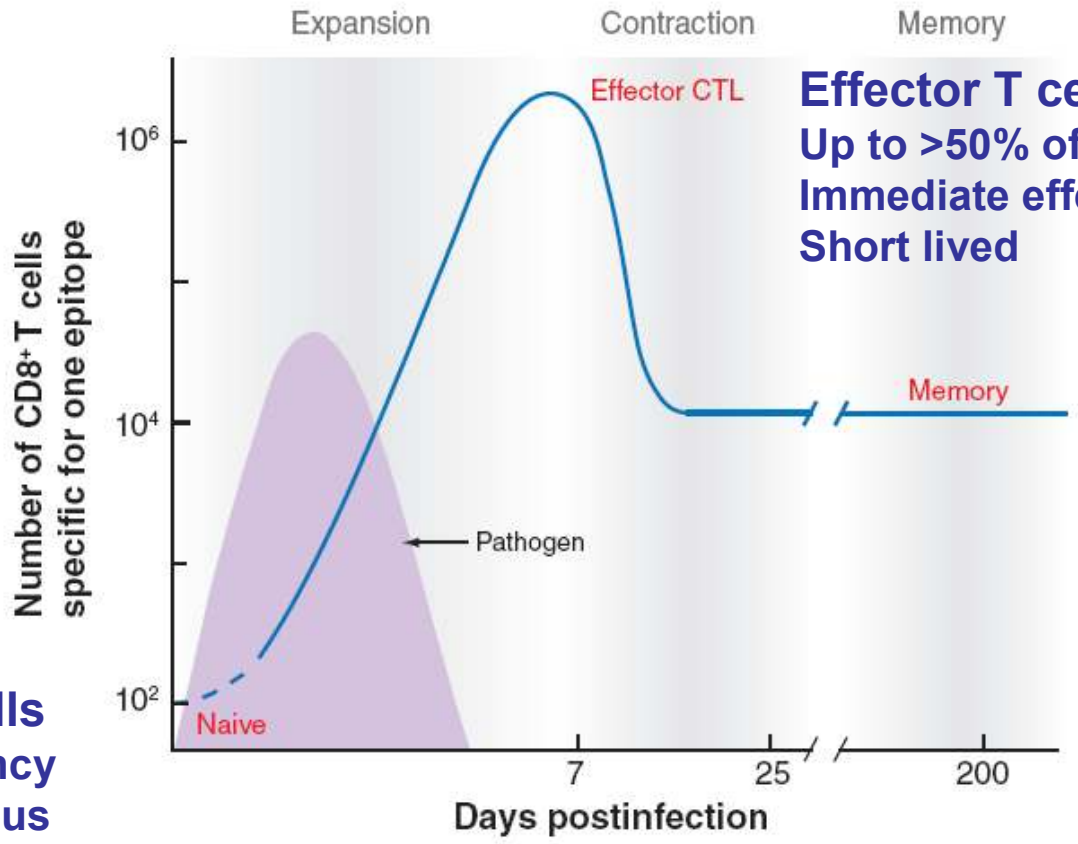
Institute for Research in Biomedicine
Bellinzona, Switzerland

lanzavecchia@irb.unisi.ch

Increased life expectancy and medical progress



From naïve to memory T cells

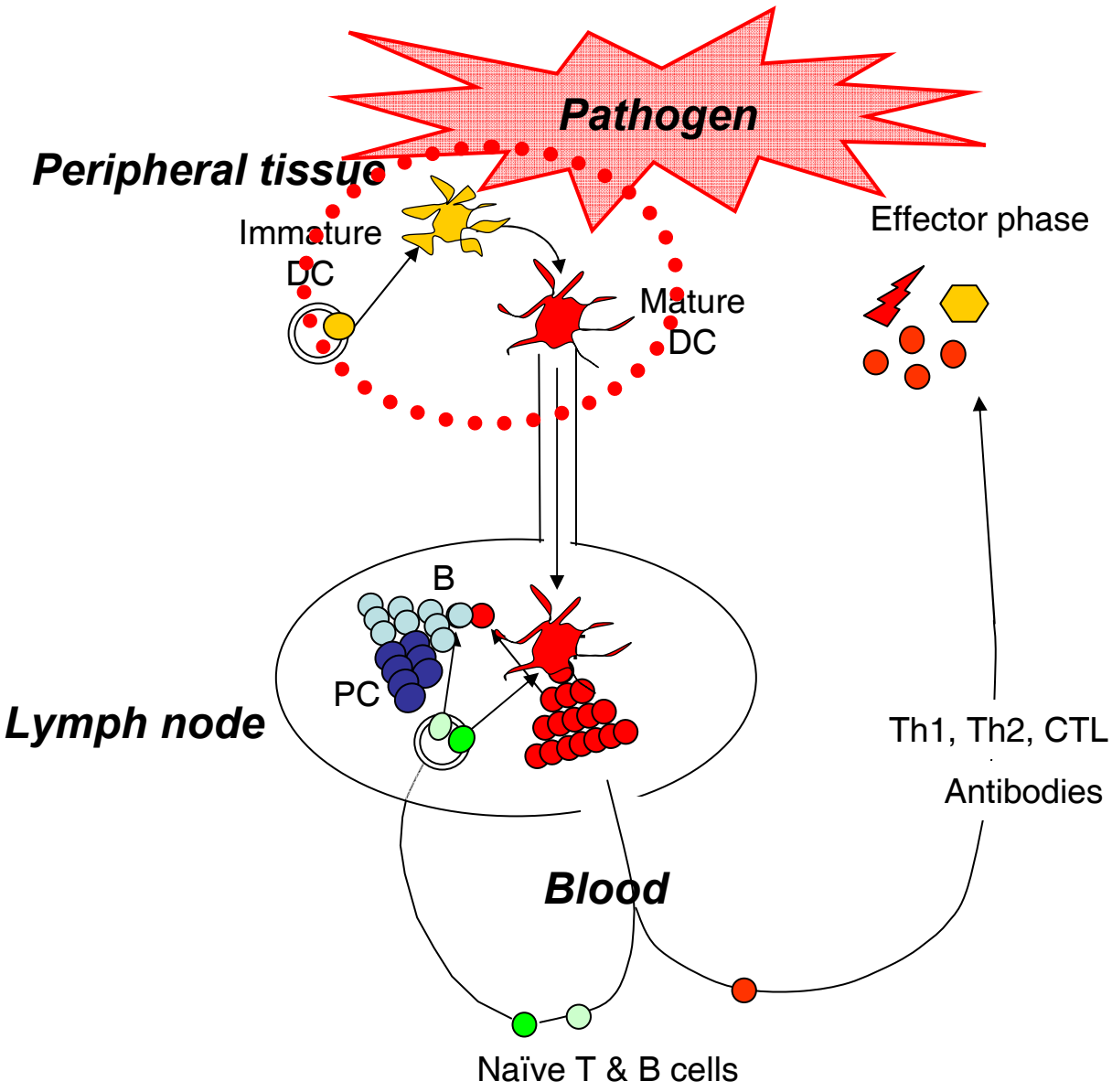


Effector T cells
- Up to >50% of circulating T cells
- Immediate effector function
- Short lived

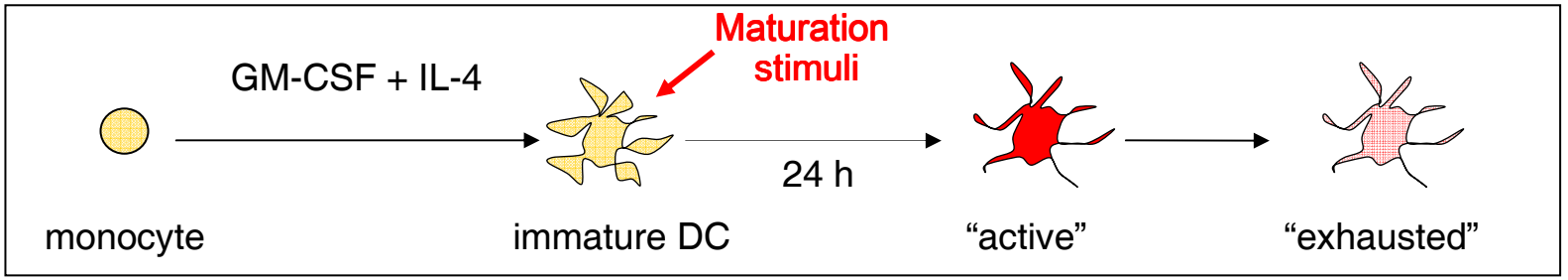
Memory T cells
- Higher frequency
- Heterogeneous
- Slowly dividing
- Highly responsive

Naïve T cells
- Low frequency
- Homogeneous
- Resting “spores”
- Poorly responsive

Cell migration in the immune response



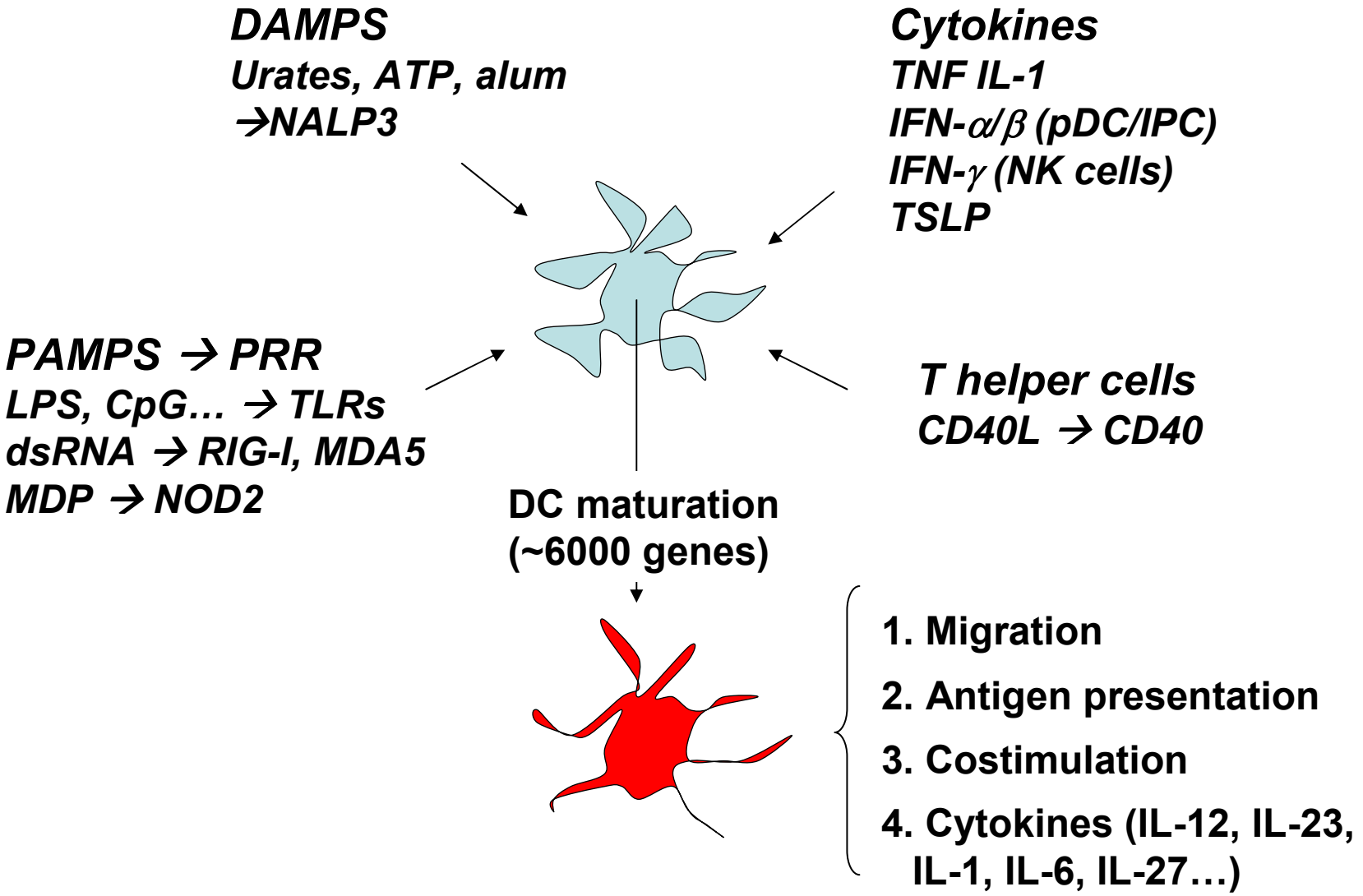
Dendritic cell maturation in vitro



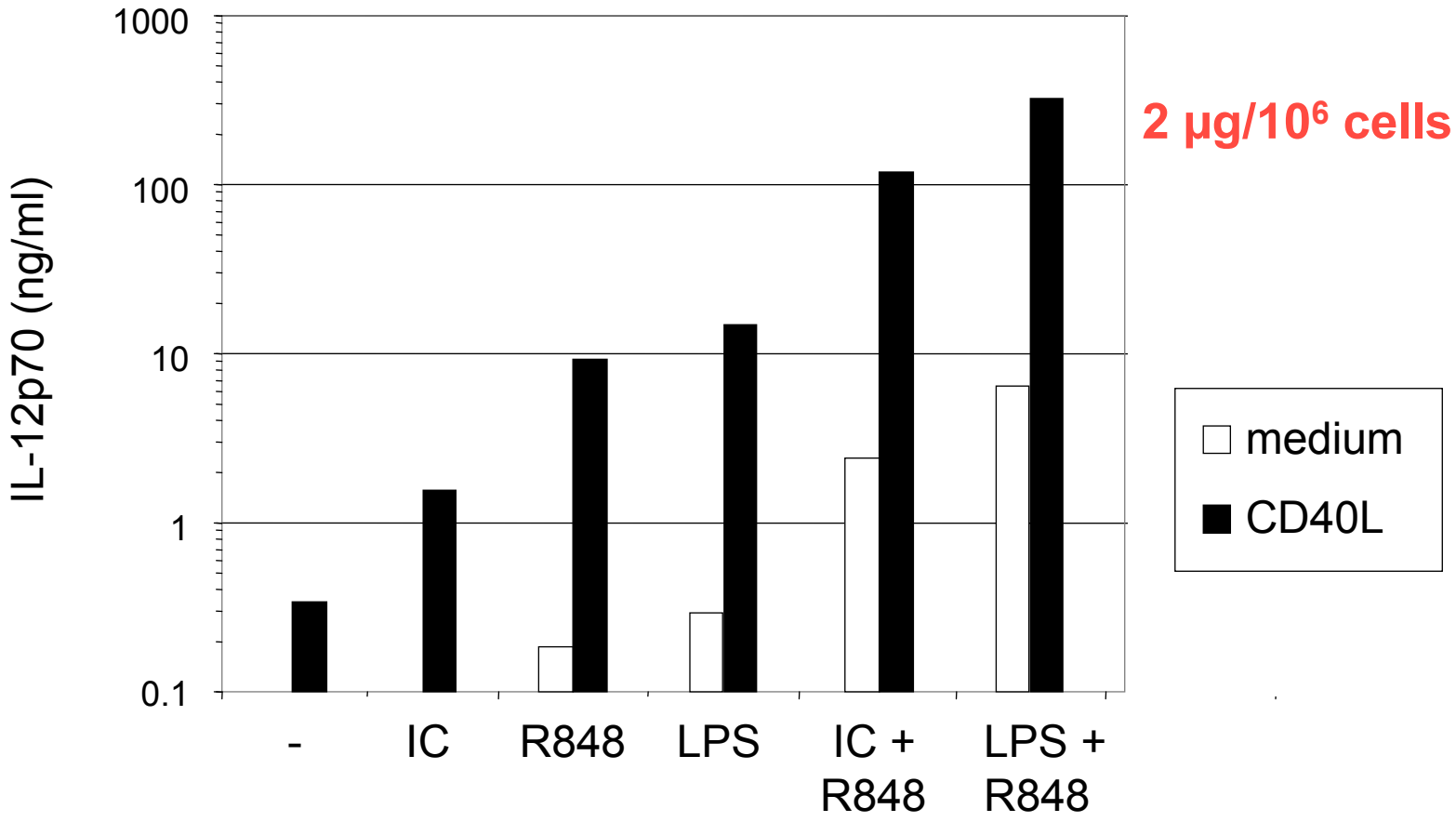
Antigen capture[1]	Macropinocytosis	++	+	-
	Mannose R	++	+	-
Antigen presentation[2]	MHC II synthesis	+	++	-
	MHC II halflife	10 h		>100 h
	MHC I synthesis	+	++	++
Costimulation[1]	B7	-	+	+
Migration[4]	CCR5	+	(+)	-
	CCR7	-	+	++
Cytokines[5]	TNF IL-6 IL-1	-	+	-
	IFN-I	-	+	-
	IL-12	-	+/-	-

1) Sallusto et al *JEM* 1994; JEM 1995; 2) Cella et al *Nature* 1997; 3) Cella et al *JEM* 1999; 4) Sallusto et al *EJI* 1998; 5) Langenkamp et al *Nat Immunol* 2000

DC activation: integration of multiple stimuli



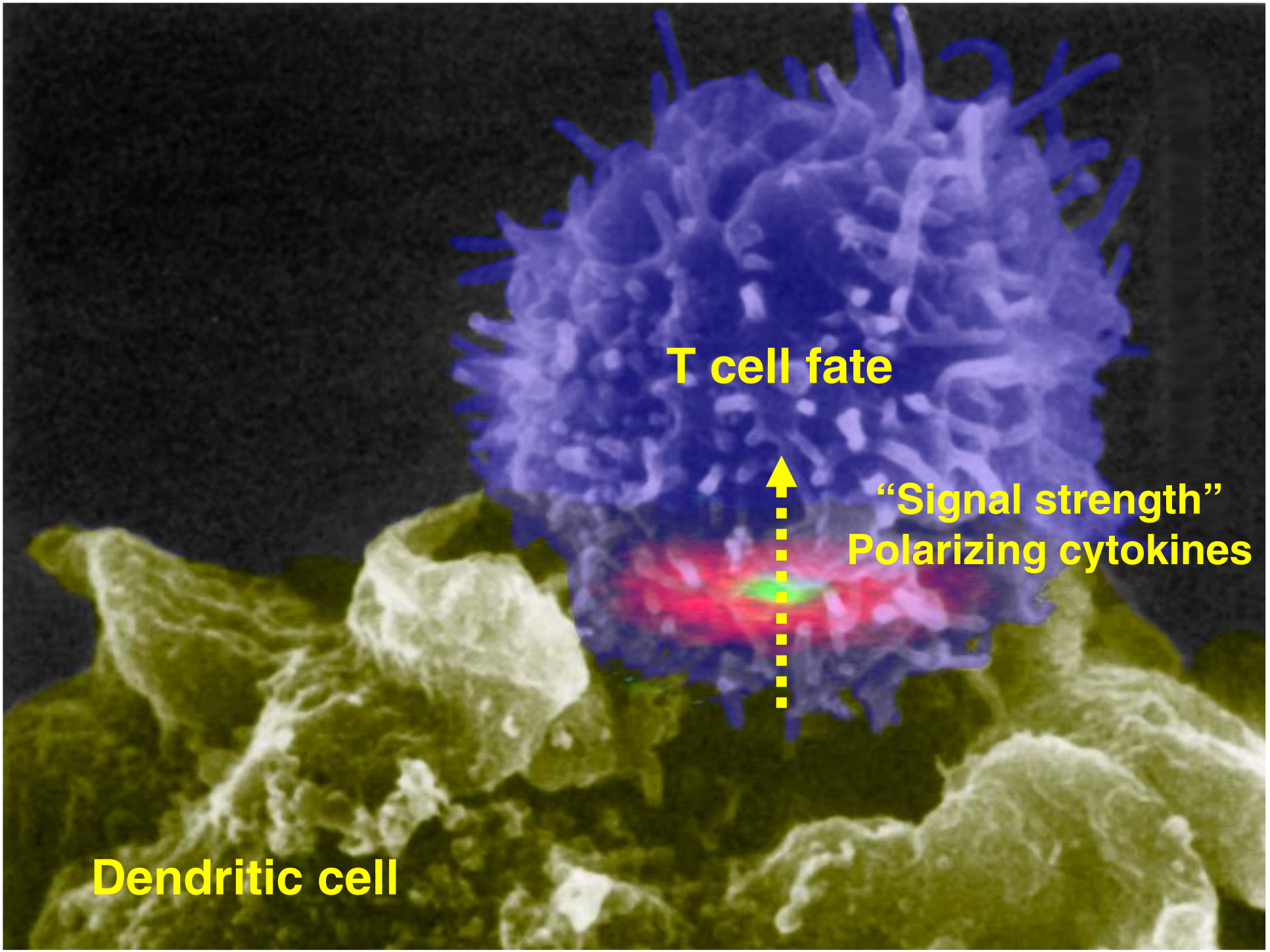
IL-12p70 production is triggered by synergic TLR stimulation and is further boosted by CD40L

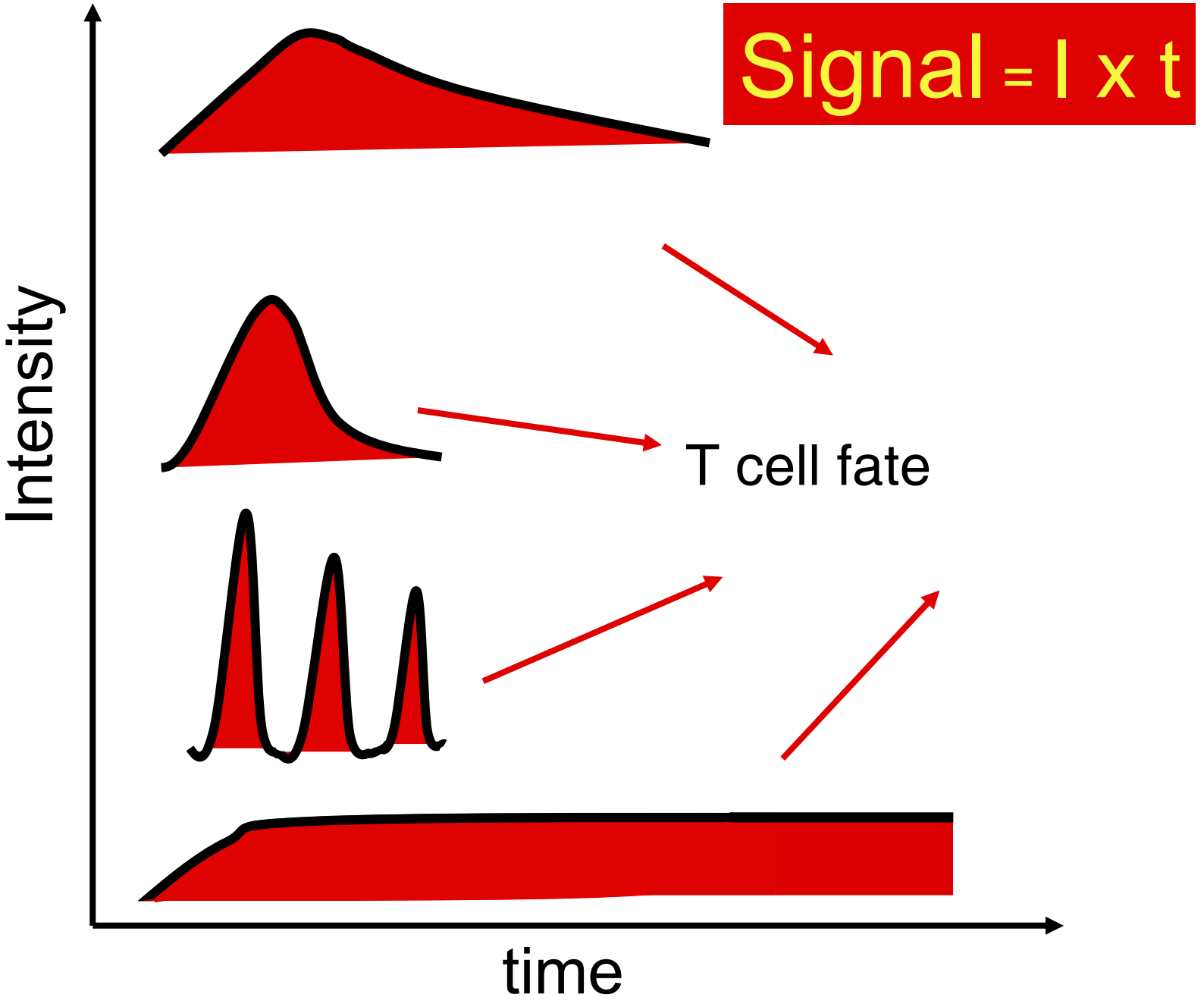


1. T cell activation and fate determination

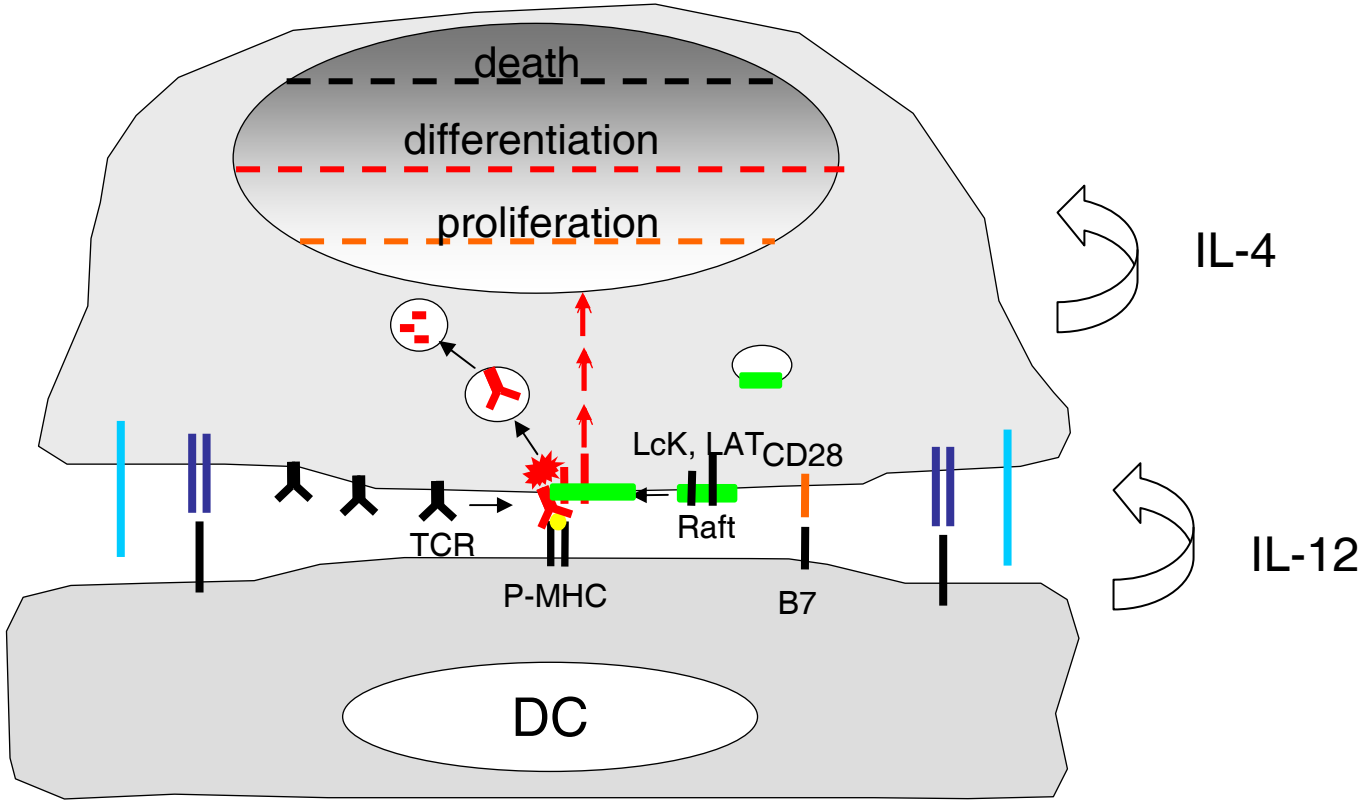
2. Human effector/memory T cell subsets
   (T_{CM}, T_{EM}, Th1, Th2, Th17, etc)

3. T cell traffic in steady state and inflammatory
   conditions (mouse)


T cell fate
“Signal strength”
Polarizing cytokines
Dendritic cell

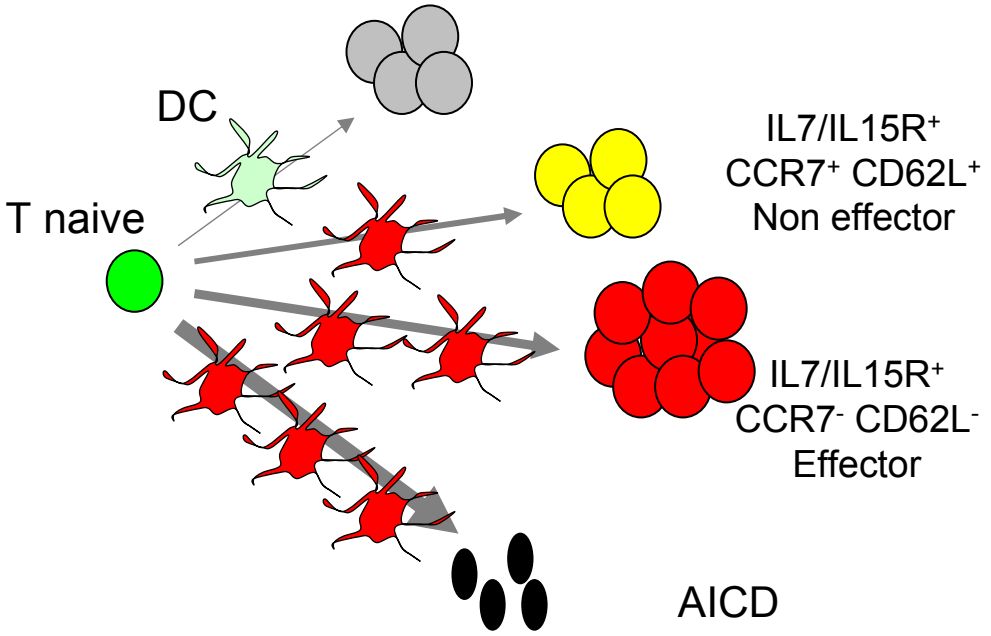

Signal = I x t
Intensity
T cell fate
time

Signal strength and activation thresholds



1. [peptide-MHC]	Rate of TCR triggering
2. [B7]	Amplification
3. Stability of the synapse	Duration of signalling
4. [Polarizing cytokines]	Differentiation

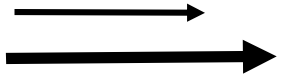
The strength of stimulation determines T cell fate



IL7/IL15R^{-}, BCL-X_L^{-} — *"Unfit" Die by neglect*

IL7/IL15R^{+} CCR7^{+} CD62L^{+} Non effector — *"Fit" Migrate to secondary lymphoid organs*

IL7/IL15R^{+} CCR7^{-} CD62L^{-} Effector — *Migrate to inflamed tissues*



Strength / duration of stimulation
DC maturation and density, antigen concentration, stochastic T-DC interactions and T-T competition
Polarizing cytokines

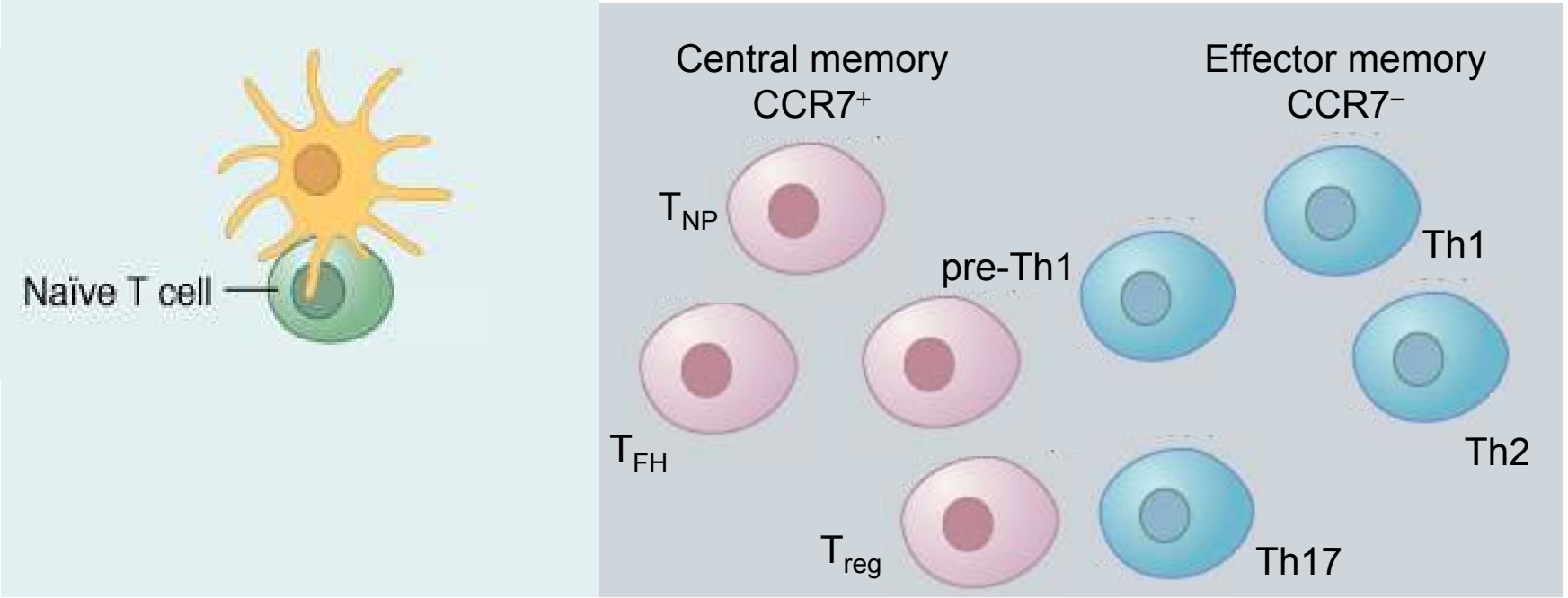
Iezzi et al, *Immunity* 1998
Iezzi et al, J*EM* 2000
Langenkamp et al, *Nat Immunol* 2000
Langenkamp et al, *EJI* 2002
Gett et al, *Nat Immunol* 2003
Messi et al, *Nat Immunol* 2003

1. T cell activation and fate determination

2. **Human effector/memory T cell subsets (T_{CM}, T_{EM}, Th1, Th2, Th17, etc)**

3. T cell traffic in steady state and inflammatory conditions (mouse)

Dynamics of T lymphocyte responses: intermediates, effectors and memory cells

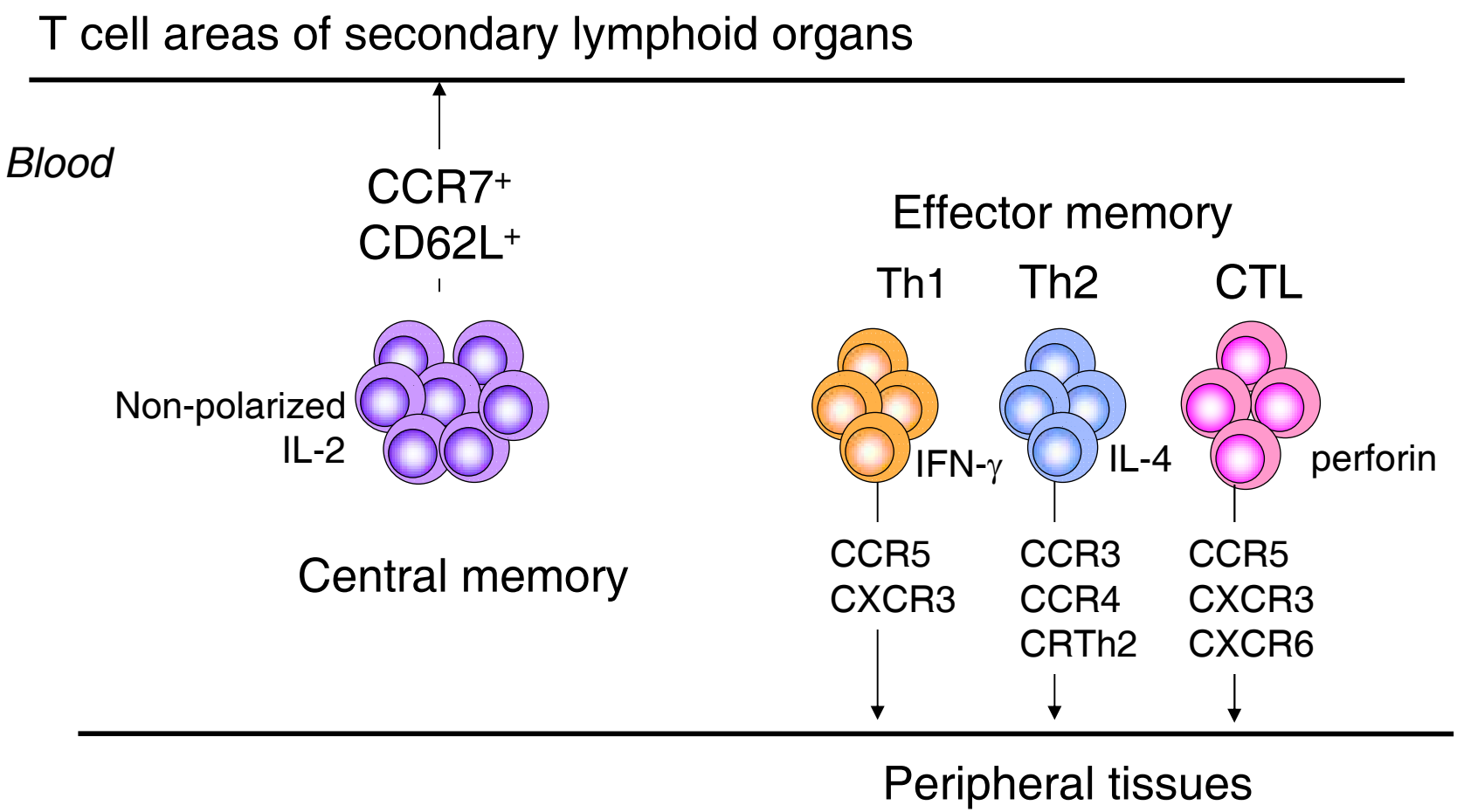


- *Strength / duration of stimulation*
- *T-DC serial encounters*
- *Polarizing cytokines*
- *Asymmetric division*

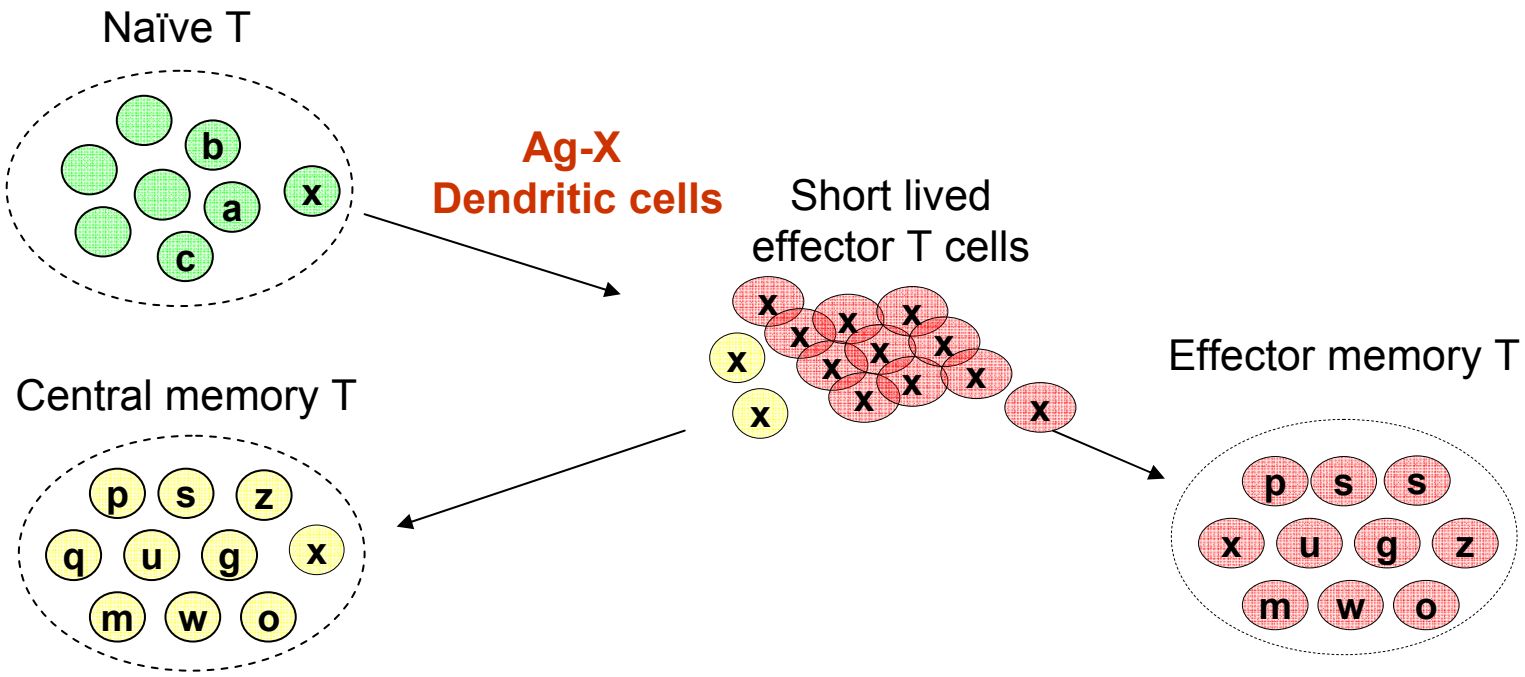
- *Migratory capacity*
- *Effector function*
- *Differentiation potential*
- *Survival*

Two subsets of memory T cells with distinct migratory capacity and effector function

Sallusto et al. *Nature 401: 708 (1999)*

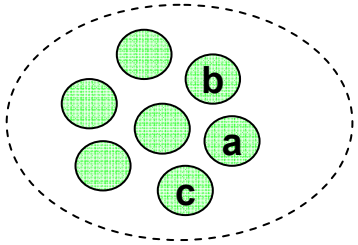


T cell clonal dynamics: primary responses

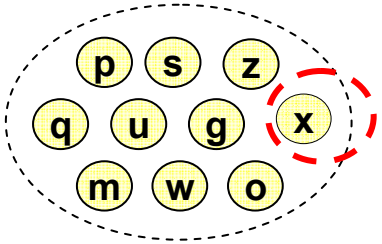


T cell clonal dynamics: memory phase

Naïve T

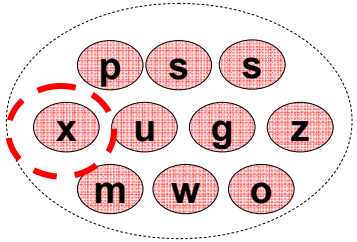


Central memory T



Secondary response

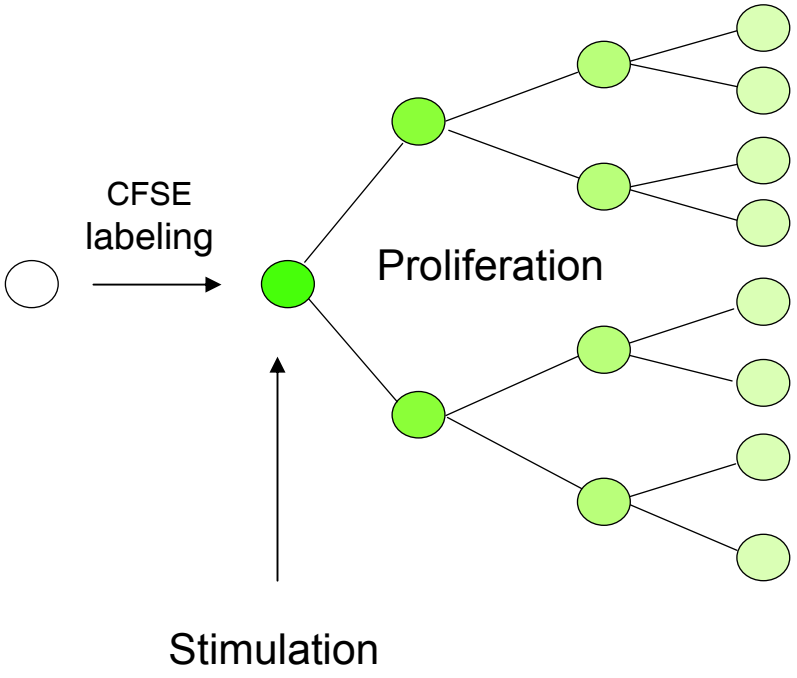
Effector memory T

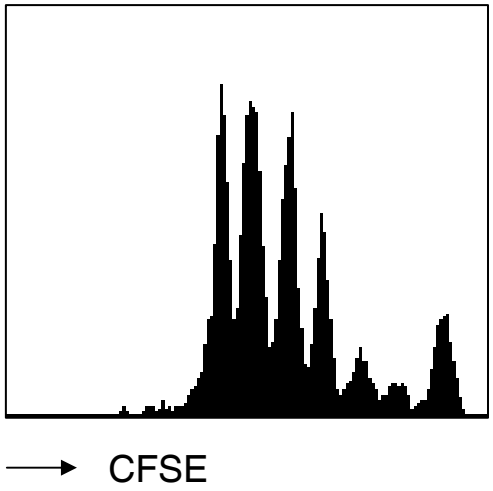


Immediate protection

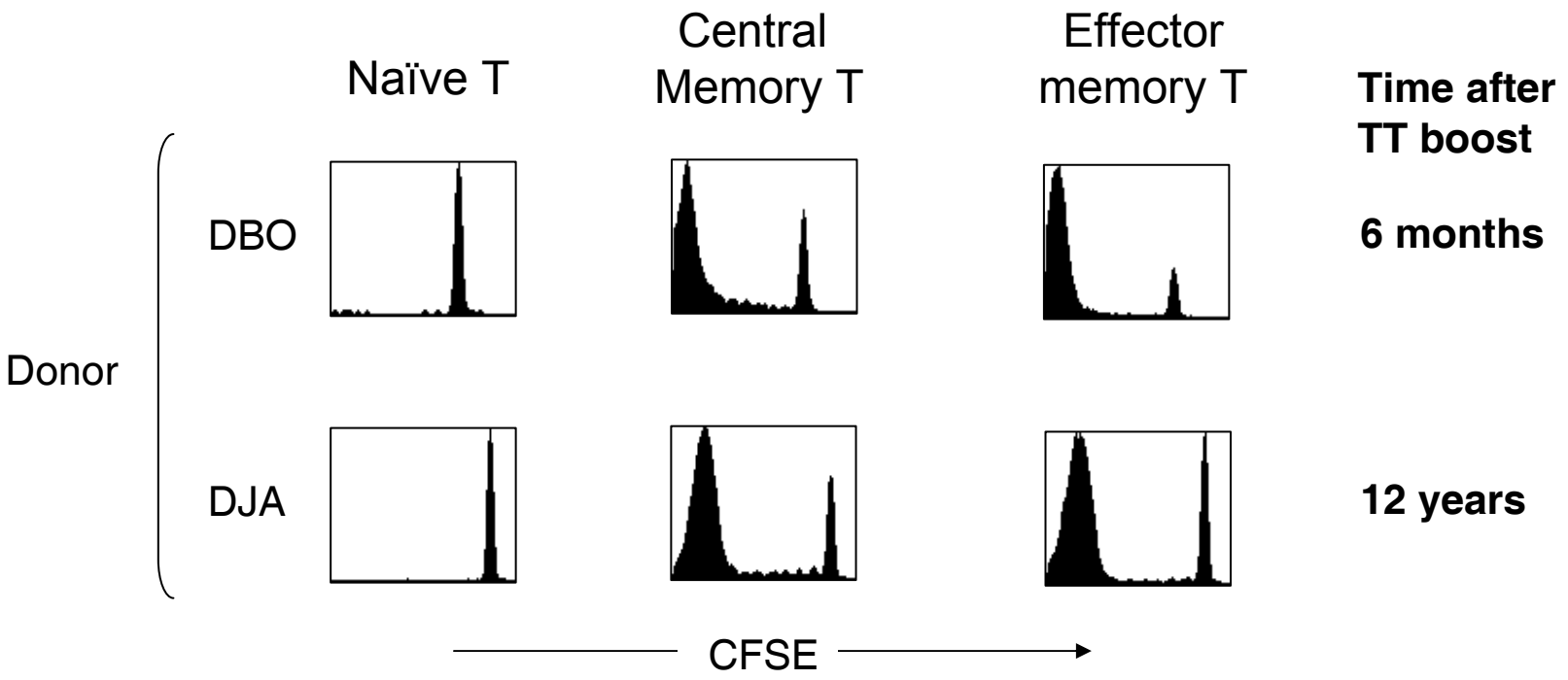
Central and effector memory T cells are stable years

Tracking T cell division by CFSE dilution (Lyons & Parish)





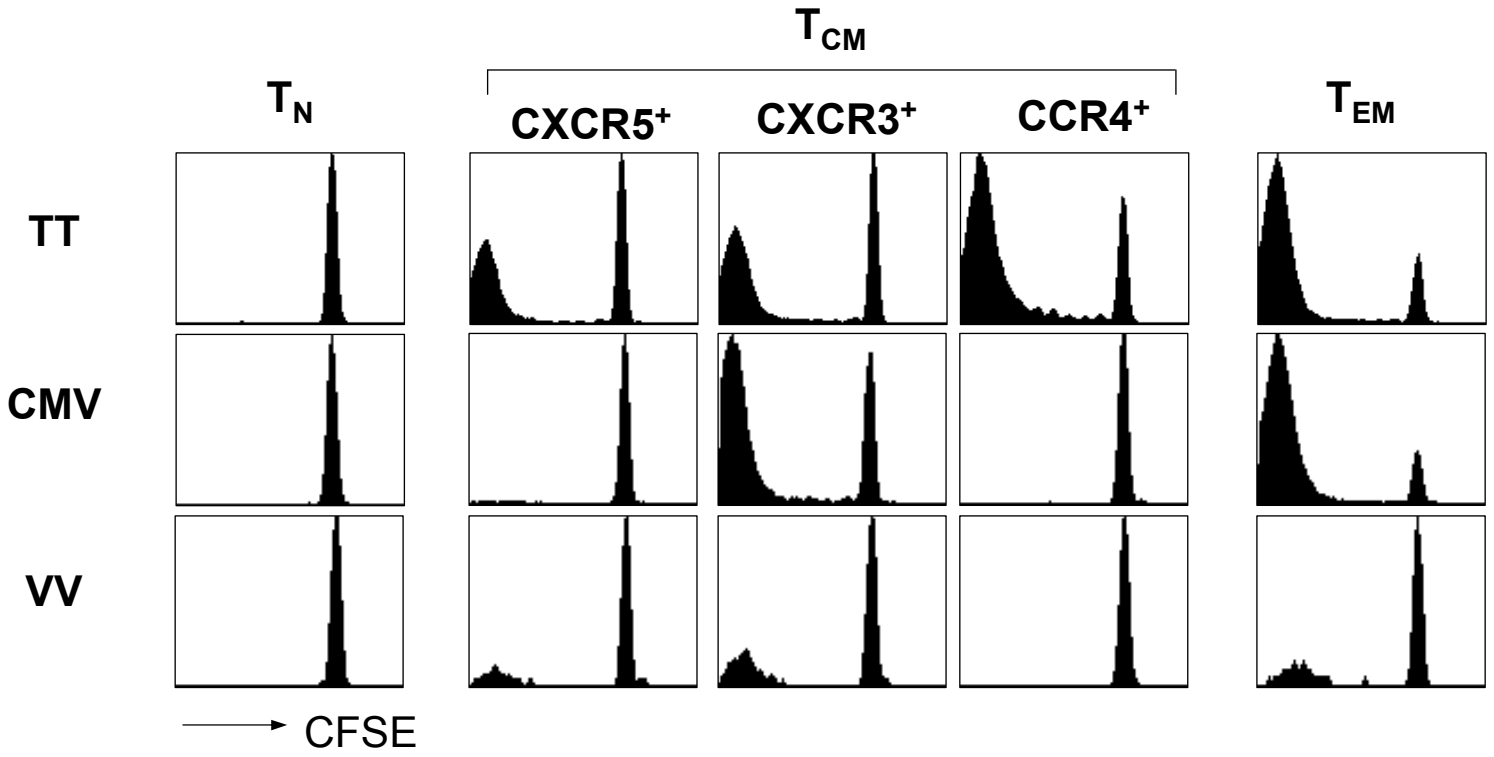
Stability of central memory and effector memory T cells



CFSE-labeled T cells stimulated by antigen-loaded autologous monocytes

Rivino et al, J Exp Med 2004

Antigen-specific T cells are differentially distributed in memory subsets



Rivino et al, J Exp Med 2004

Division of labor among memory T cells

Central memory T (T_{CM})

T_{CM} circulate through secondary lymphoid tissues

Secrete IL-2 but little IFN-γ and no perforin

Robust proliferation capacity and transition into effector cells that can migrate to non-lymphoid tissue

Differentiation intermediates

Effector memory T (T_{CM})

T_{EM} circulate through and can reside in non-lymphoid tissue

Secrete IFN- γ and perforin but little IL-2

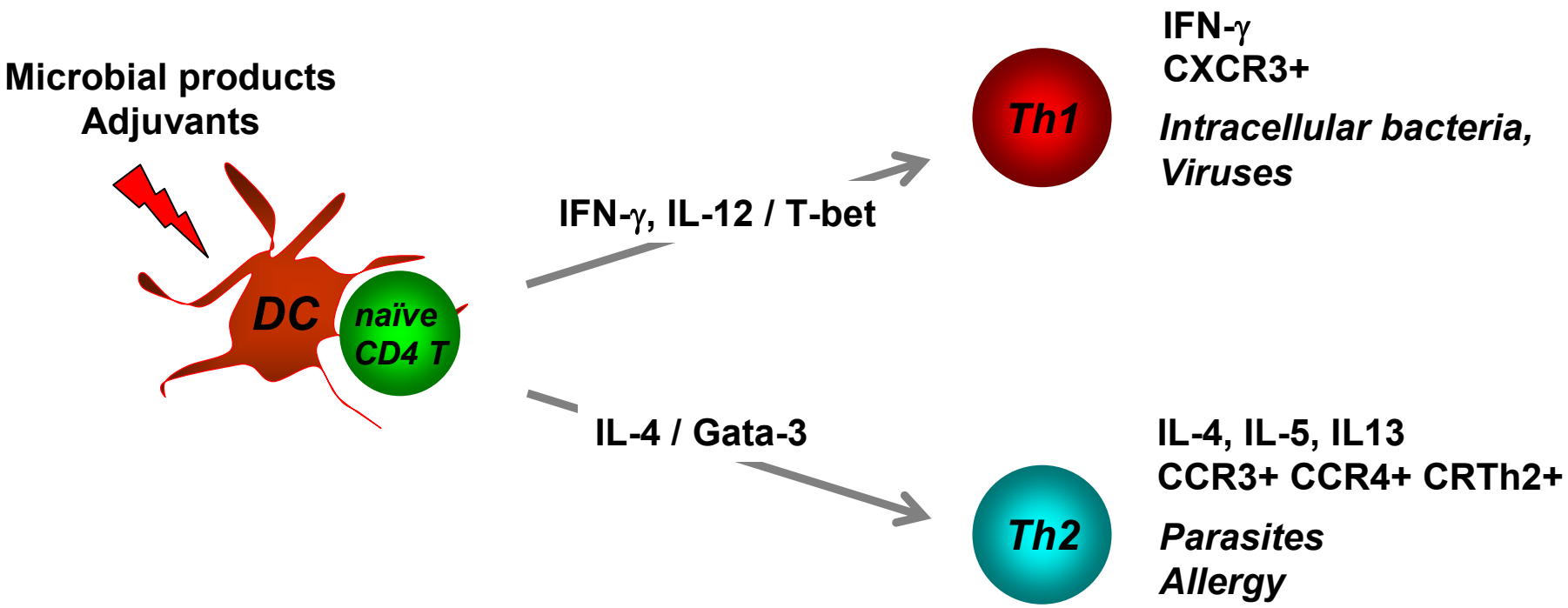
Limited proliferation capacity but display of immediate effector function

Terminally differentiated

Secondary responses

Immediate protection

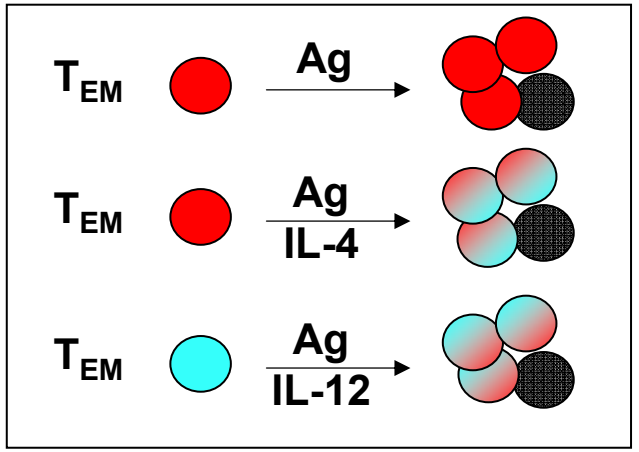
The Th1 / Th2 paradigm



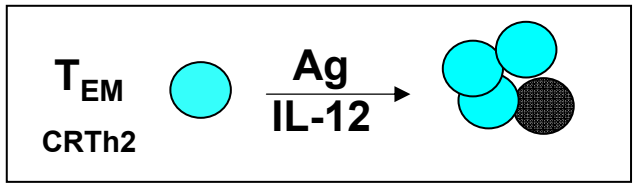
Plasticity of cytokine gene expression in T_{EM}

Chromatin configuration:

	CCR7	Cytokine production	Open chromatin
TH1	-	IFN-γ	*ifng*
TH2	-	IL-4	*il4*
TH0	-	IL-4+IFN-γ	*Ifng* and *il4*



Most

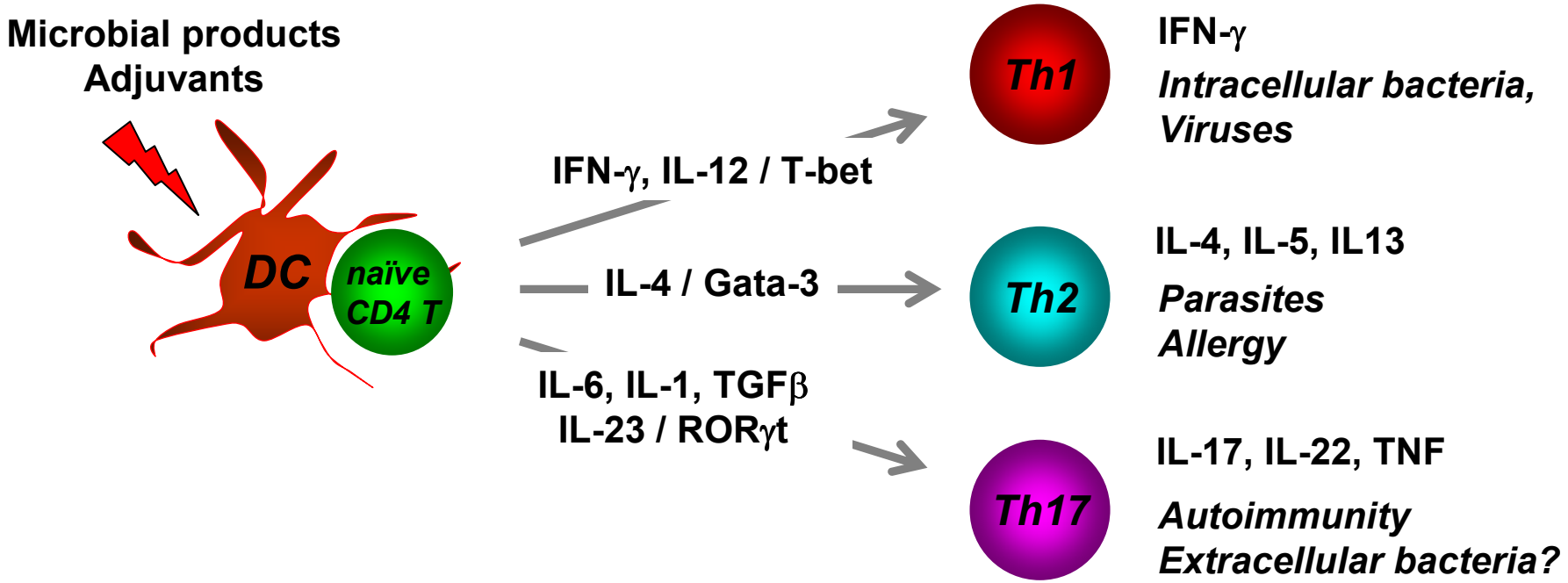


Some

Subset specific, loss of T-bet

Messi et al, Nat Immunol 2003

Th17: a novel subset of CD4 effector T cells



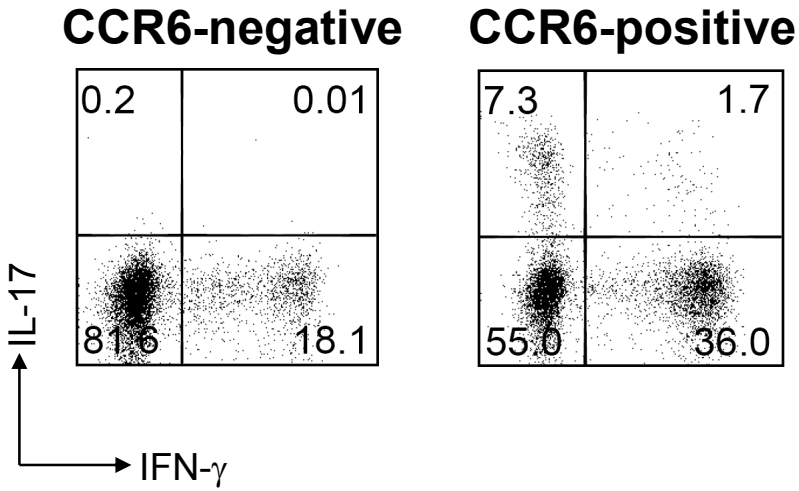
Can we identify Th17 cells using surface markers?

Which are the pathogens that trigger Th17 responses?

IL-17 production is characteristic of a CCR6^{+} subset

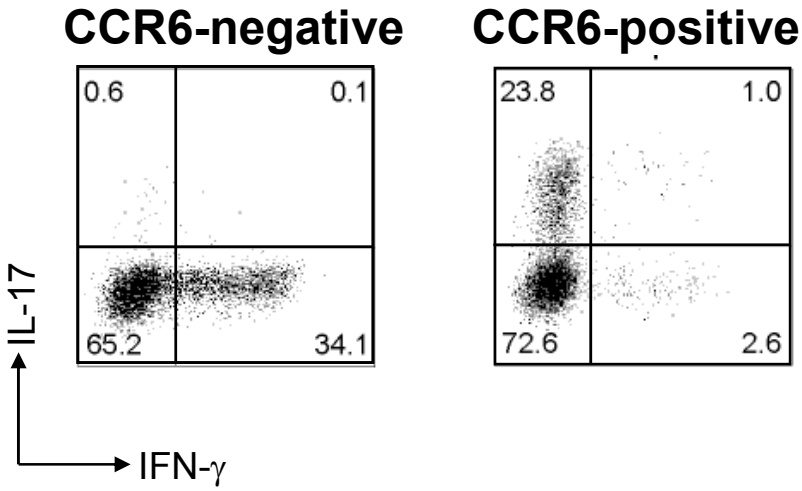
Human memory T cells
(CD4^{+} CD45RA^{-} CD25^{-})

Ex-vivo from blood



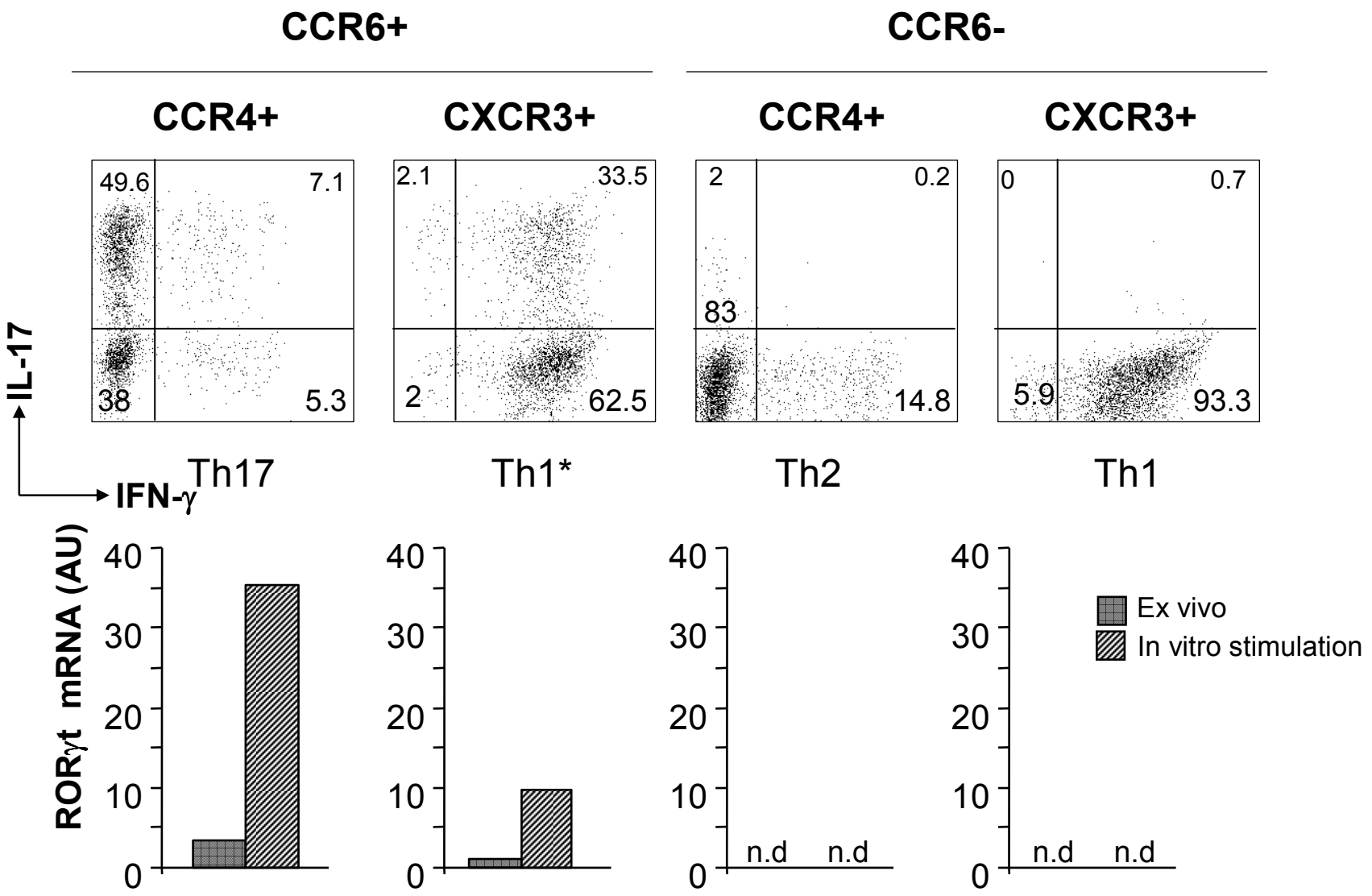
Mouse memory T cells
(CD4^{+} CD25^{-} CD44^{hi})

Ex-vivo from spleen

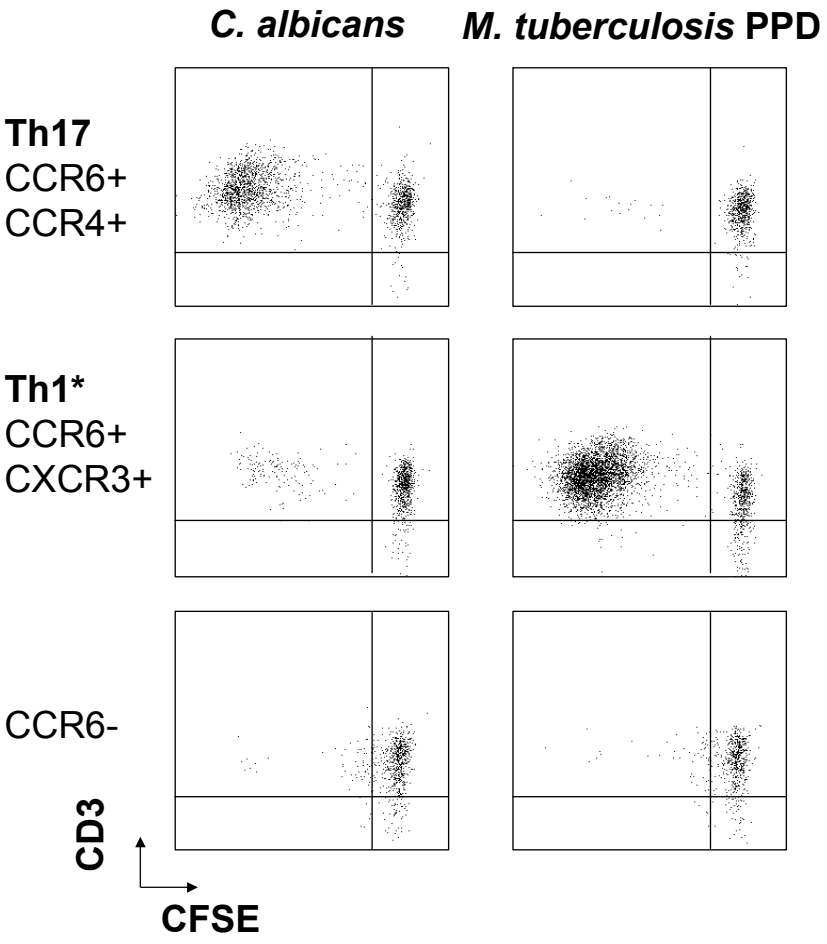


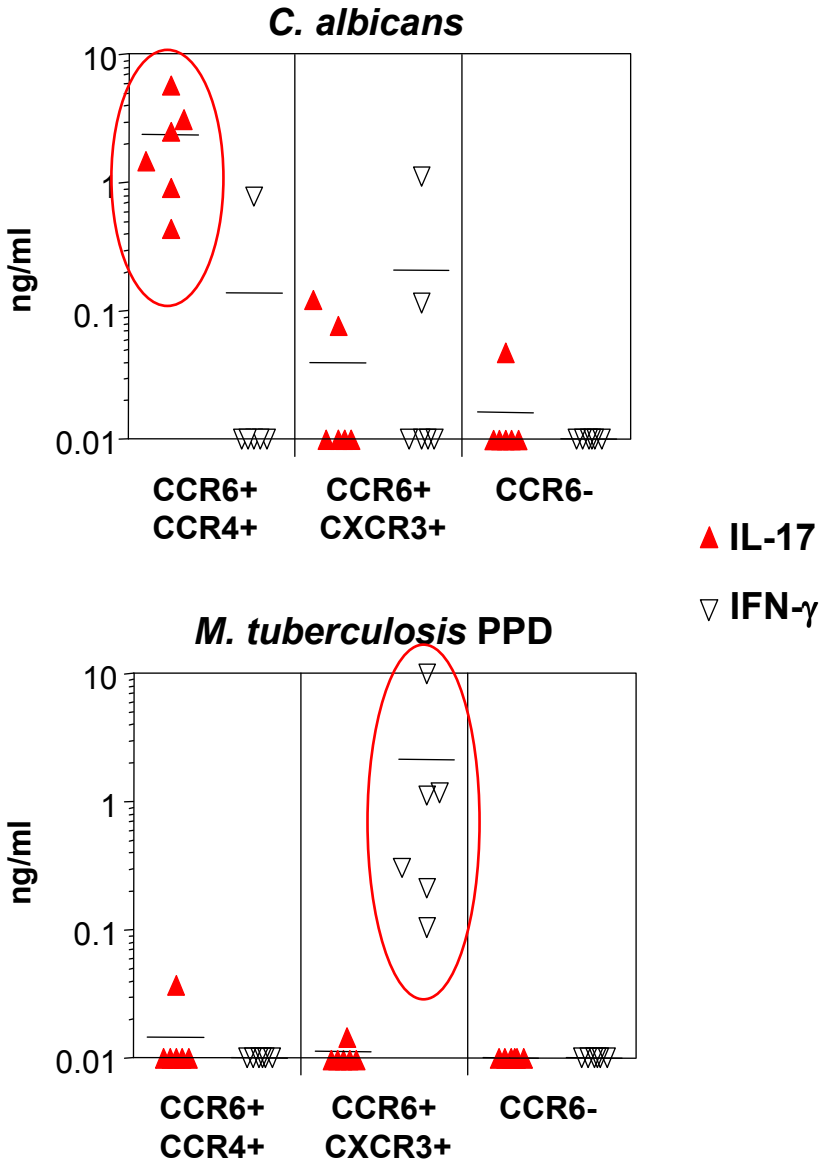
Acosta-Rodriguez et al, Nat Immunol, 2007; Reboldi et al, Nat Immunol, 2009

Human Th17 memory cells co-express CCR6 and CCR4 and express RORγt



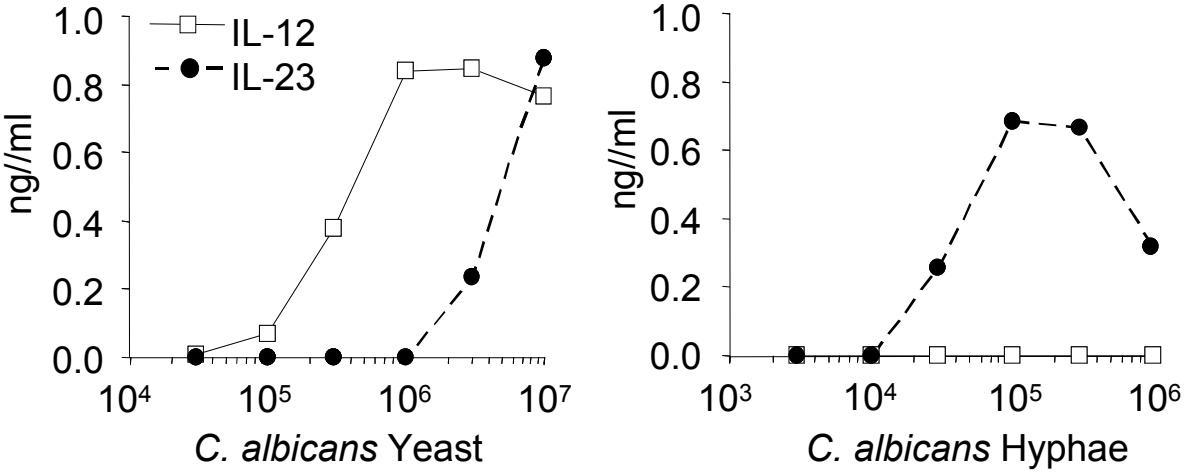
C. albicans specific memory cells are Th17



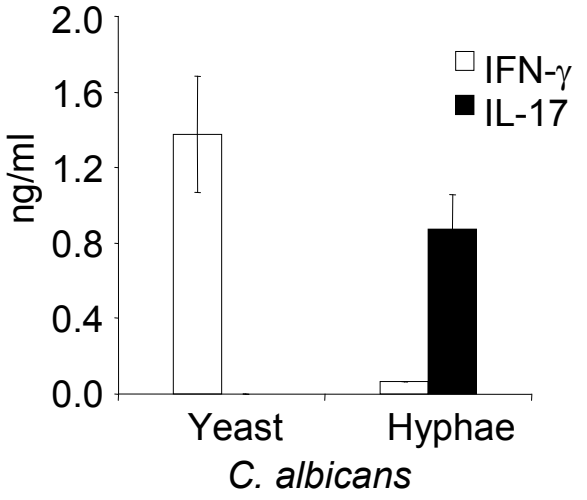


C. albicans hyphae selectively induce IL-23 and prime Th17 cells

IL-12 and IL-23 production by DC



Priming naïve CD4+ T cells



Th17 and immunity to fungi

- Th17 memory cells express CCR6 and CCR4
- *C. albicans* specific memory cells are Th17
- The Hyphal form of *C. albicans* triggers IL-23, but not IL-12
- Th17 deficiency and candidiasis in STAT3 deficient patients
  (Milner et al Nature 2008; Ma et al JEM 2008)

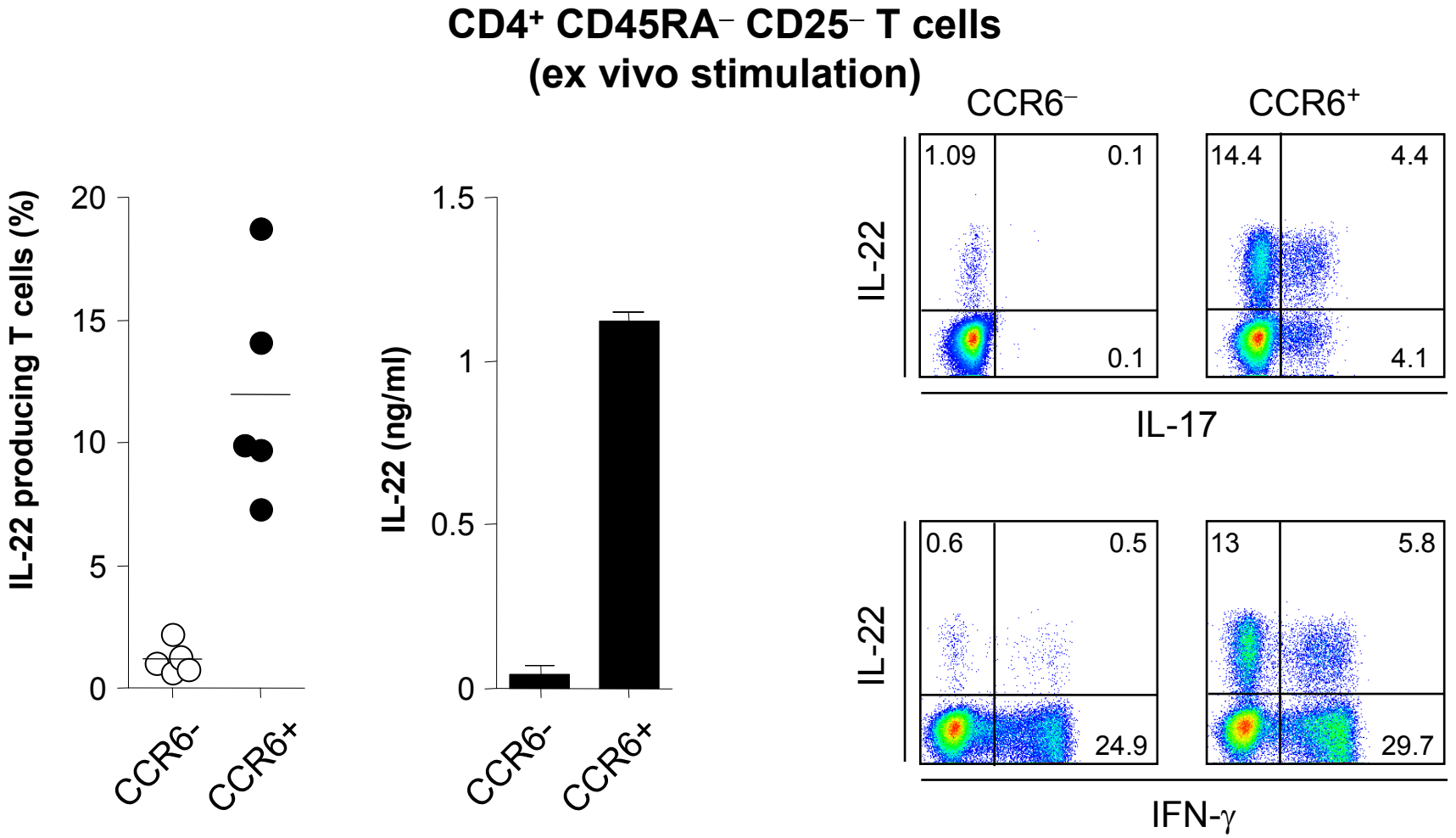
Th17 cells might recruit neutrophils thus promoting entrapment and killing of hyphae

Institute for Research in Biomedicine

Th22: a new subset of T helper cells?

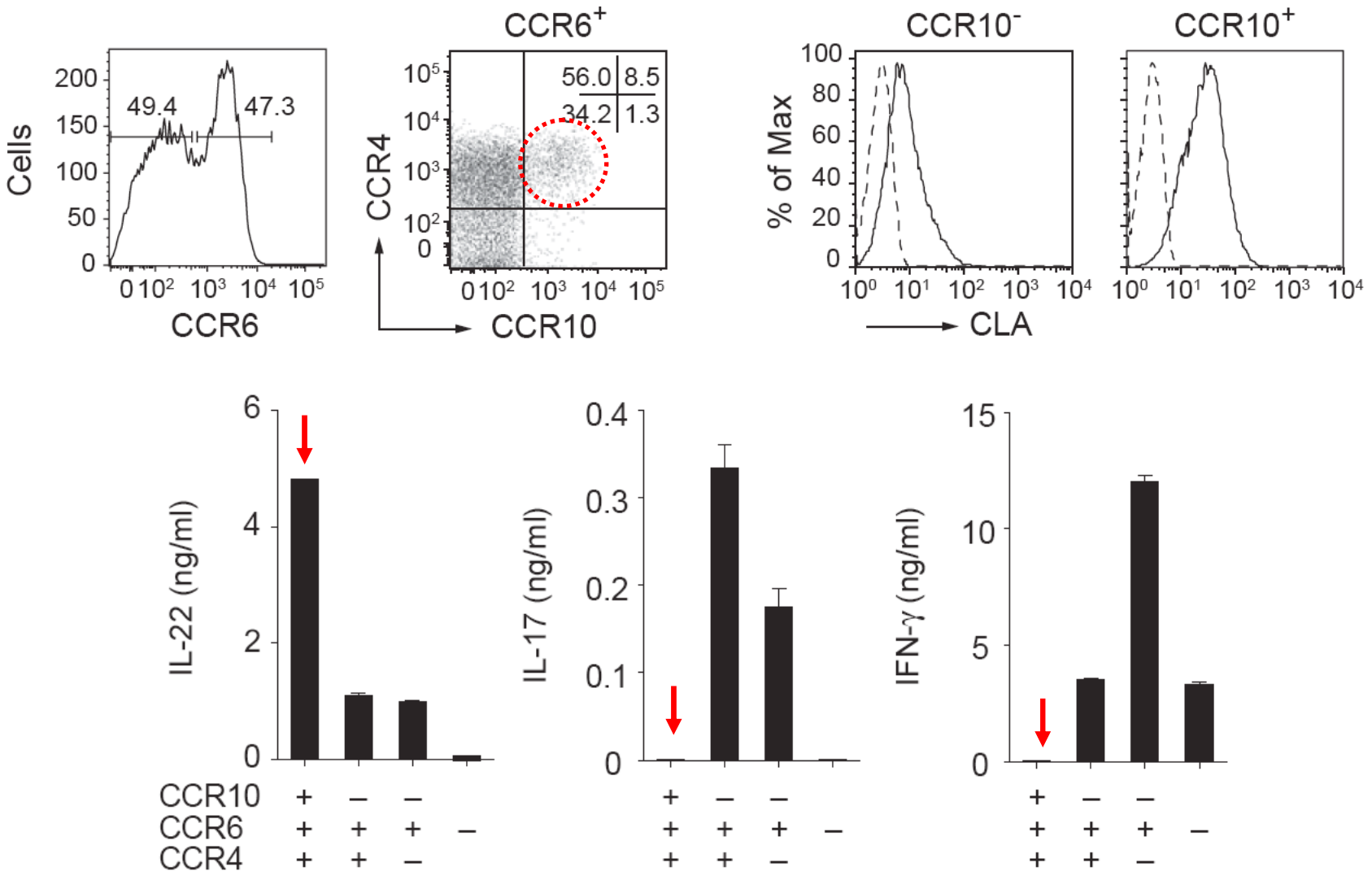
Thomas Duhen

IL-22-producing T cells are present in the CD4+ CCR6+ memory subset

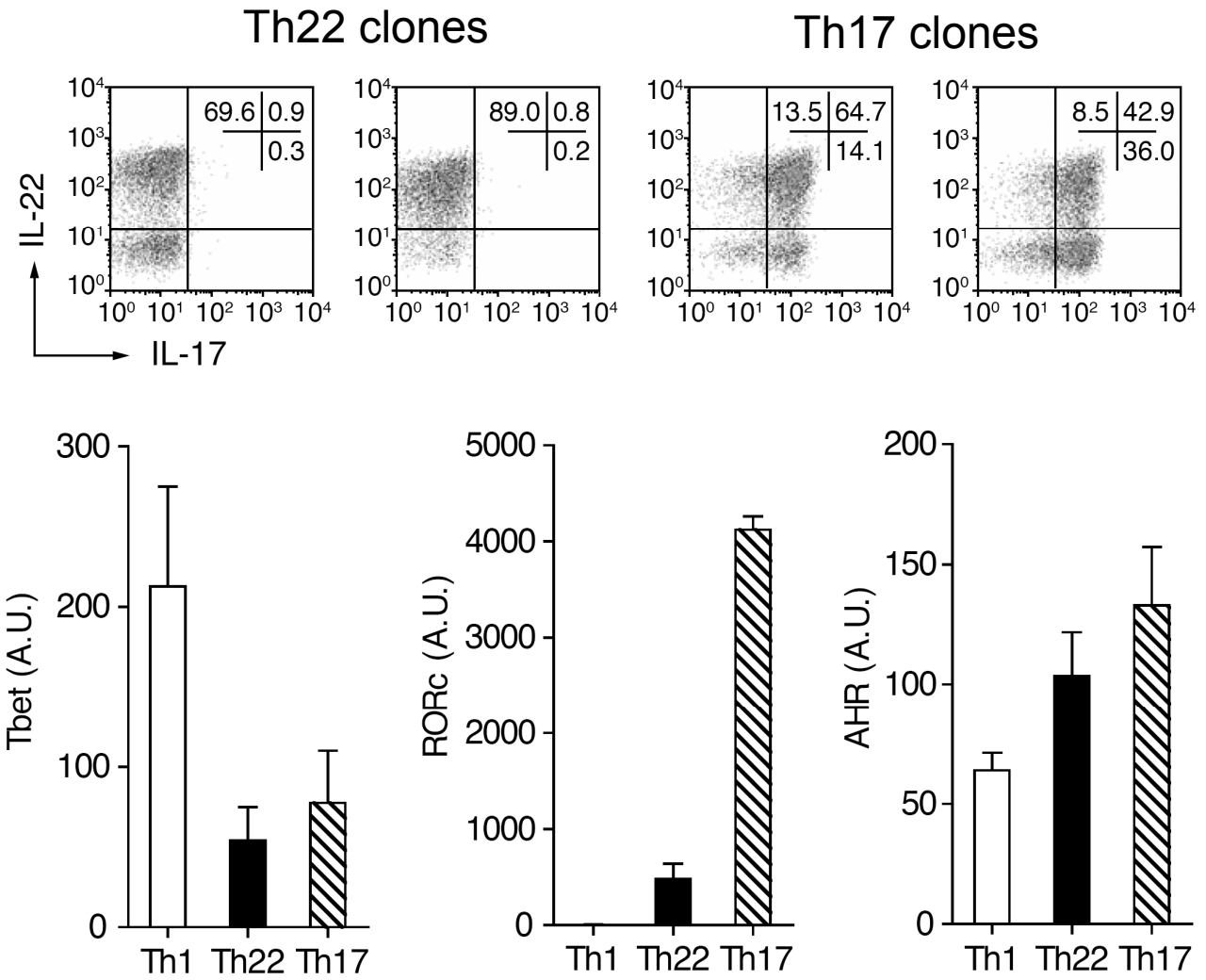


… but most IL-22-producing cells do not produce IL-17

Skin-homing T cells (CCR4+ CCR10+ CLA+) produce IL-22, but no IL-17



Th22 clones do not express ROR-c

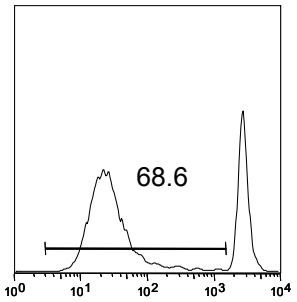


How are IL-22-only producing $CD4^+$ T cells generated?

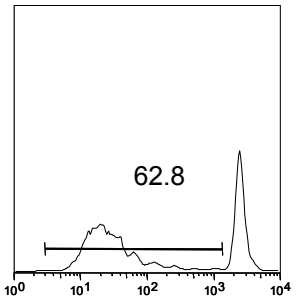
Plasmacytoid DC prime IL-22-producing T cells

CD4+ naïve T cells primed by allogeneic:

cDC

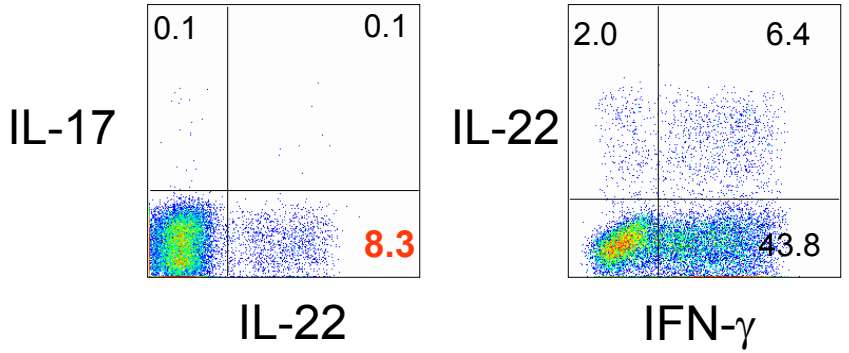


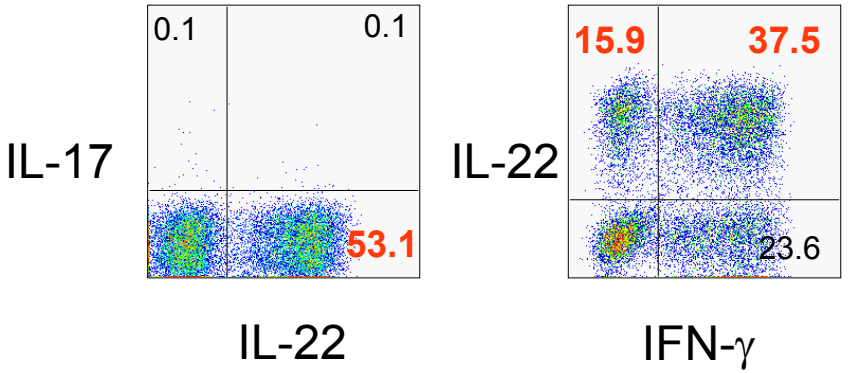
pDC



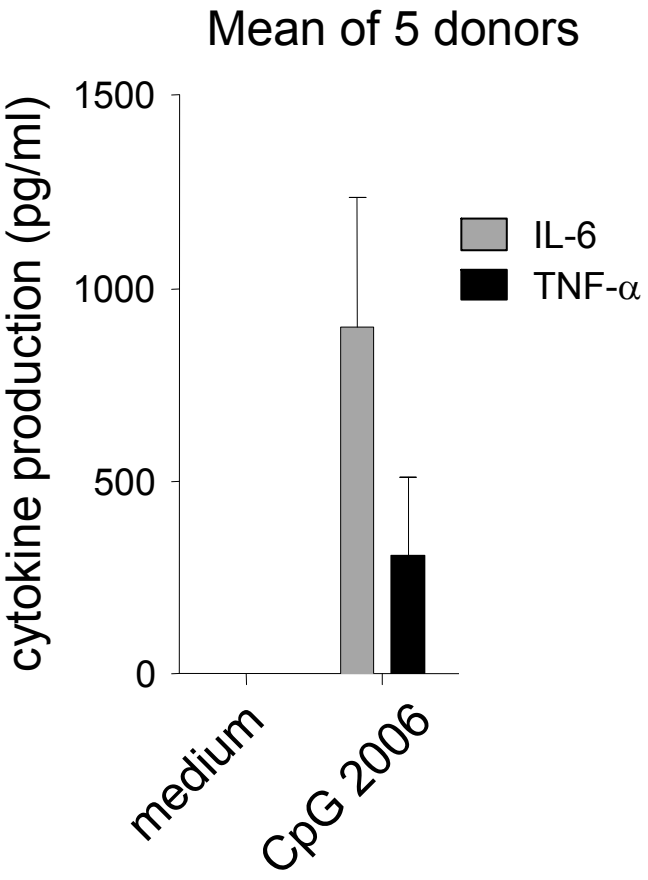
CFSE

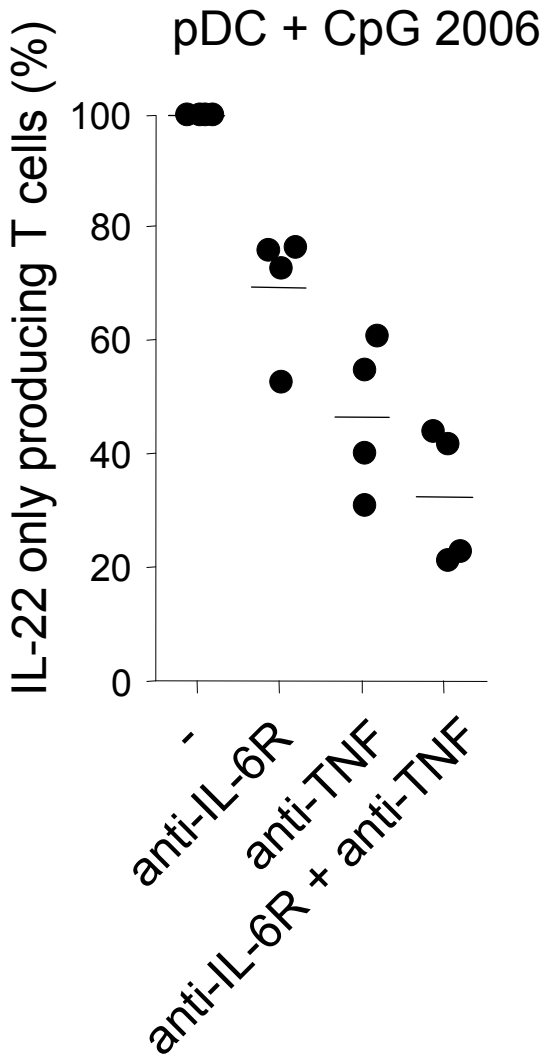
Cytokine production following PMA + ionomycin



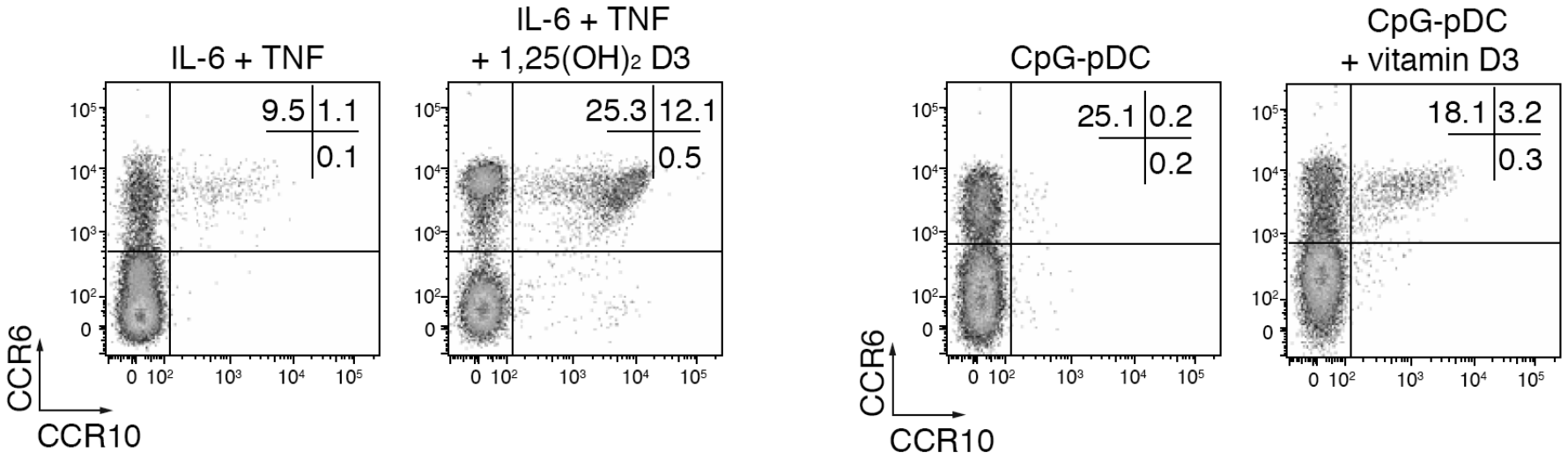


IL-6 and TNF produced by pDC are required for Th22 polarization





Vitamin D3 and pDC promote expression of CCR6 and CCR10



Th22: a module of adaptive immunity dedicated to epithelial cell physiology?

Module	Polarizing cytokine(s)	Transcription factor	Homing receptor(s)	Effector cytokine(s)	Target cell	Function
T_H1	IL-12. IFN	T-bet	CXCR3	IFN-γ	Macrophages	**Bacteria**
T_H2	IL-4	GATA-3	CCR4/CRTh2	IL-4, IL-5, IL-13	Eosinophils	**Parasites**
T_H17	IL-6,IL-1β.TGF-β	ROR-γt	CCR6 / CCR4	IL-17, IL-22	Neutrophils	**Fungi**
Treg	?	FOXP3	CCR7 / CCR6	TGF-β	DC / T cells	**Regulation**
T_{FH}	IL-21	Bcl-6	CXCR5	IL-21	B cells	**Antibodies**
Tr1	IL-10	?	CCR7 / CCR6	IL-10	T cells	**Regulation**
T_H22	IL-6, TNF	?	CCR6 / CCR10	IL-22	Keratinocytes	**?**

Institute for Research in Biomedicine

Human T cell repertoire analysis using amplified T cell libraries

Rebekka Geiger

Challenges in analyzing the human naïve T cell repertoire

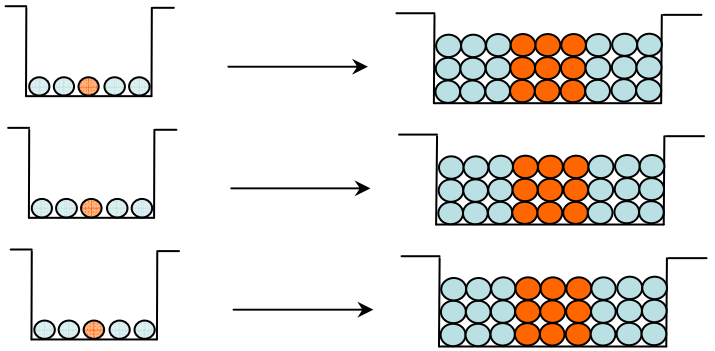
- Low frequency of antigen-specific naïve T cells
- High activation threshold of naïve T cells
- Broad spectrum of avidities
- Limitations of peptide-based and tetramer-based approaches
- Need to measure T cell responses to complex naturally processed antigens

Analysis of naïve and memory T cell repertoires using amplified T cell libraries

1st step: Amplification

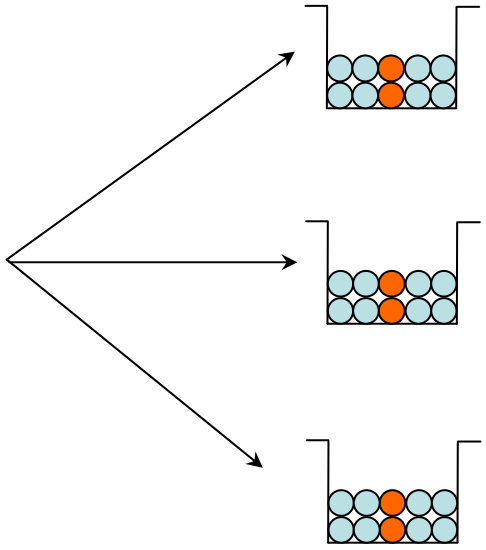
Multiple cultures (96 up to 384) each containing 1000 cells are expanded with PHA and IL-2

1 naïve T cell → 5,000 T cell blasts



2nd step: Interrogation

Each culture is tested for proliferation in response to Ag and autologous APC



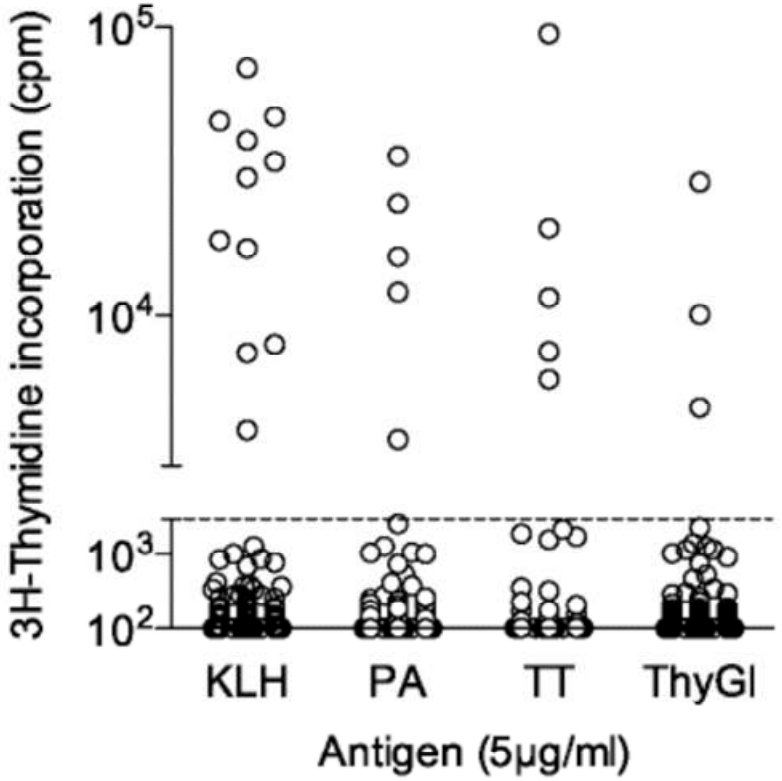
KLH, Anthrax… (primary antigens)

Thyroglobulin… (self antigens)

TT, CMV, PPD… (recall antigens)

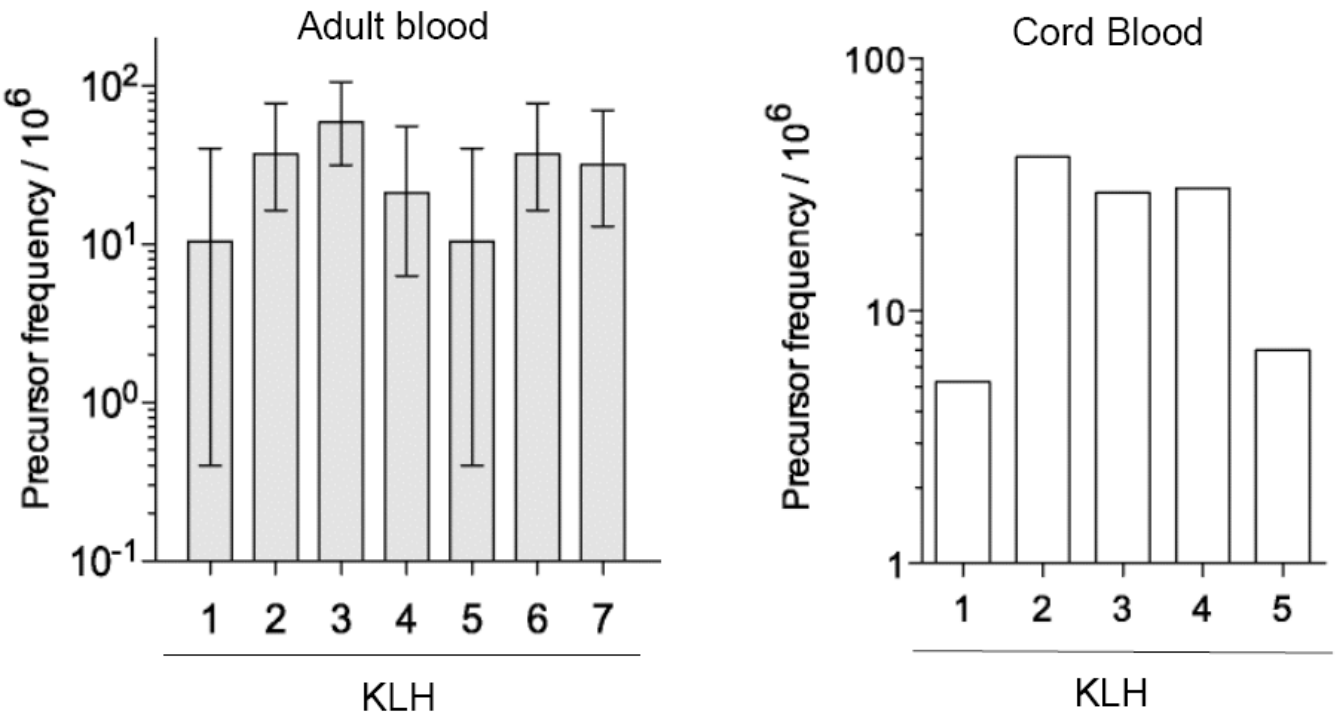
Geiger et al JEM 2009

Antigen-specific CD4+ T cells in the human naïve repertoire

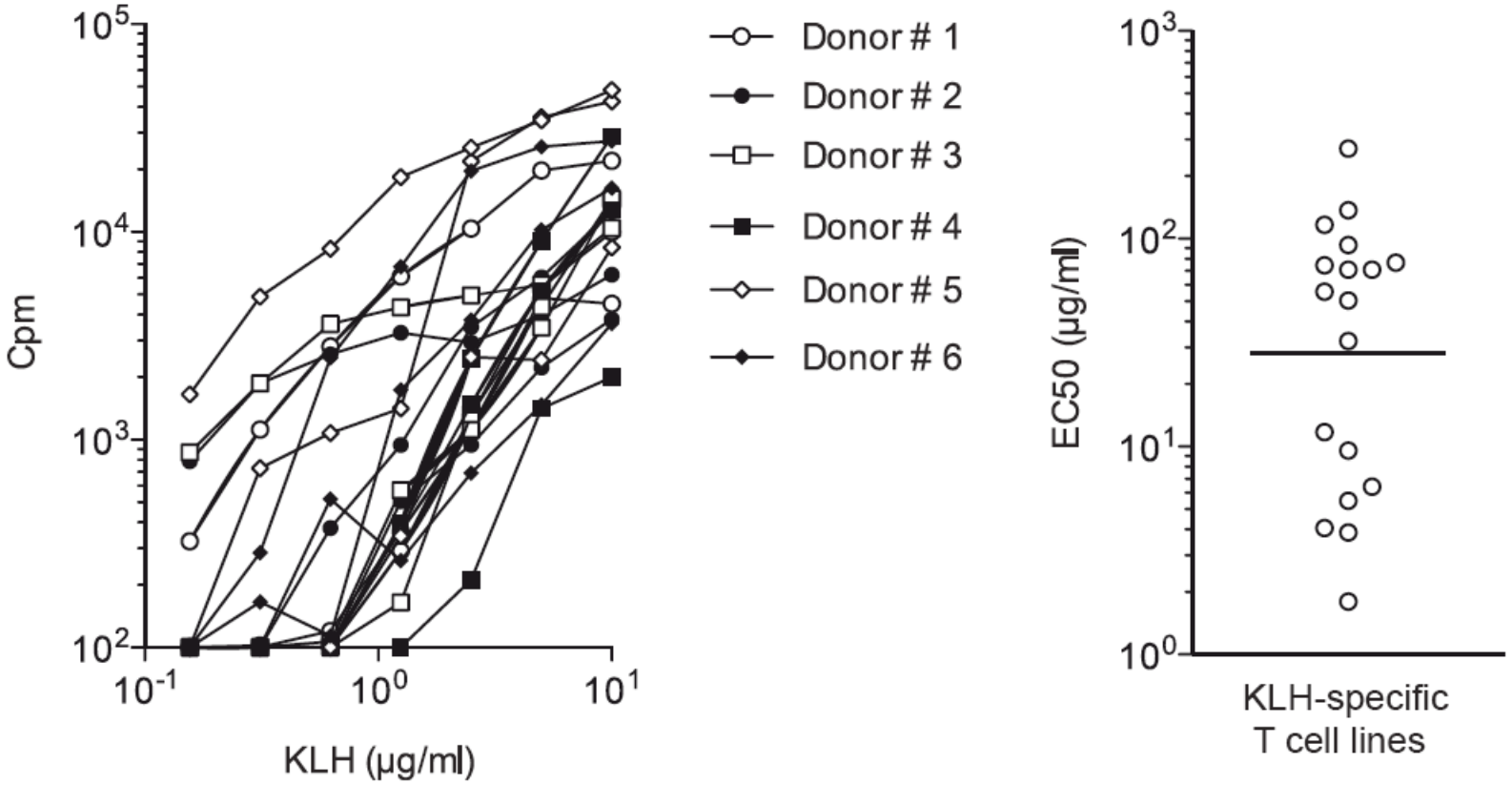


Frequency of KLH-specific naïve T cells

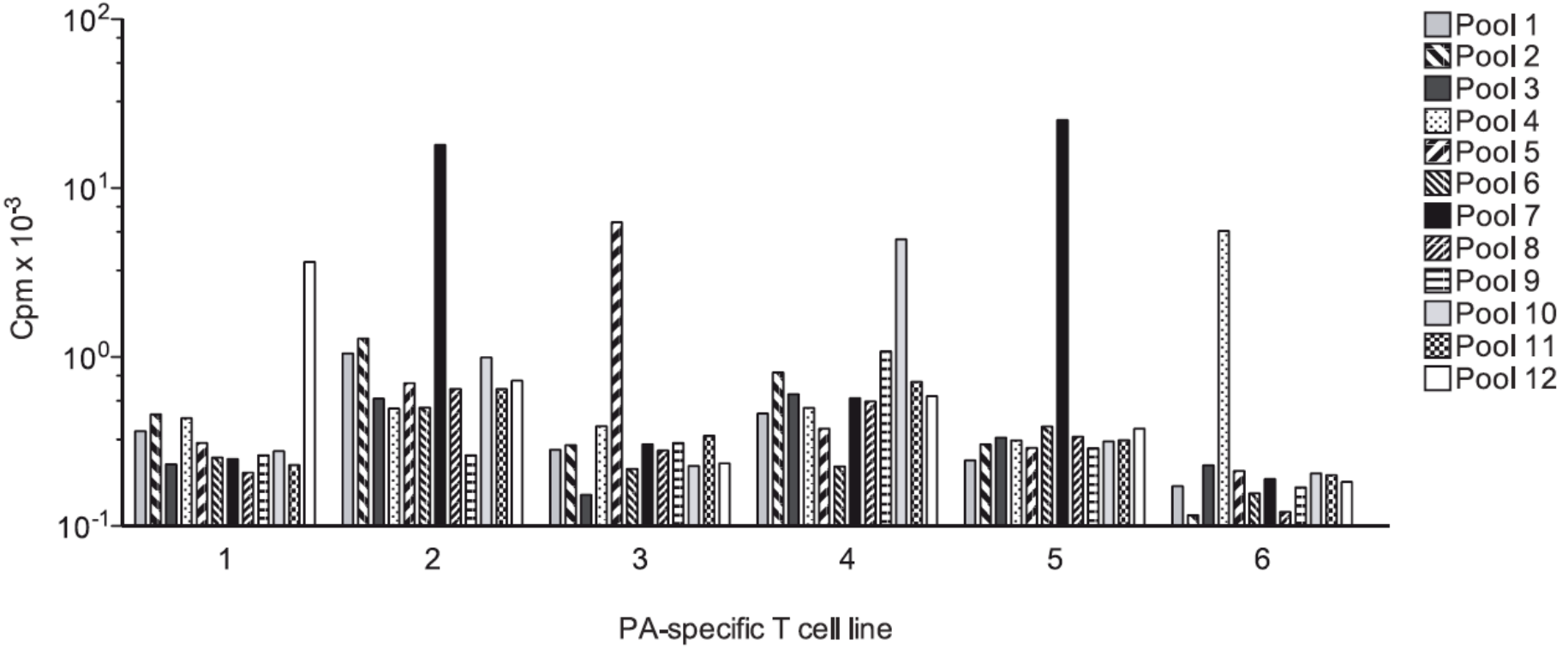
"Amplified T cell libraries" from naïve CD45RA$^+$ CD45RO$^-$ CCR7$^+$ CD4$^+$ T cells



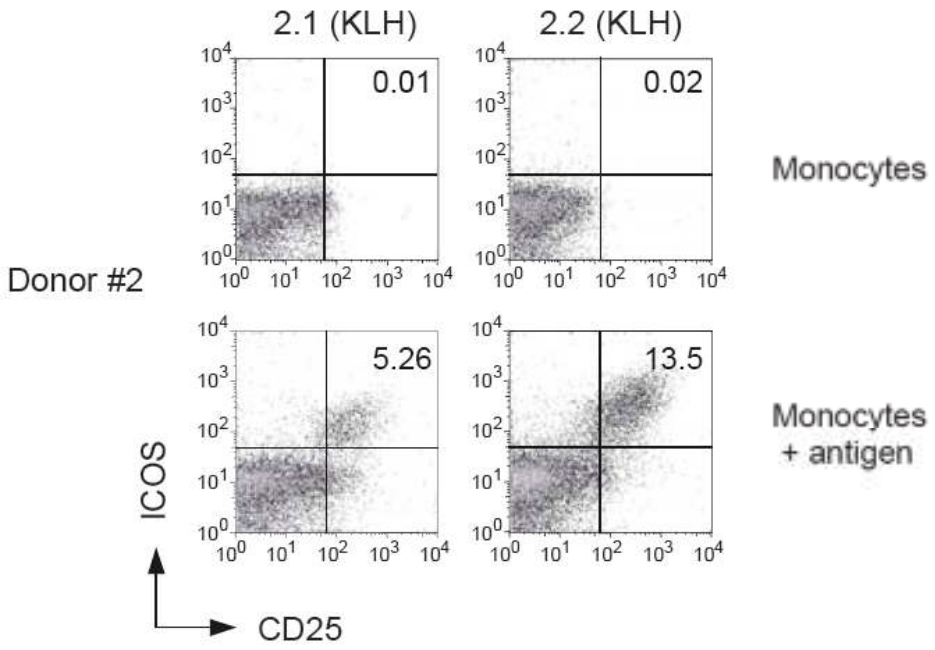
Broad range of responsiveness

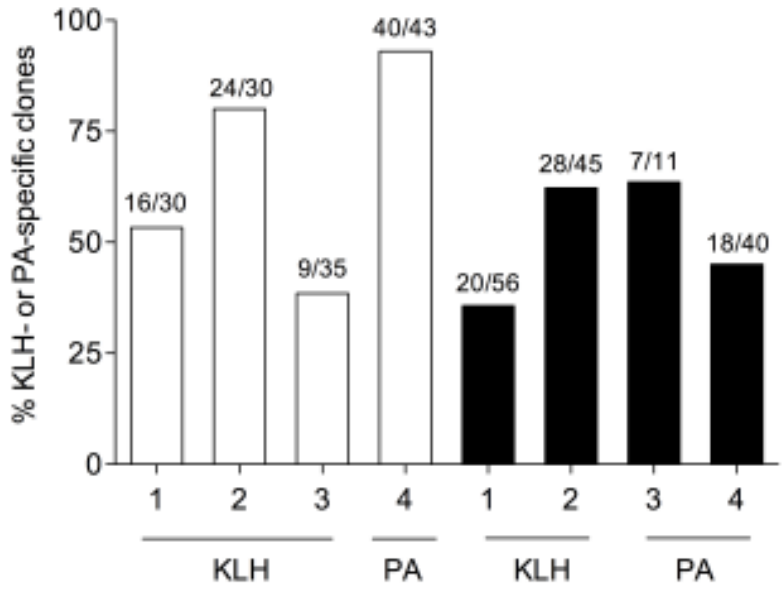


Broad range of epitope specificities

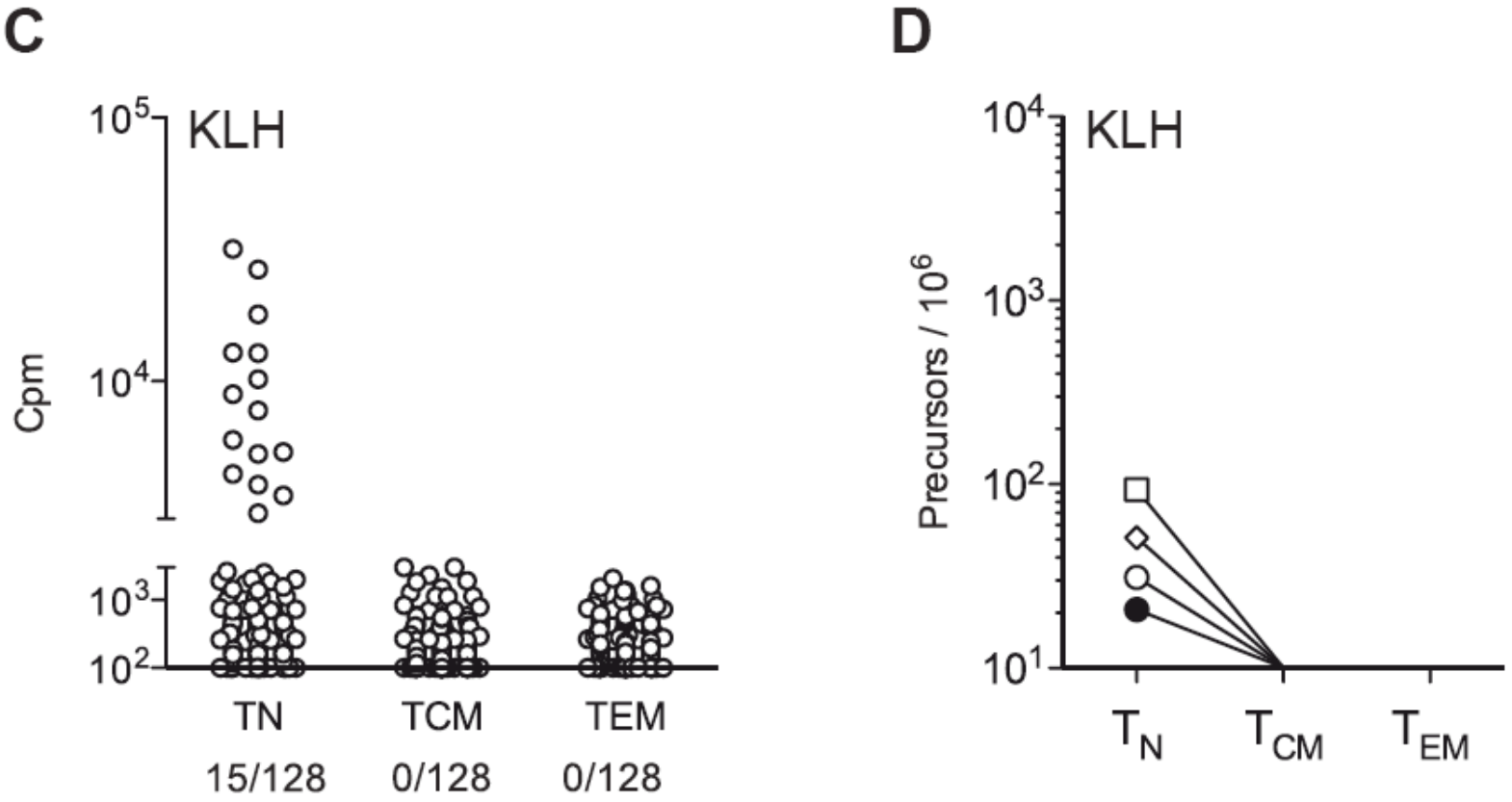


Isolation of Ag-specific T cell clones from the naïve repertoire

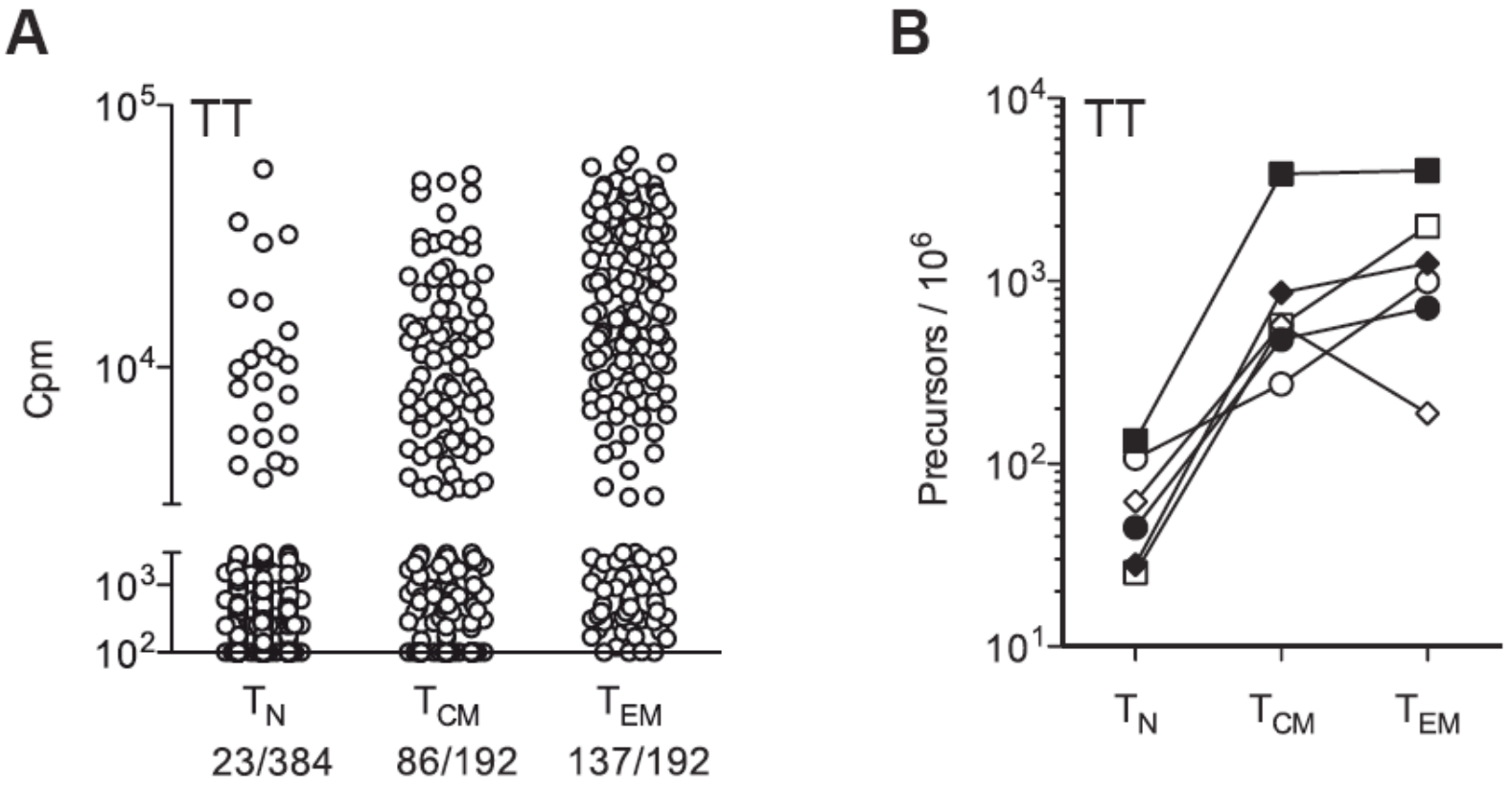




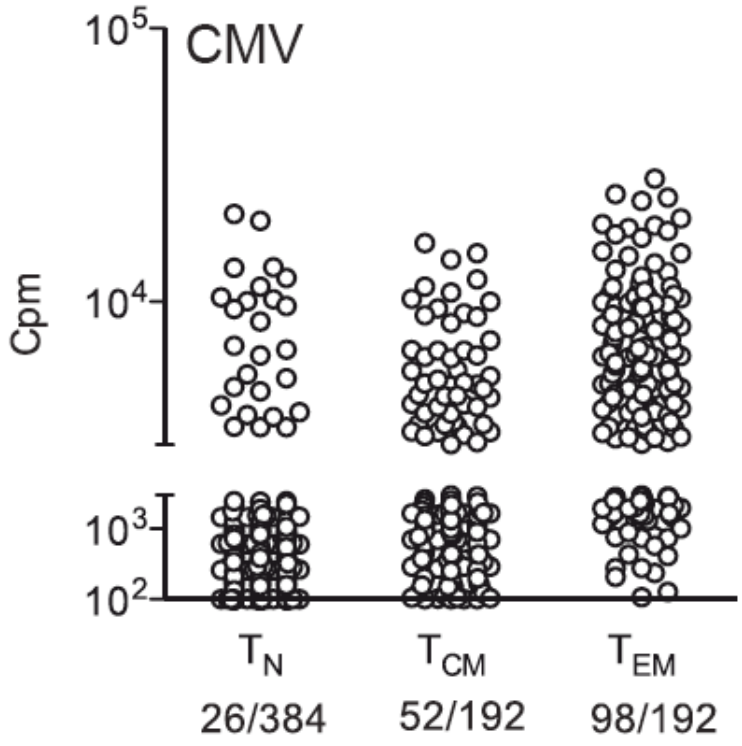
Analysis of memory repertoires

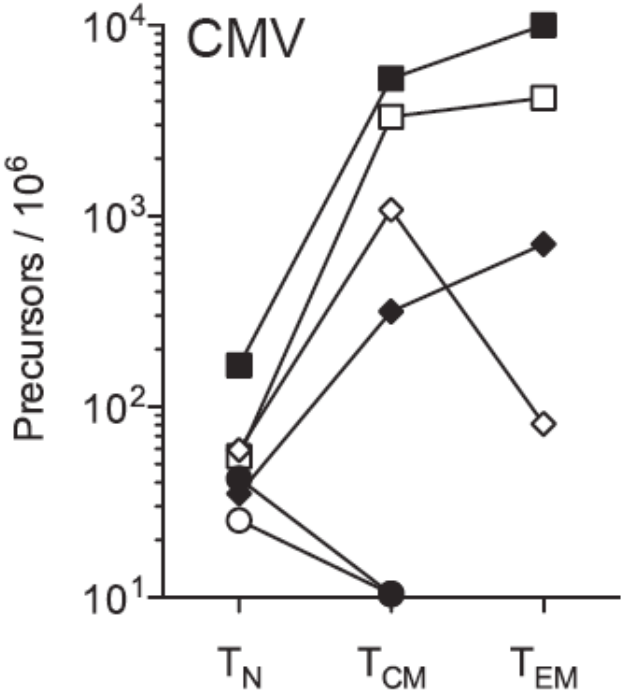


Increased frequencies in the memory compartment (Tet Tox)

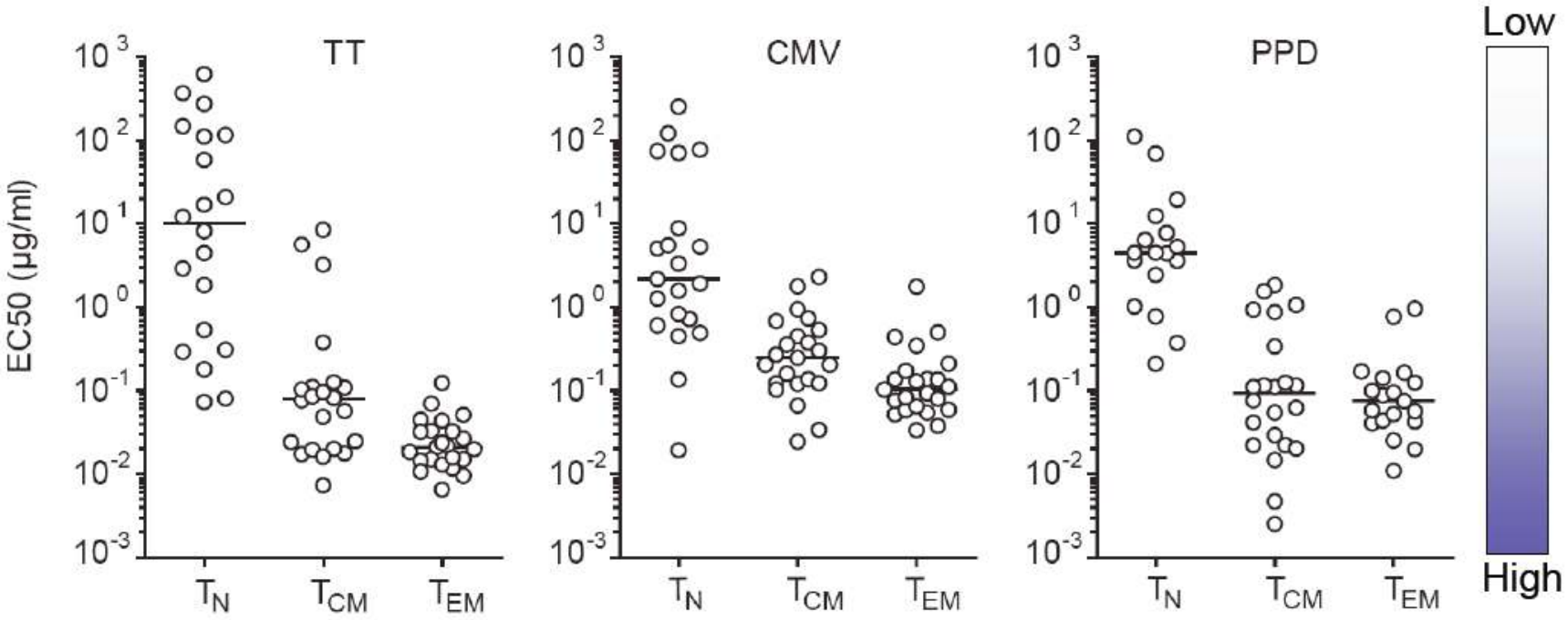


Increased frequencies in the memory compartment (CMV)





Selection of high avidity T cells in the memory pool



Applications

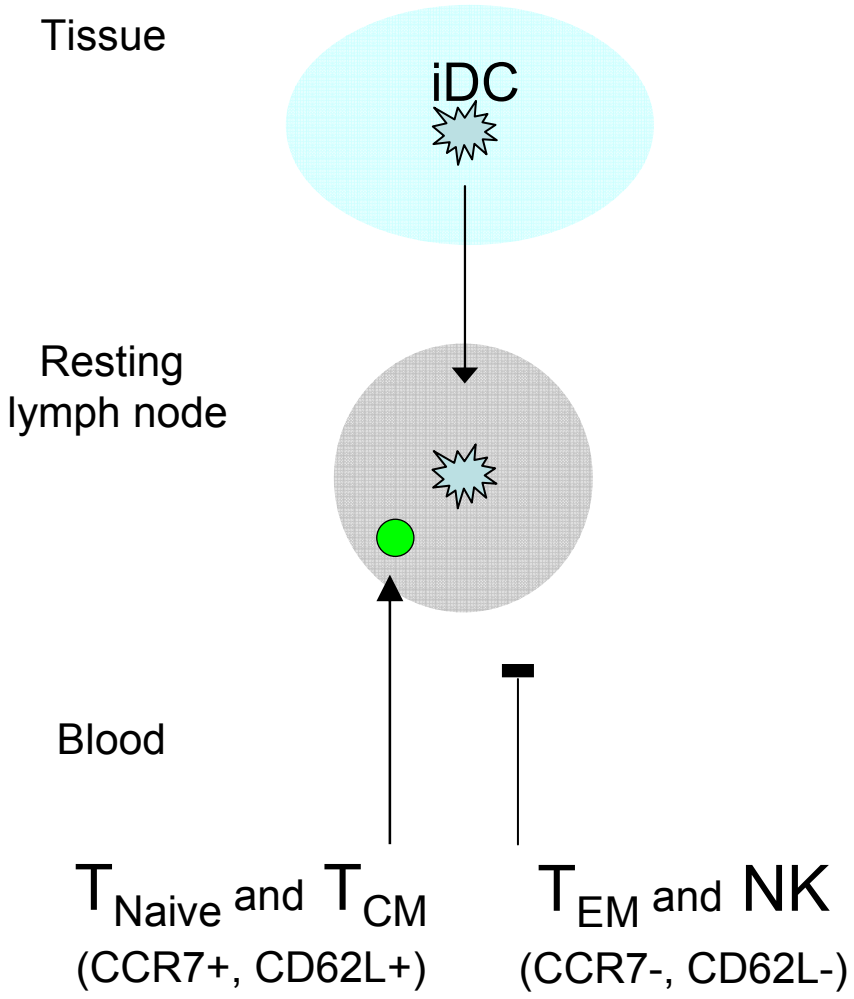
- Predict antigenicity of complex molecules (even whole pathogens)
- Assess immunocompetence (elderly, HIV)
- Assess memory in different T cell subsets
- Generate T cells for cellular immunotherapy

1. T cell activation and fate determination

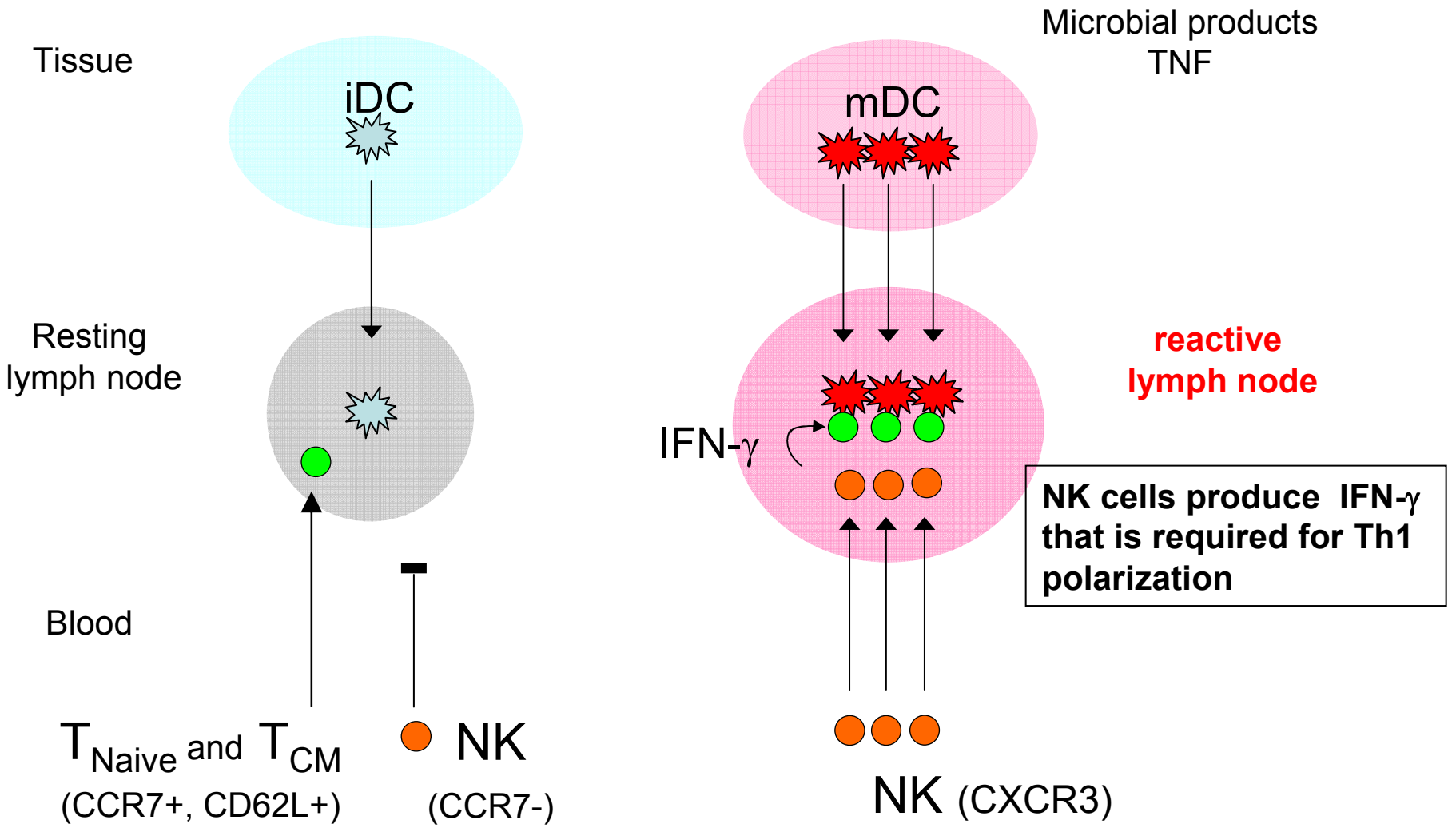
2. Human effector/memory T cell subsets
   (T_{CM}, T_{EM}, Th1, Th2, Th17, etc)

3. T cell traffic in steady state and inflammatory conditions (mouse)

Paradigm: migration from blood to lymph nodes requires CCR7 and CD62L

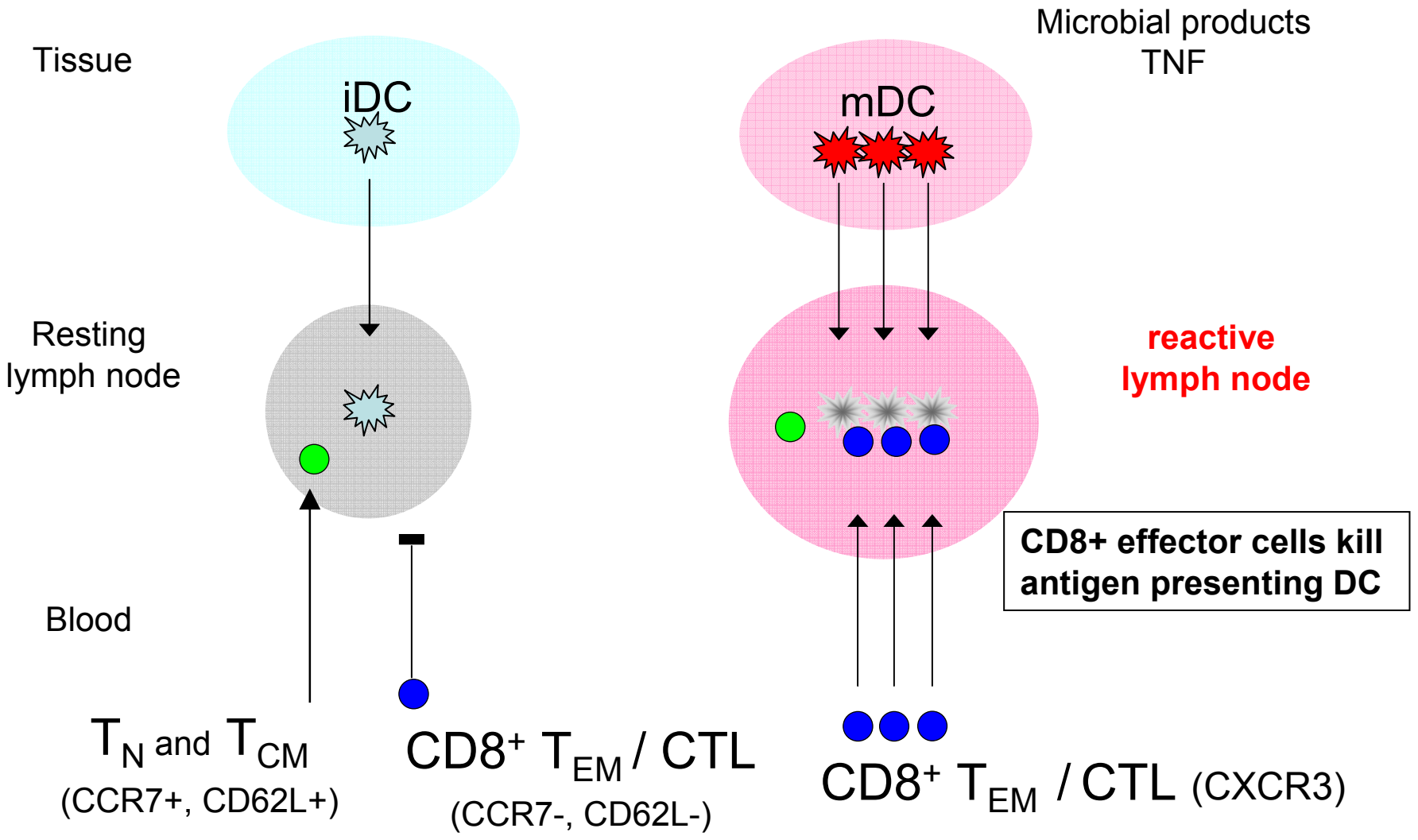


NK cells migrate to reactive lymph nodes in a CXCR3-dependent fashion



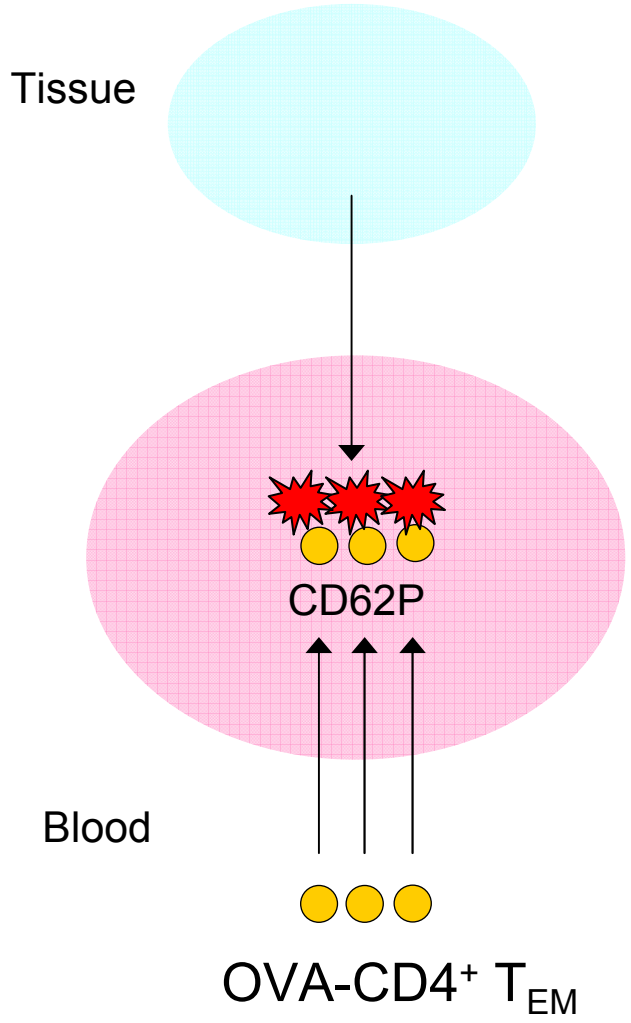
Martin-Fontecha et al *Nat Immunol* 2004

Effector and effector memory CD8 T cells migrate to inflamed lymph nodes in a CXCR3 dependent fashion and kill antigen presenting dendritic cells



Guarda et al *Nat Immunol* 2007

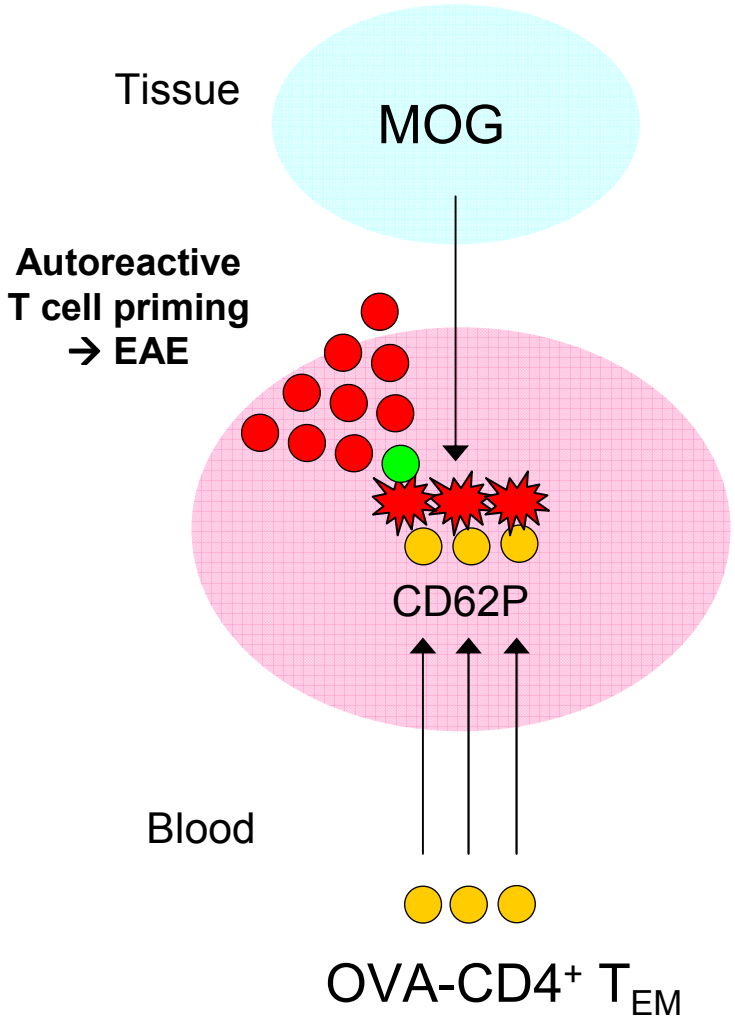
Effector memory CD4 T cells license DC in chronically inflamed lymph nodes



CD4+ effector memory T cells

- **migrate to chronically reactive lymph nodes via CD62P**
- **Constitutively express CD40L**
- **Trigger DC maturation**

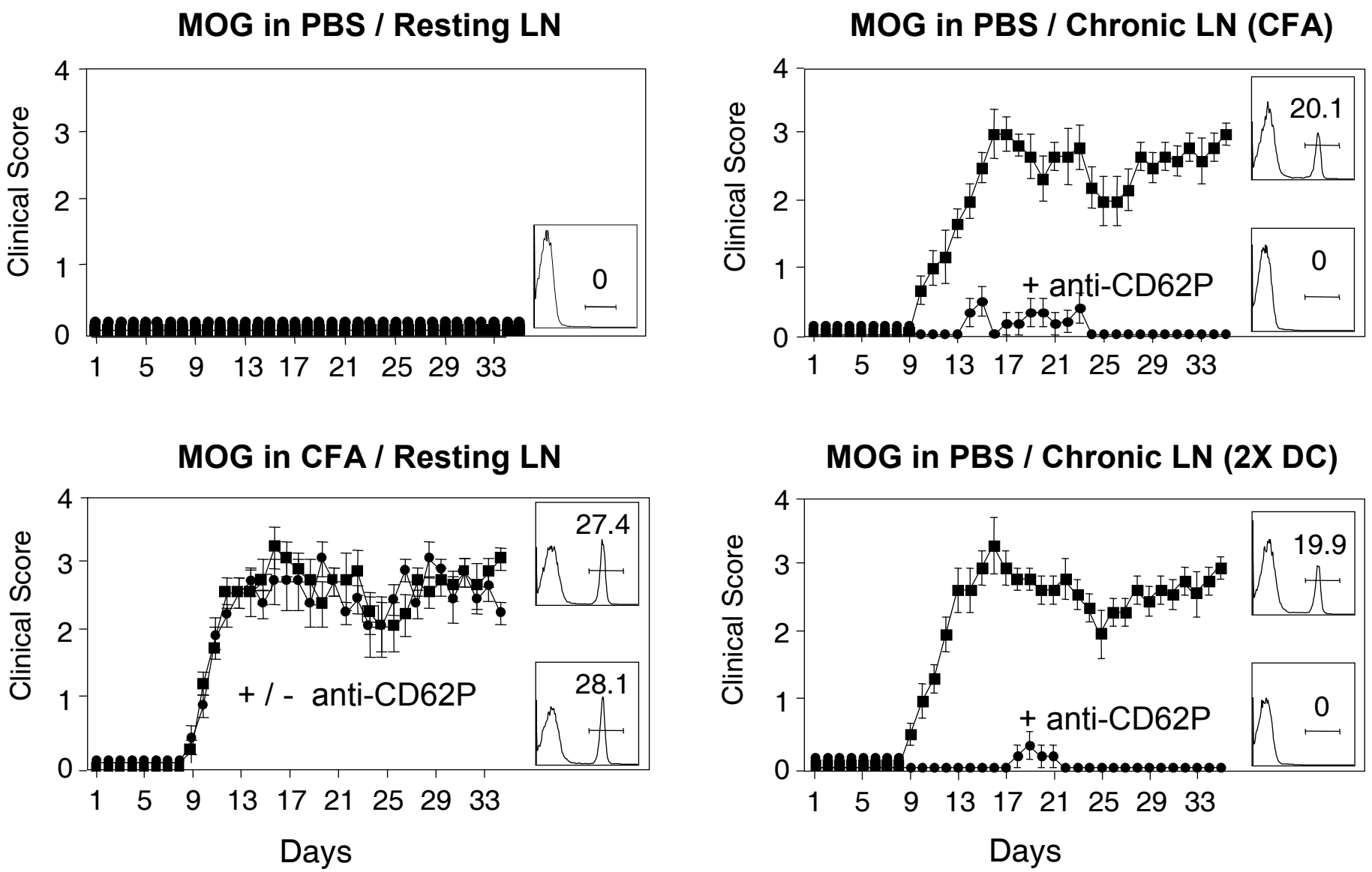
Effector memory CD4 T cells license DC in chronically inflamed lymph nodes



CD4+ effector memory T cells

- **migrate to chronically reactive lymph nodes via CD62P**
- **Constitutively express CD40L**
- **Trigger DC maturation**
- **Cause EAE in the absence of adjuvant**

Induction of EAE by MOG in PBS draining to a chronic lymph node



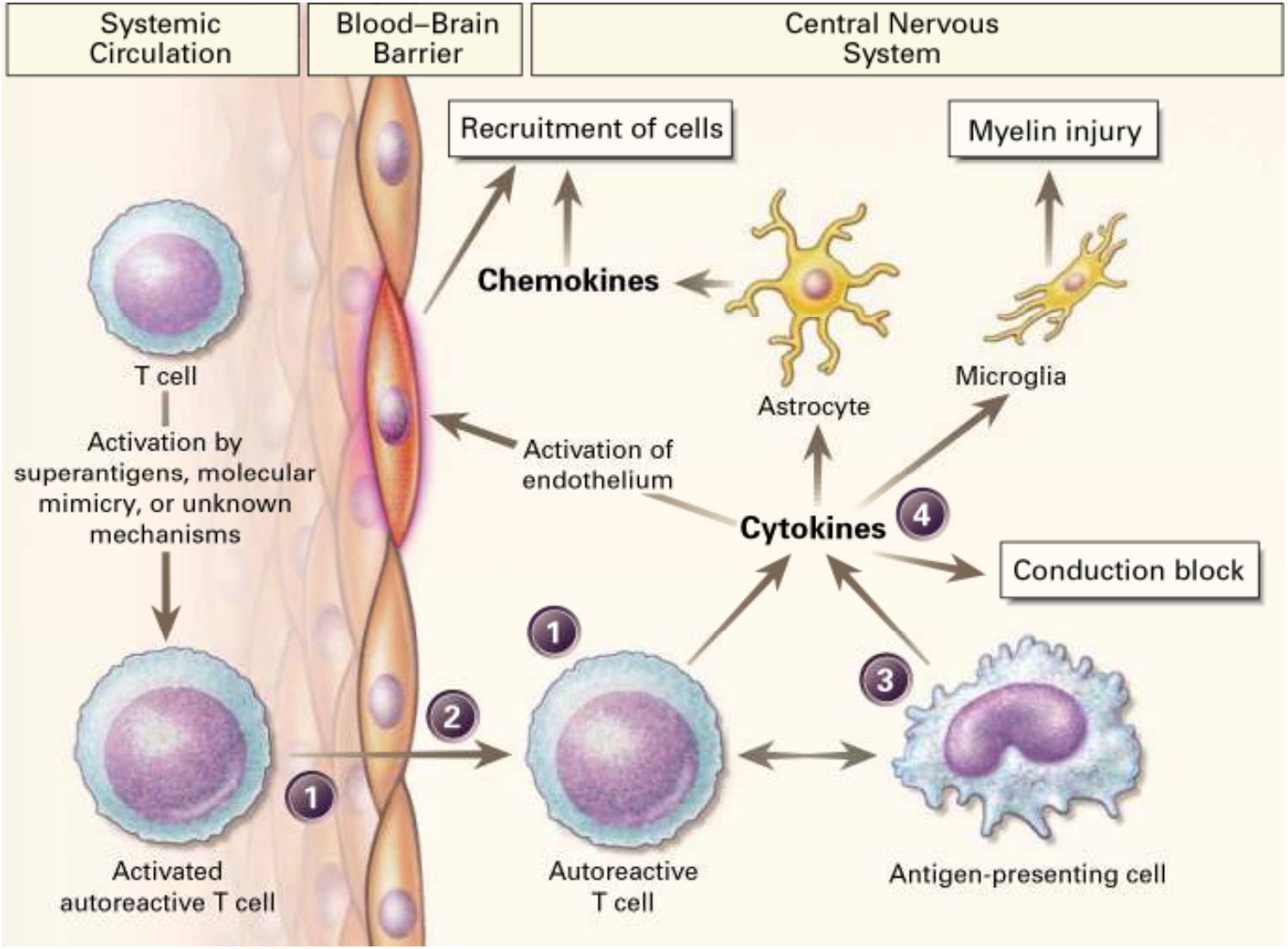
Institute for Research in Biomedicine

The role of CCR6 in experimental autoimmune encephalomyelitis

Andrea Reboldi

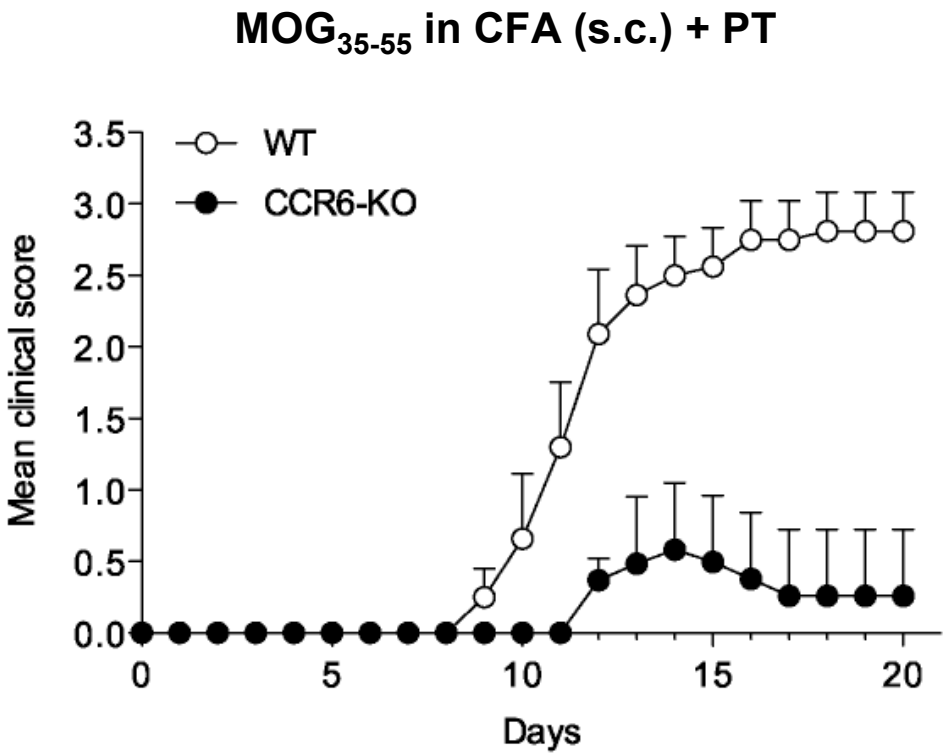
CCR6-KO mice (*Cook et al, Immunity 2000*)

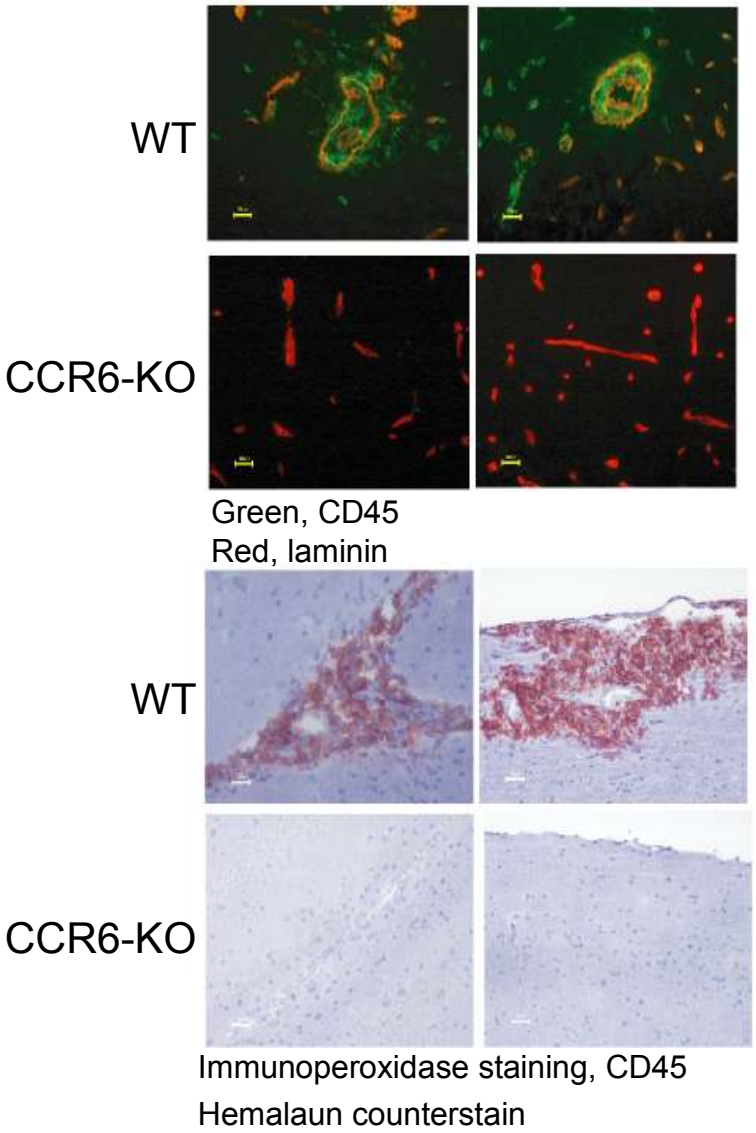
Pathogenesis of Multiple Sclerosis



Ransohoff et al

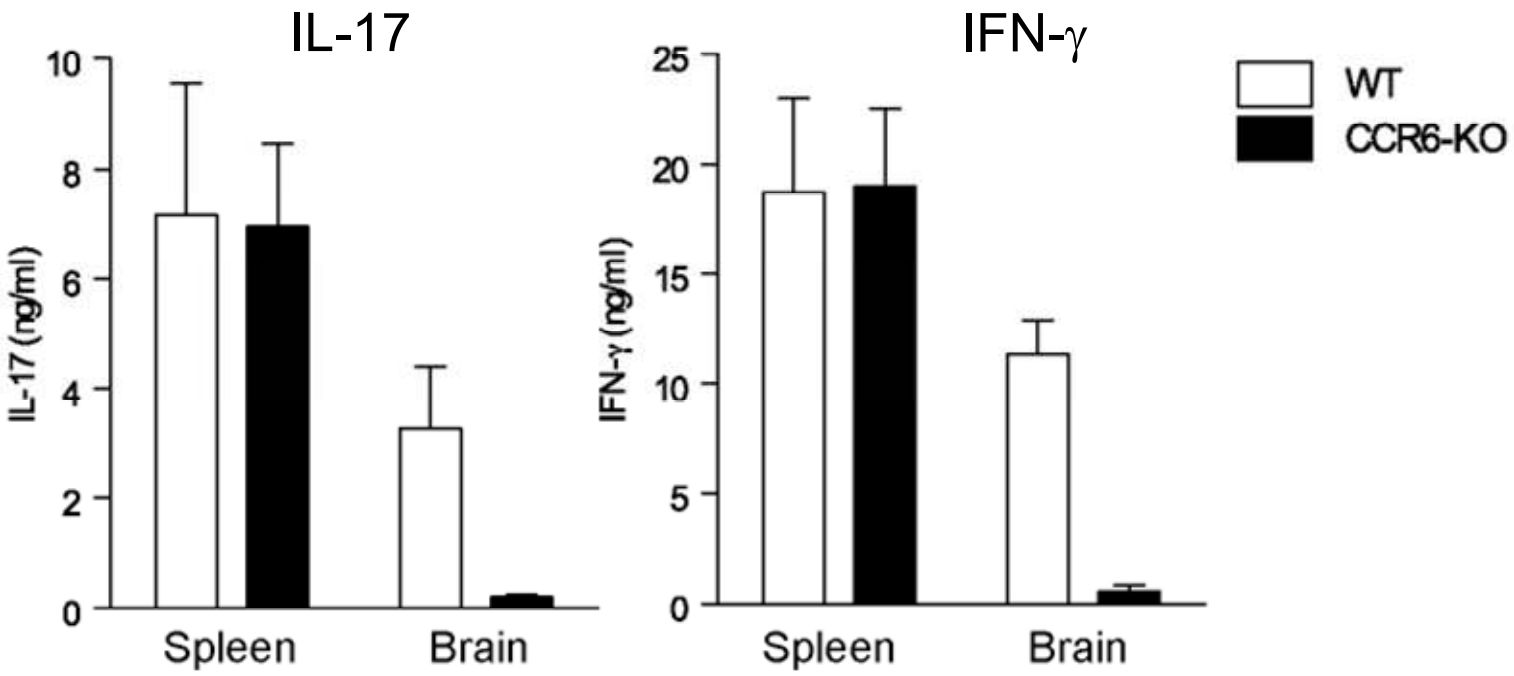
CCR6-KO mice do not develop EAE



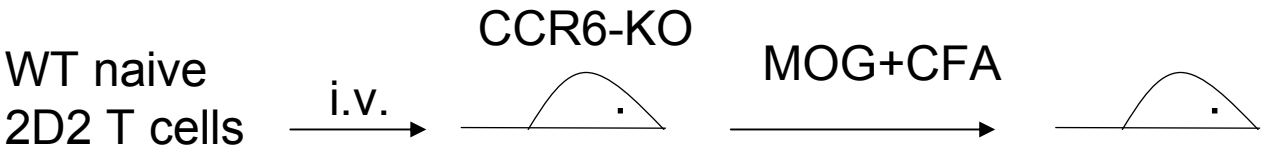


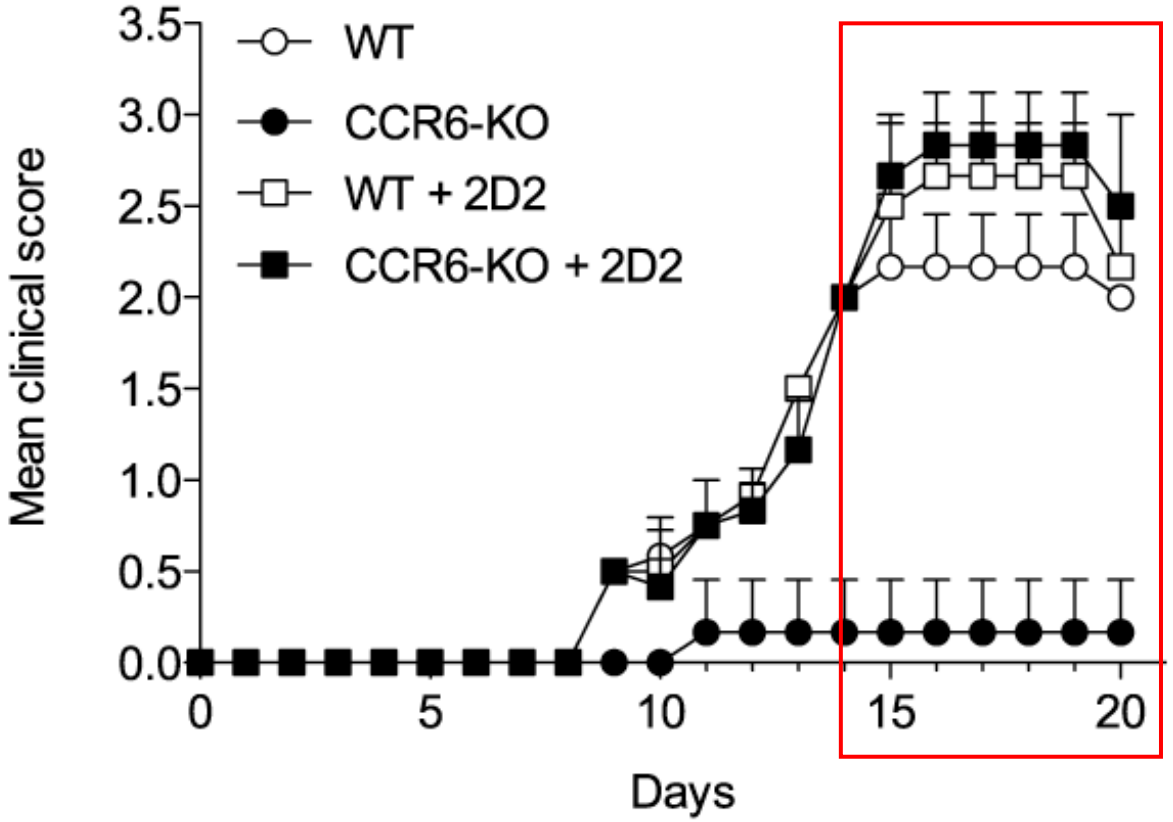
In CCR6-KO mice MOG-reactive Th17 and Th1 cells are primed but do not migrate into the CNS

Cytokine production following restimulation with MOG peptide:



Transfer of wild-type 2D2 T cells reconstitutes disease susceptibility in CCR6-KO mice



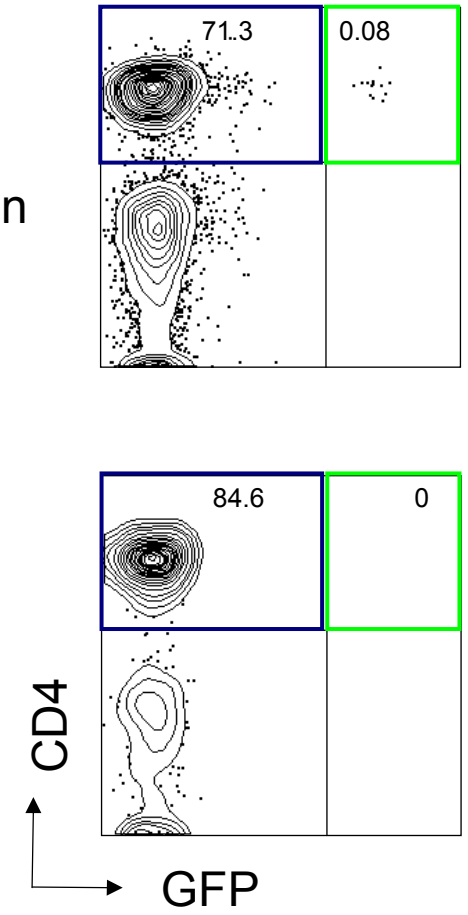


… but on day 20 the T cells in the CNS are endogenous CCR6-/- Th1 and Th17

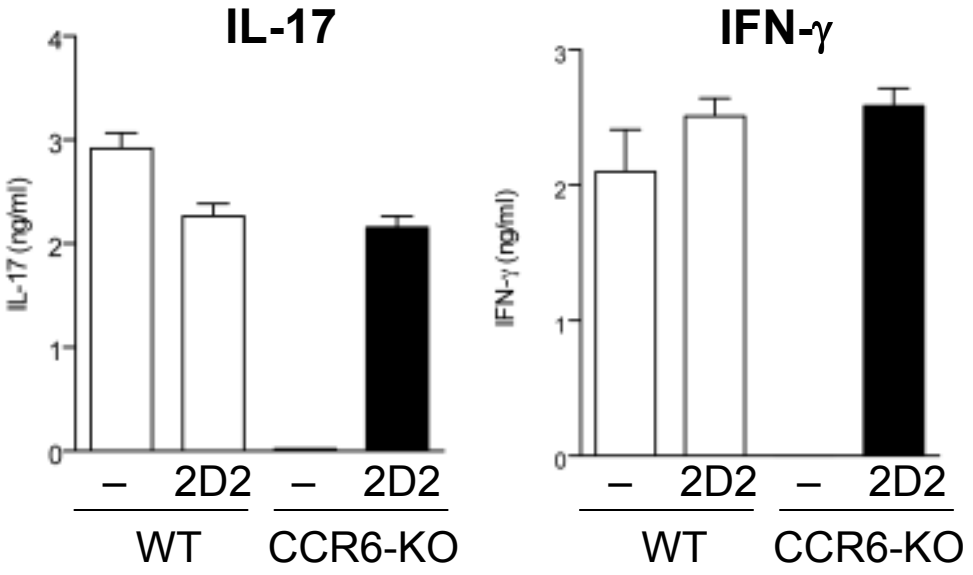
CCR6-KO mice transferred with CCR6+/+ GFP$^+$ 2D2 T cells (Day 20)

Spleen

Brain

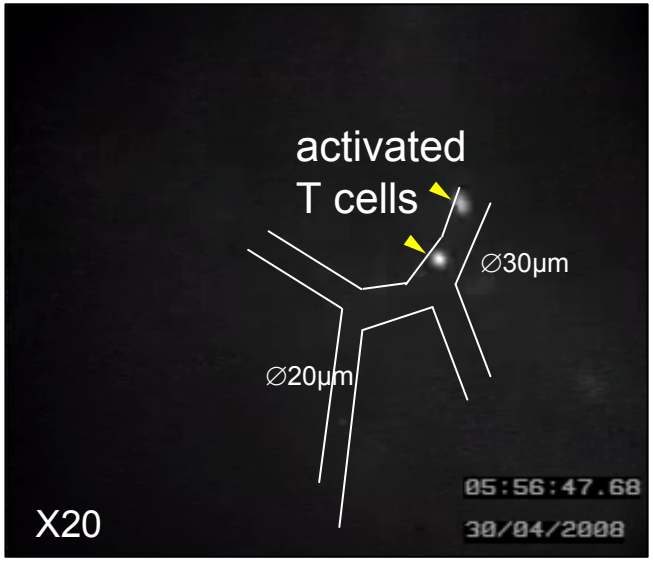


Cytokine production following restimulation with MOG peptide:

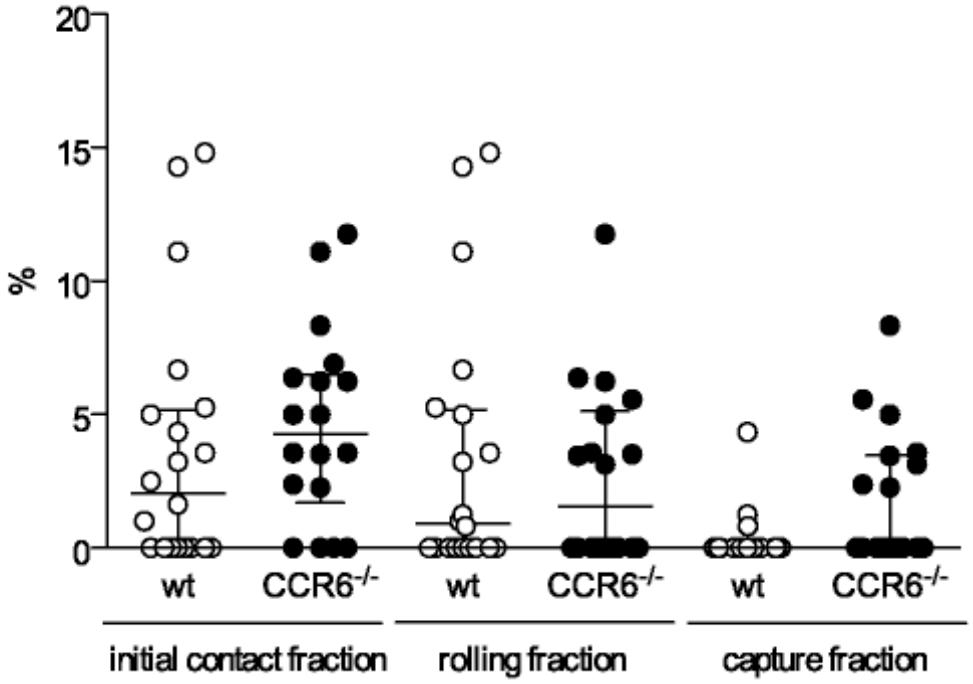


CCR6 is not required for rolling and adhesion to inflamed endothelial cells of CNS parenchyma

Intravital microscopy



Firmly adherent CCR6-KO T cells
10min after injection
in score 2 EAE mice



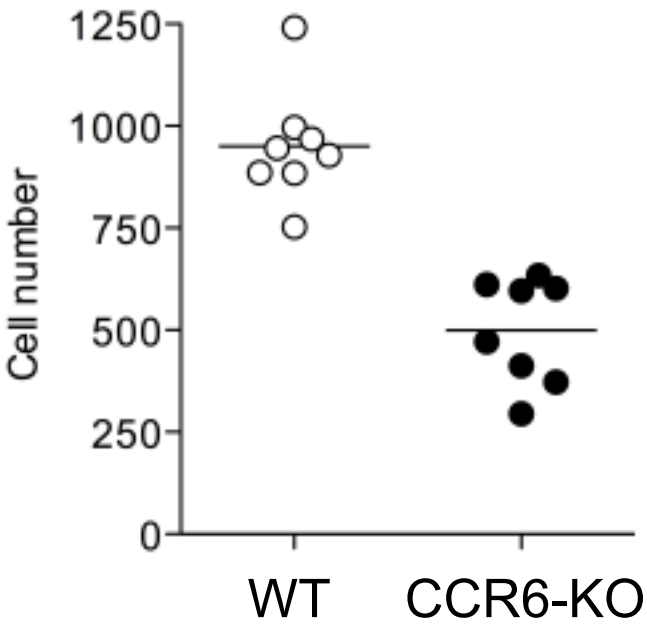
CCR6^{+} T cells as gate keepers for entry in an intact CNS?

In EAE, effector T cells generated upon immunization have to enter a normal non inflamed brain.

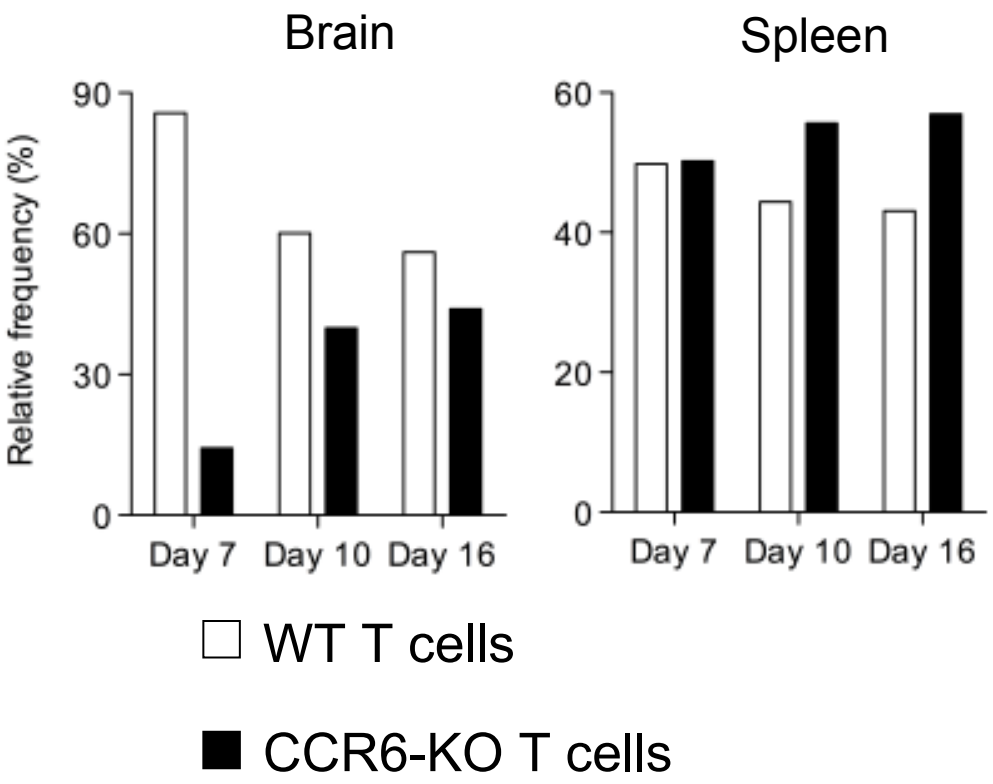
Initial entry of CCR6^{+} T cells by a constitutive pathway may be required to trigger subsequent recruitment of effector T cells by an inflammatory pathway through activated endothelial cells of the blood brain barrier.

CCR6 requirement under steady state conditions and at early time points during EAE

Brain CD4+ T cells in steady state conditions

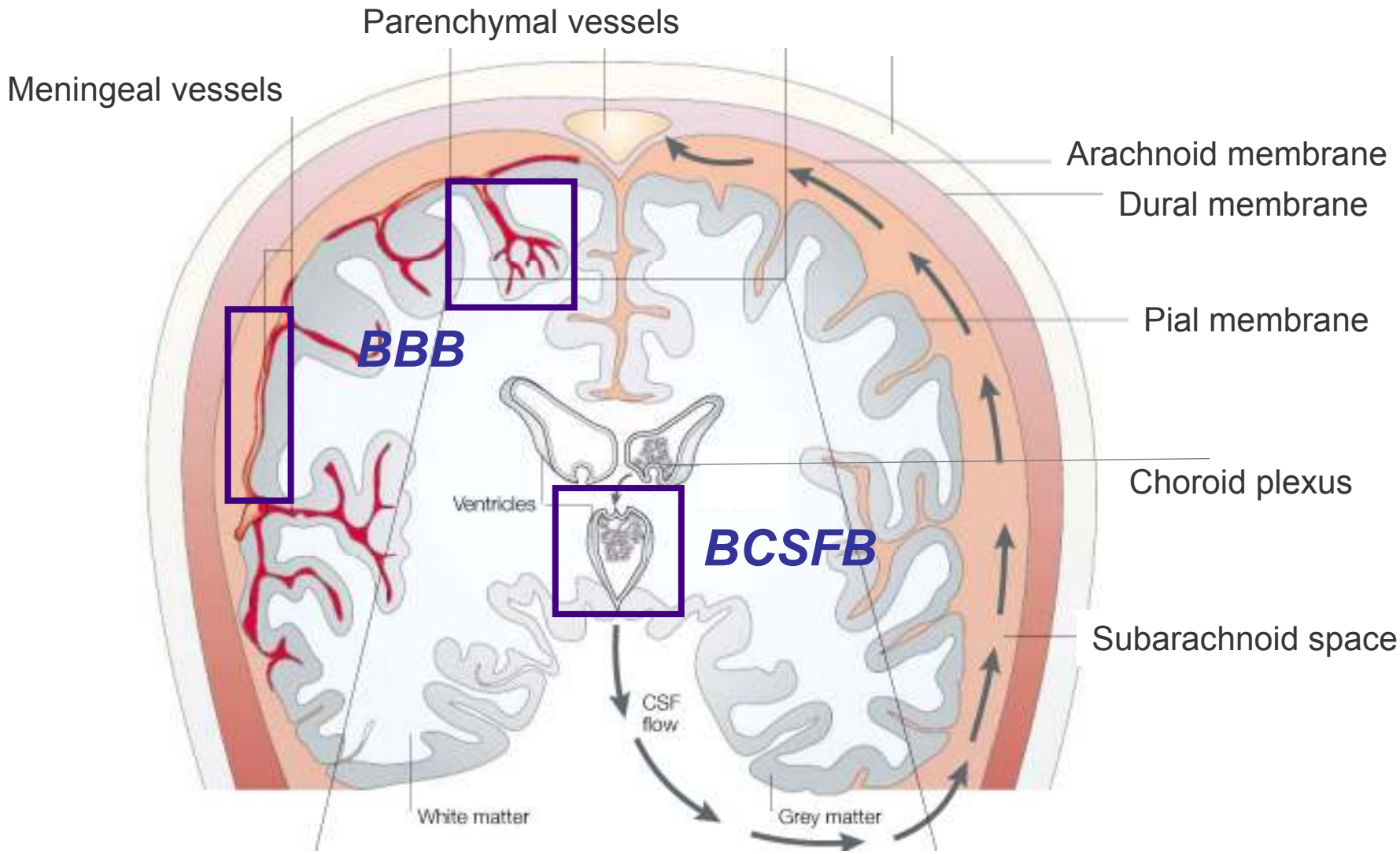


Migration of WT and CCR6-KO T cells



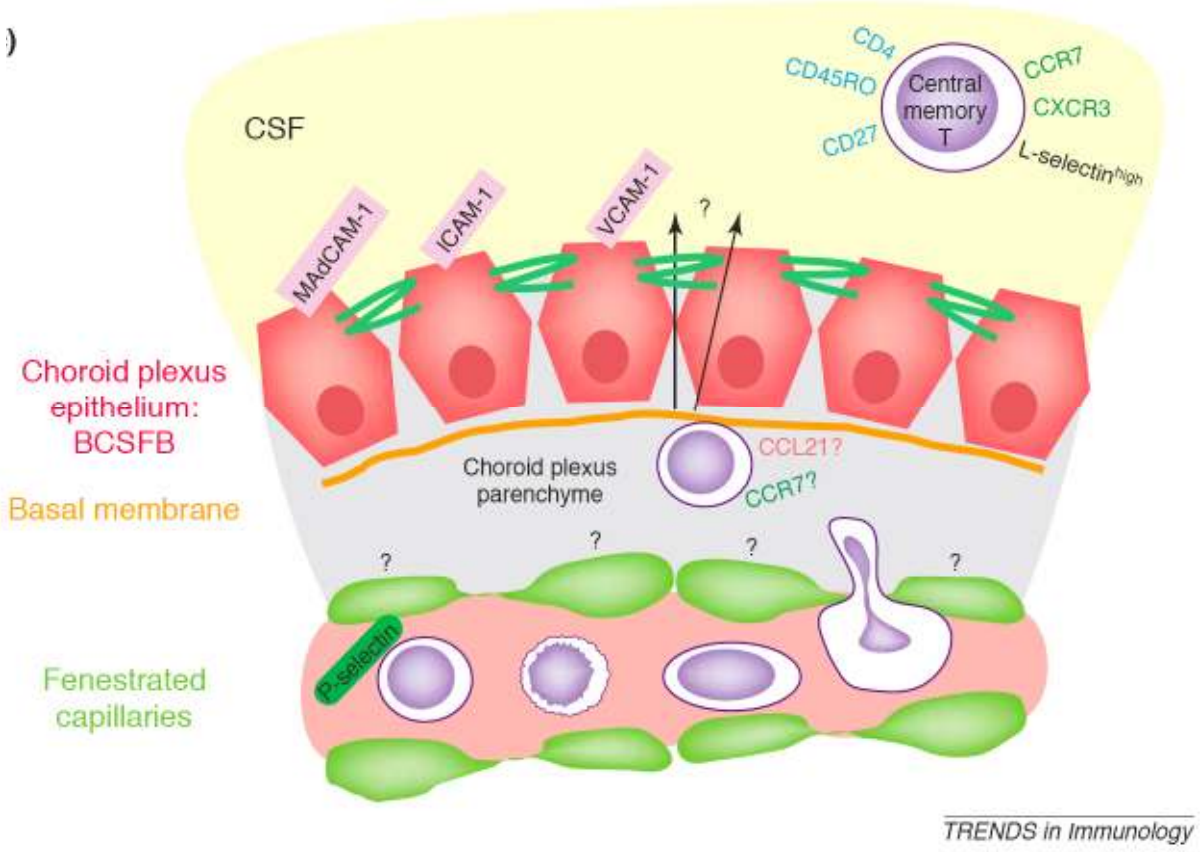
Which is the initial port of entry of CCR6^{+} T cells?

Routes for leukocyte migration into the CNS



Modified from Ransohoff et al, Nat Rev Immunol 2003

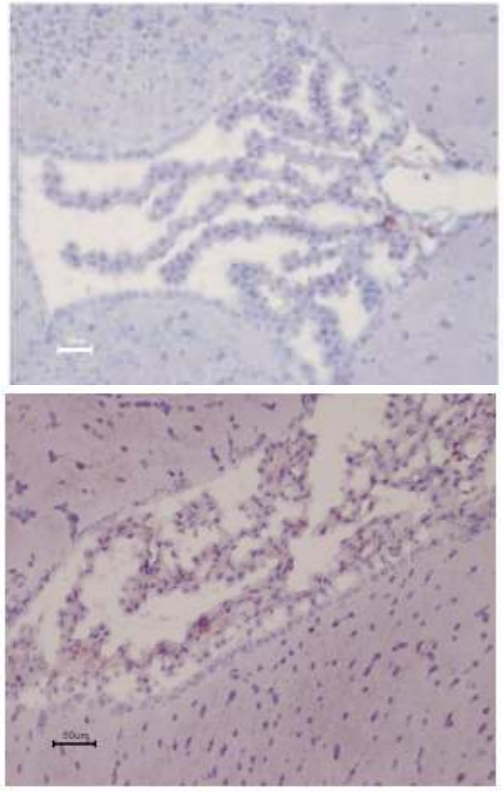
Is the choroid plexus the port of entry of CCR6^{+} Th17 cells?



Engelhardt and Ransohoff, Trends Immunol 2005

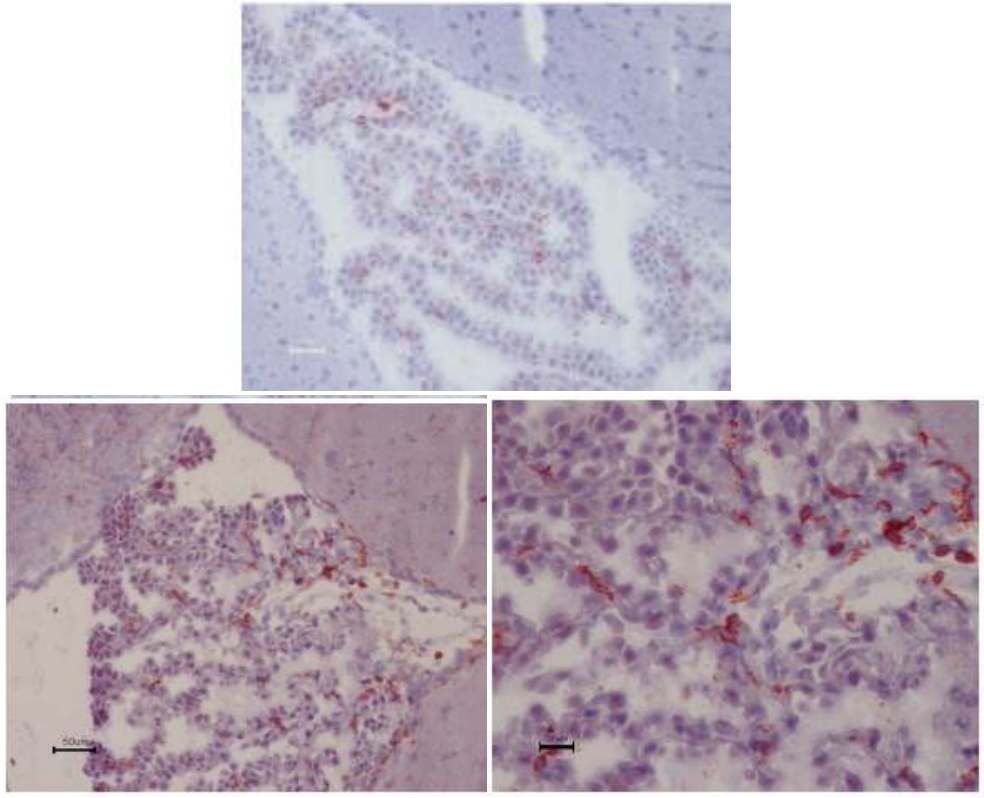
In CCR6-KO mice immunized with MOG+CFA lymphocytes are trapped in the choroid plexus

Wild-type



CD45 Ab

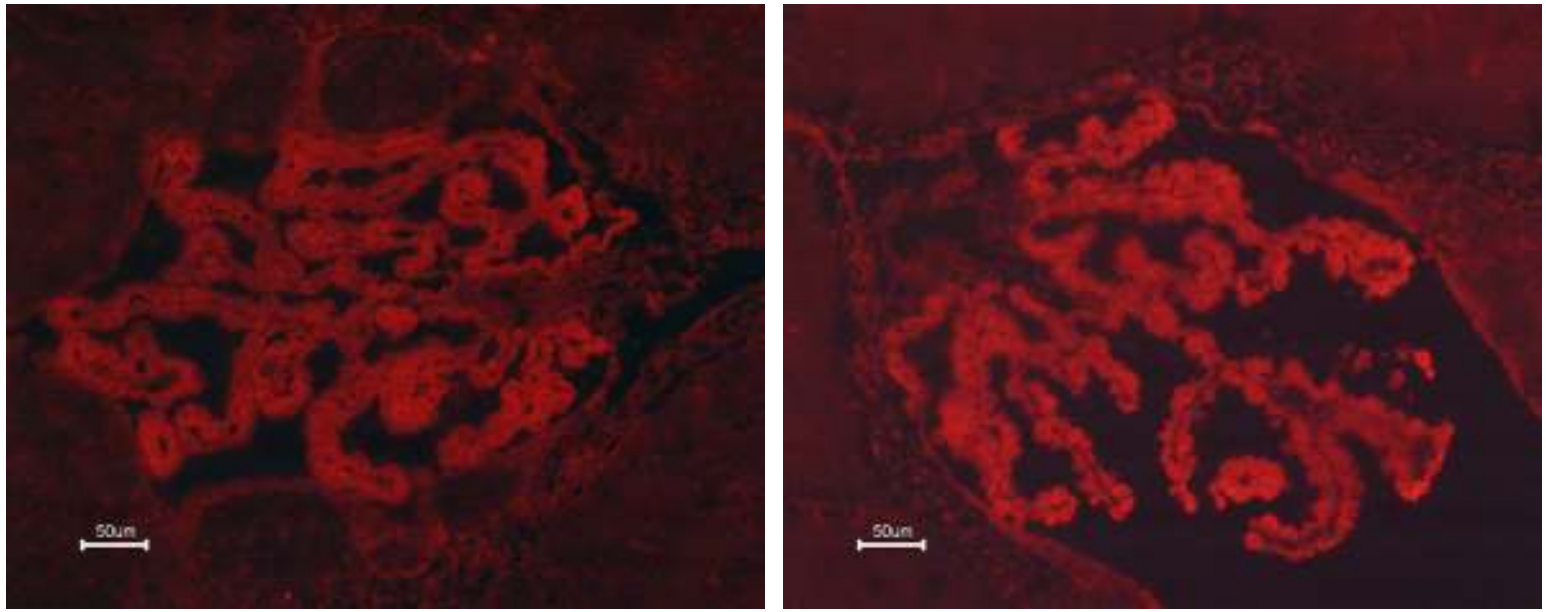
CCR6-KO



CD45 Ab

The CCR6 ligand CCL20 is highly expressed in epithelial cells of the mouse choroid plexus

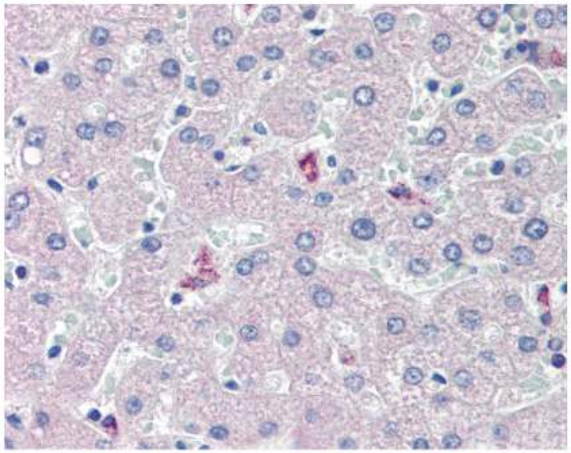
Wild-type **CCR6-KO**



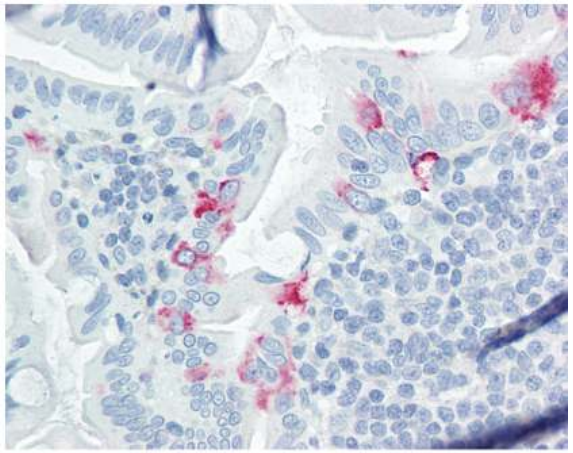
CCL20 Ab

The CCR6 ligand CCL20 (LARC) is constitutively expressed in human choroid epithelium

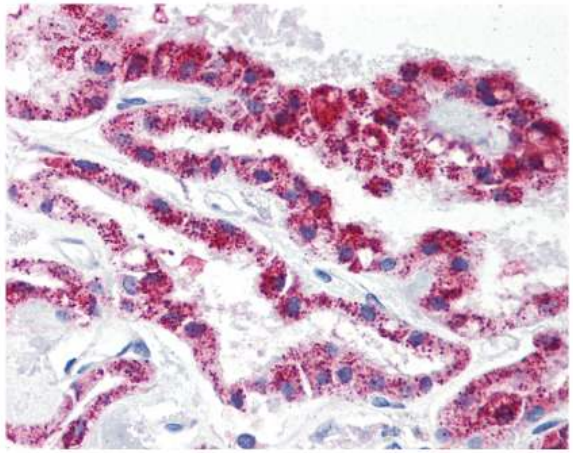
Liver



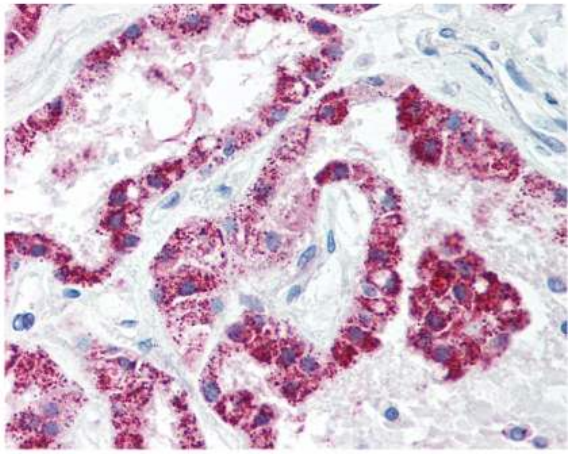
Ileum



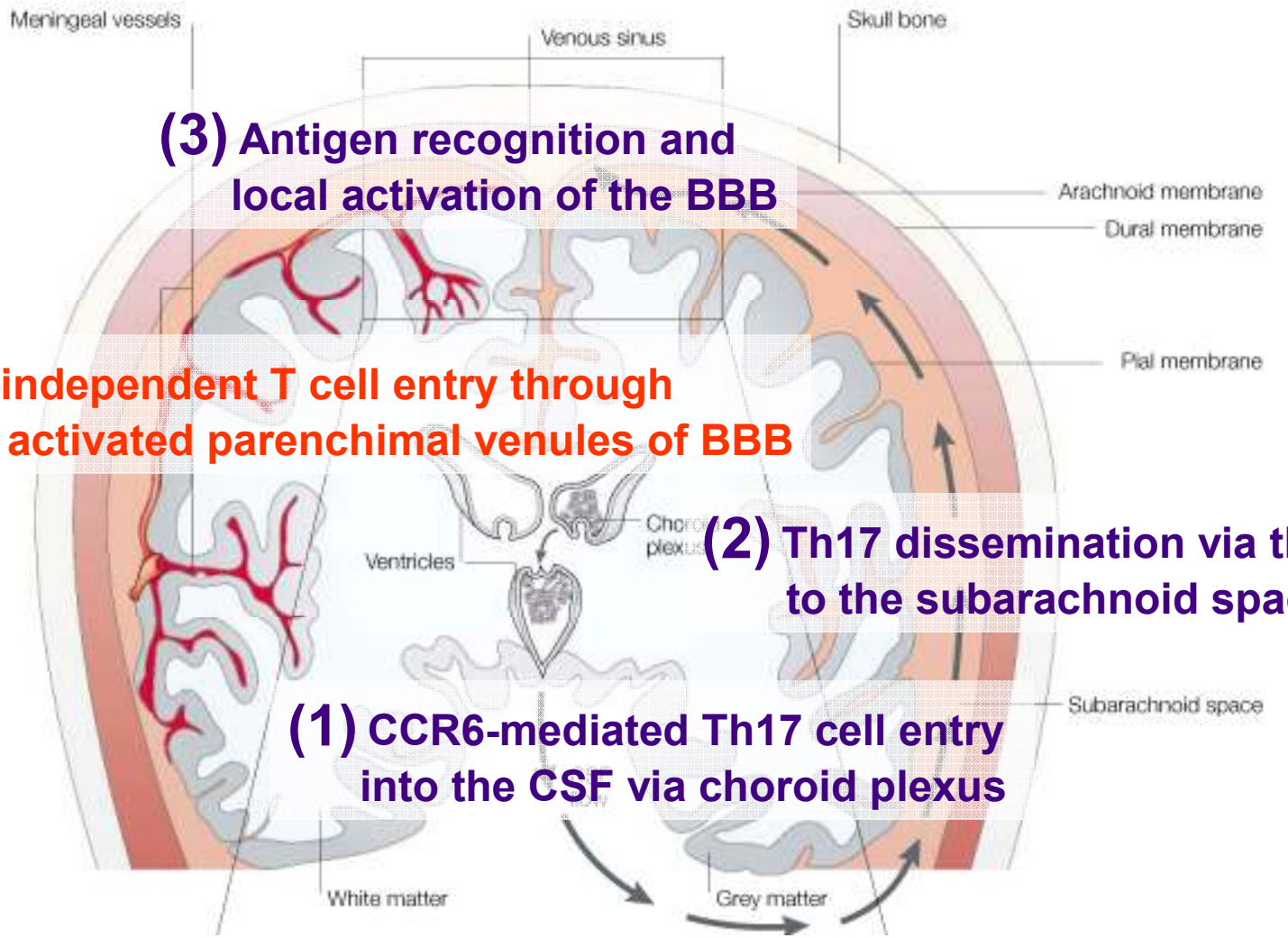
Choroid plexus



Choroid plexus



Constitutive and **inflammatory** routes of entry into the CNS: a two step model of EAE pathogenesis



(3) **Antigen recognition and local activation of the BBB**

(4) **CCR6-independent T cell entry through locally activated parenchimal venules of BBB**

(2) **Th17 dissemination via the CSF to the subarachnoid space**

(1) **CCR6-mediated Th17 cell entry into the CSF via choroid plexus**

CCR6+ T cells as gate keepers for CNS entry

- *How do CCR6^{+} T cells trigger leukocyte recruitment?*
- *Which are the chemokine receptors involved in late EAE?*
- *What is the relative contribution of Th17 and Th1 cells at different stages of the disease?*
- *Does CCR6-blockade have any therapeutic effect (relapsing-remitting SJL model)?*
- *Do CCR6^{+} T cells play a role in surveillance of the CNS?*

Institute for Research in Biomedicine

Human T cell repertoire analysis using amplified T cell libraries

Rebekka Geiger

Challenges in analyzing the human naïve T cell repertoire

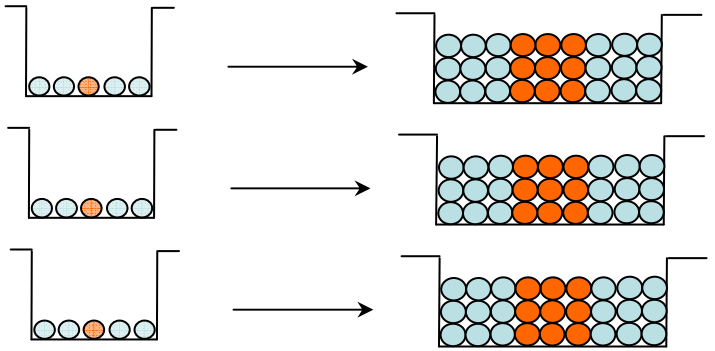
- Low frequency of antigen-specific naïve T cells
- High activation threshold of naïve T cells
- Broad spectrum of avidities
- Limitations of peptide-based and tetramer-based approaches
- Need to measure T cell responses to complex naturally processed antigens

Analysis of naïve and memory T cell repertoires using amplified T cell libraries

1st step: Amplification

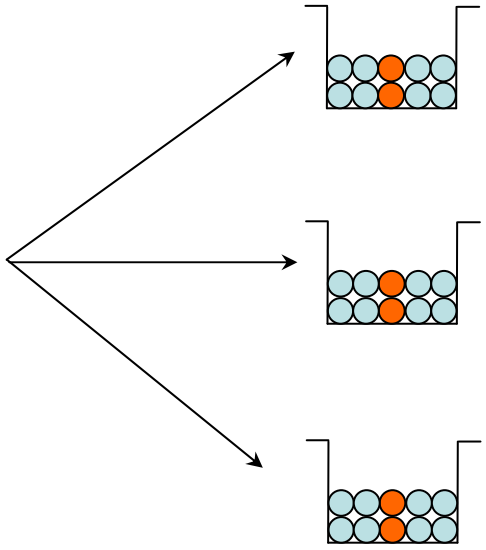
Multiple cultures (96 up to 384) each containing 1000 cells are expanded with PHA and IL-2

1 naïve T cell → 5,000 T cell blasts



2nd step: Interrogation

Each culture is tested for proliferation in response to Ag and autologous APC



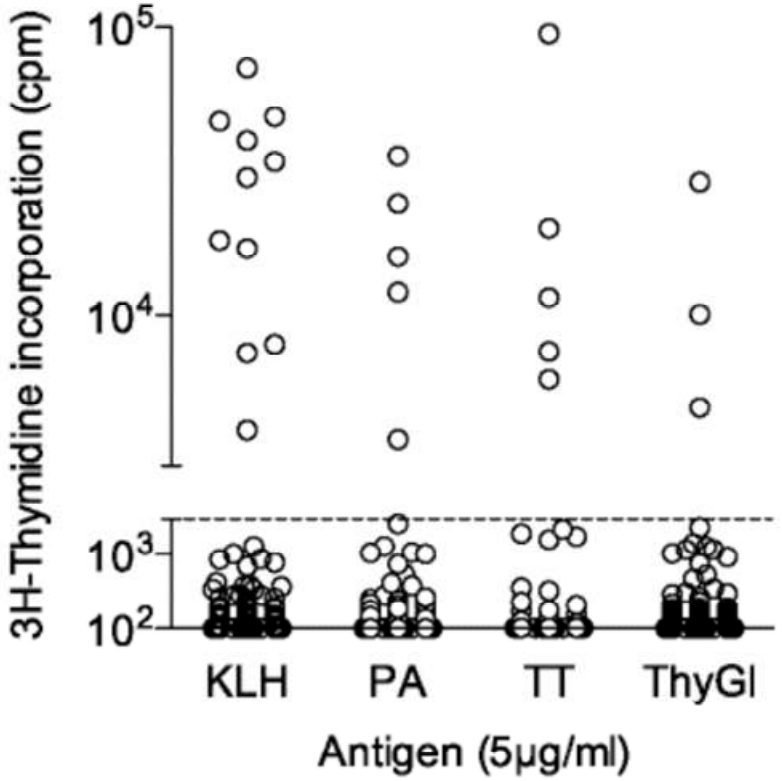
KLH, Anthrax… (primary antigens)

Thyroglobulin… (self antigens)

TT, CMV, PPD… (recall antigens)

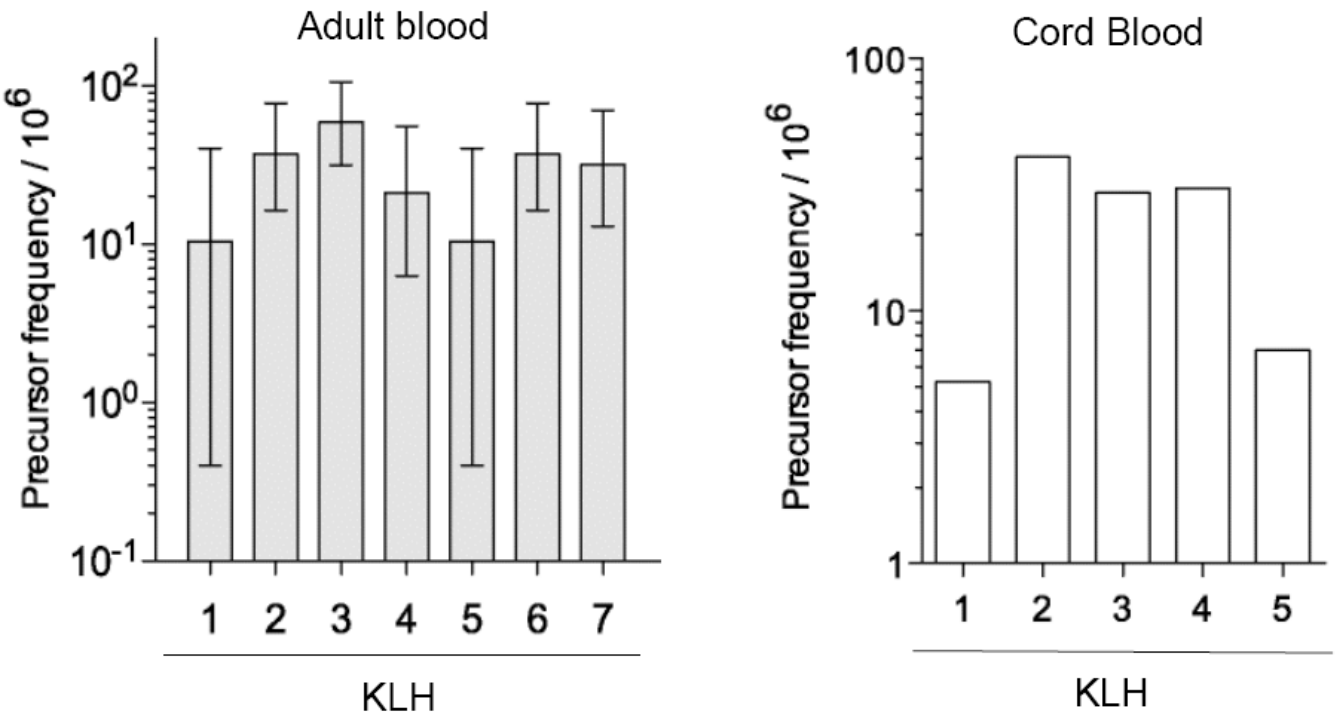
Geiger et al JEM 2009

Antigen-specific CD4+ T cells in the human naïve repertoire

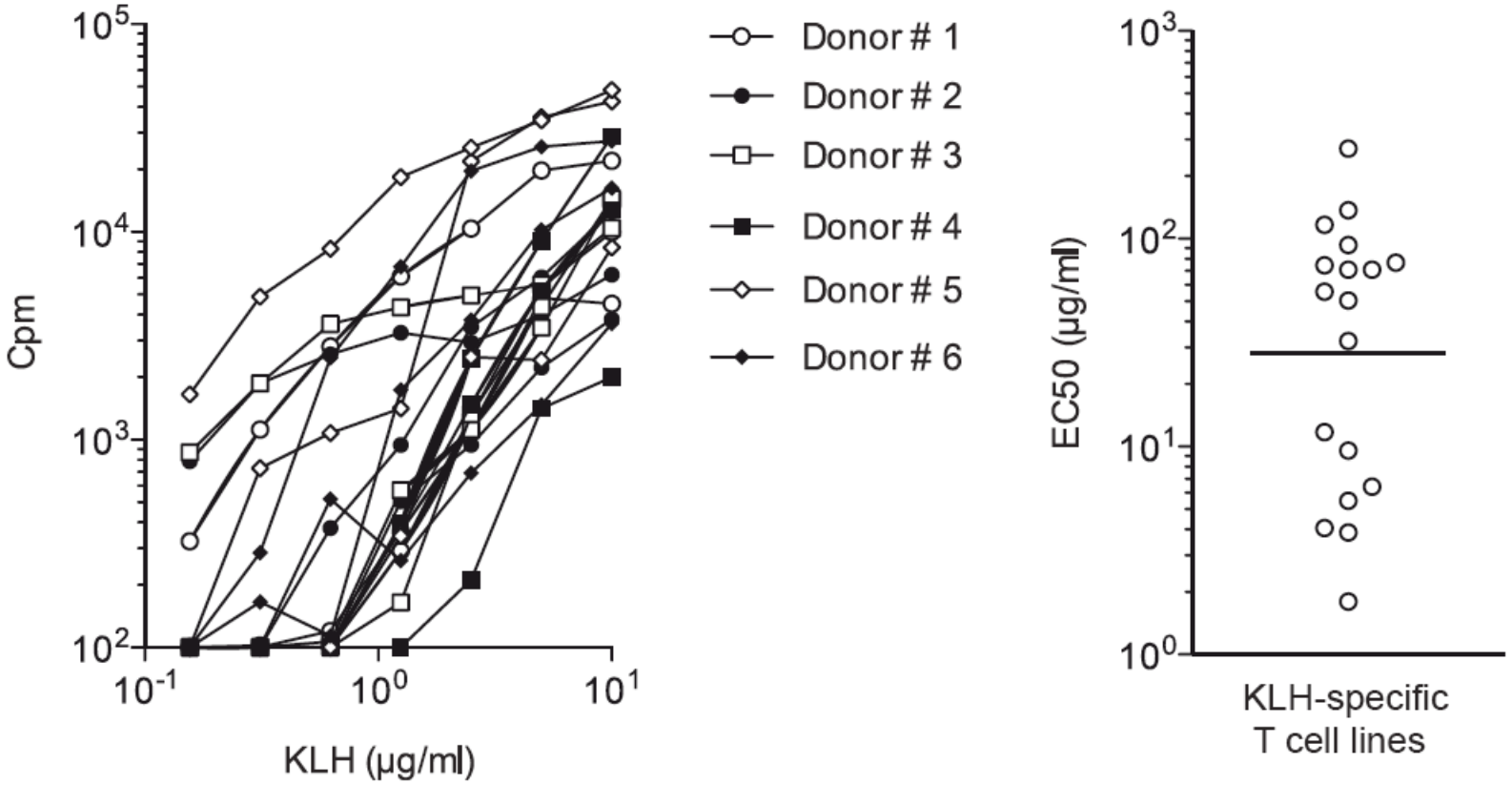


Frequency of KLH-specific naïve T cells

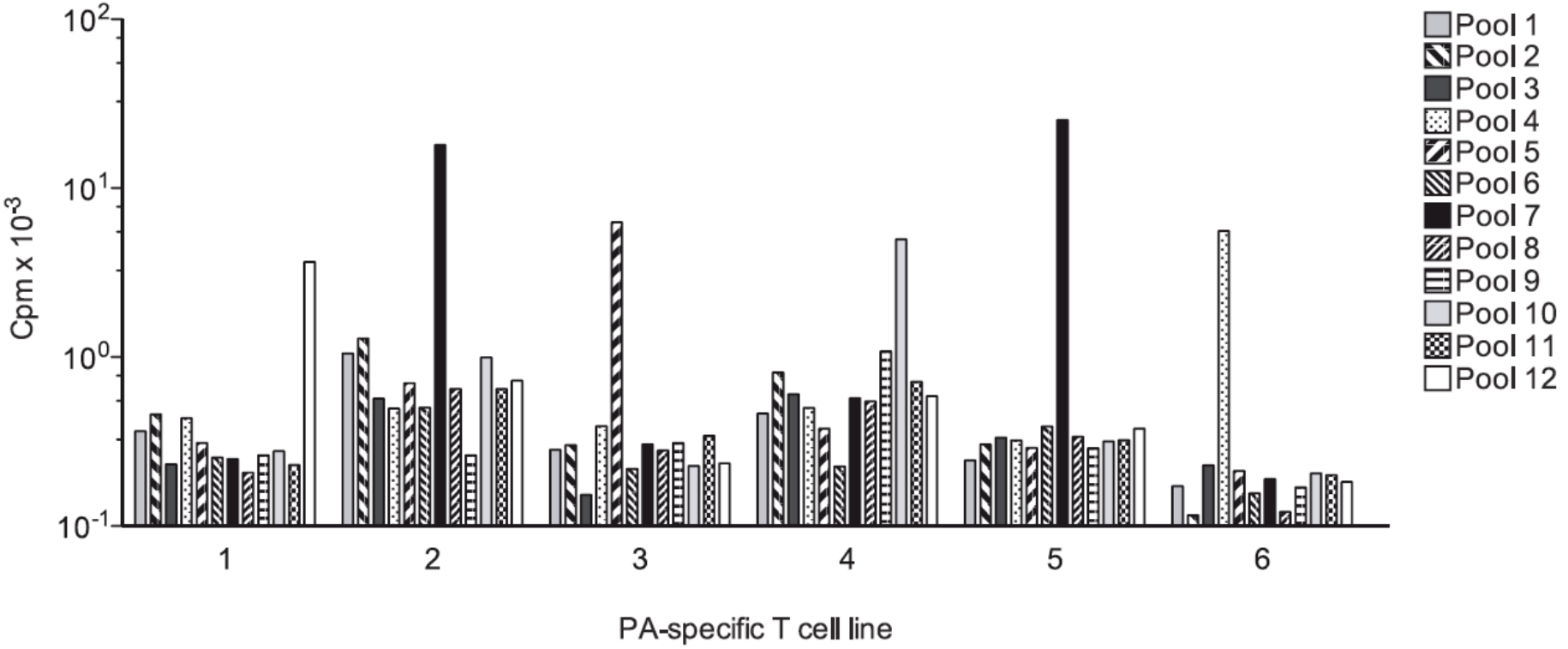
"Amplified T cell libraries" from naïve CD45RA$^+$ CD45RO$^-$ CCR7$^+$ CD4$^+$ T cells



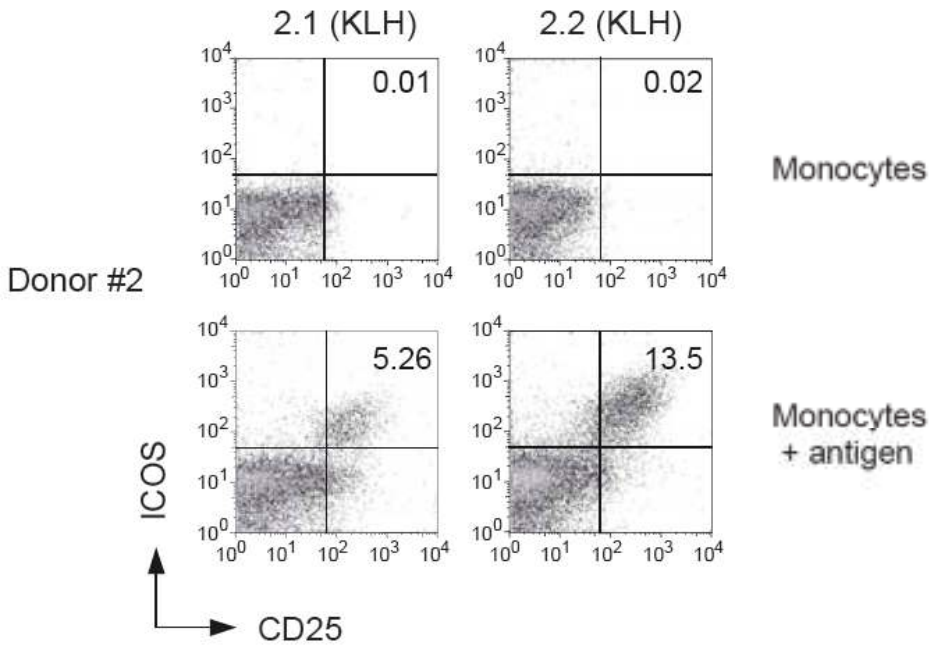
Broad range of responsiveness

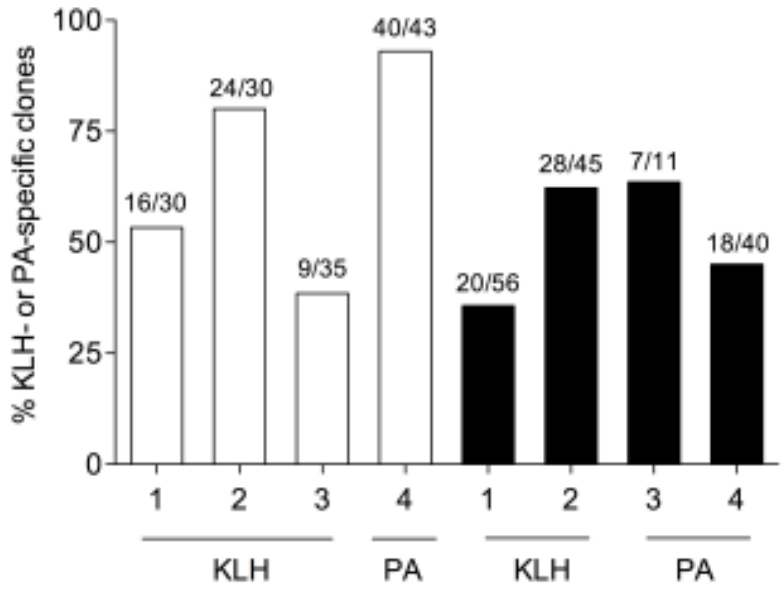


Broad range of epitope specificities

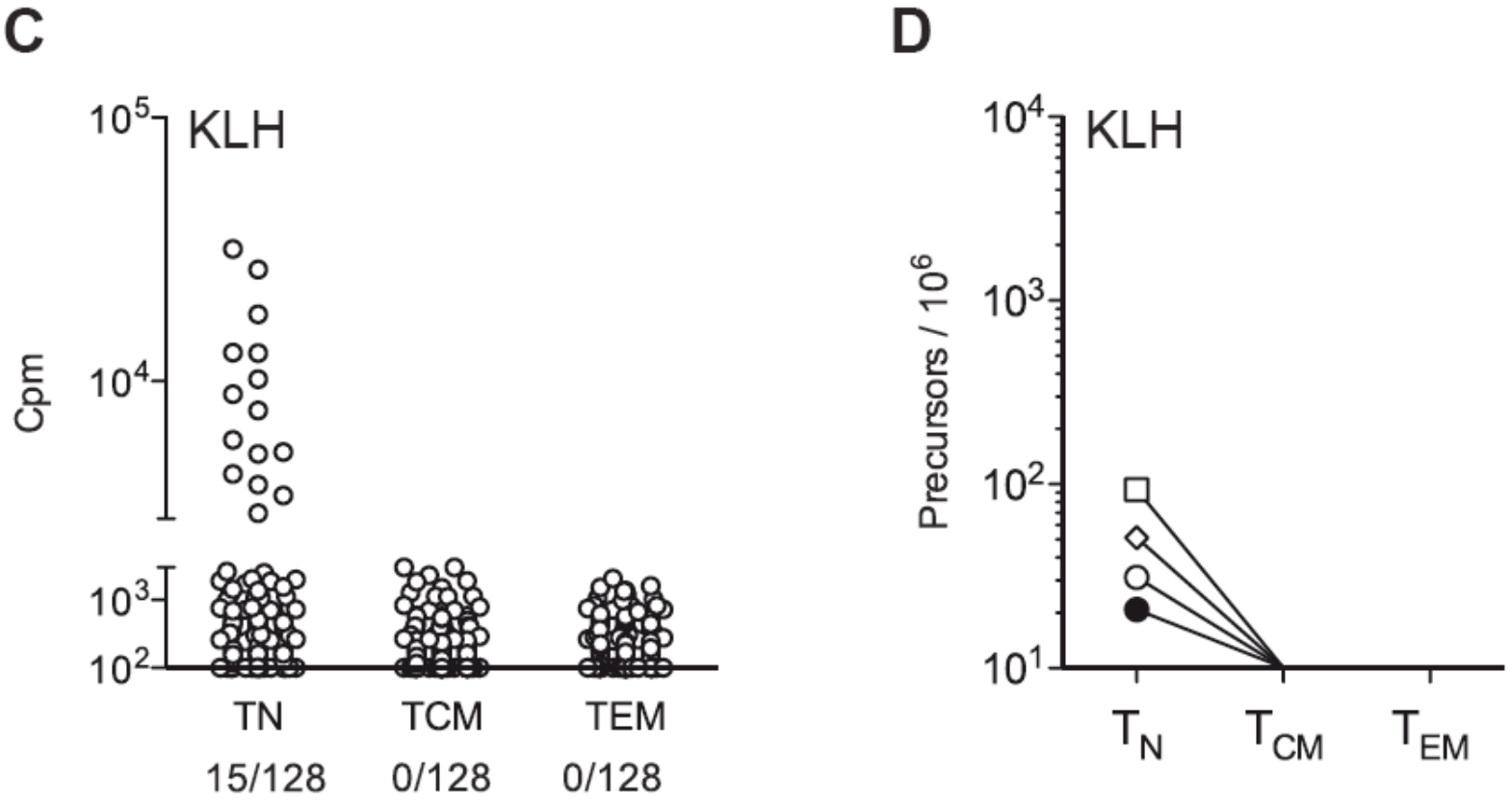


Isolation of Ag-specific T cell clones from the naïve repertoire

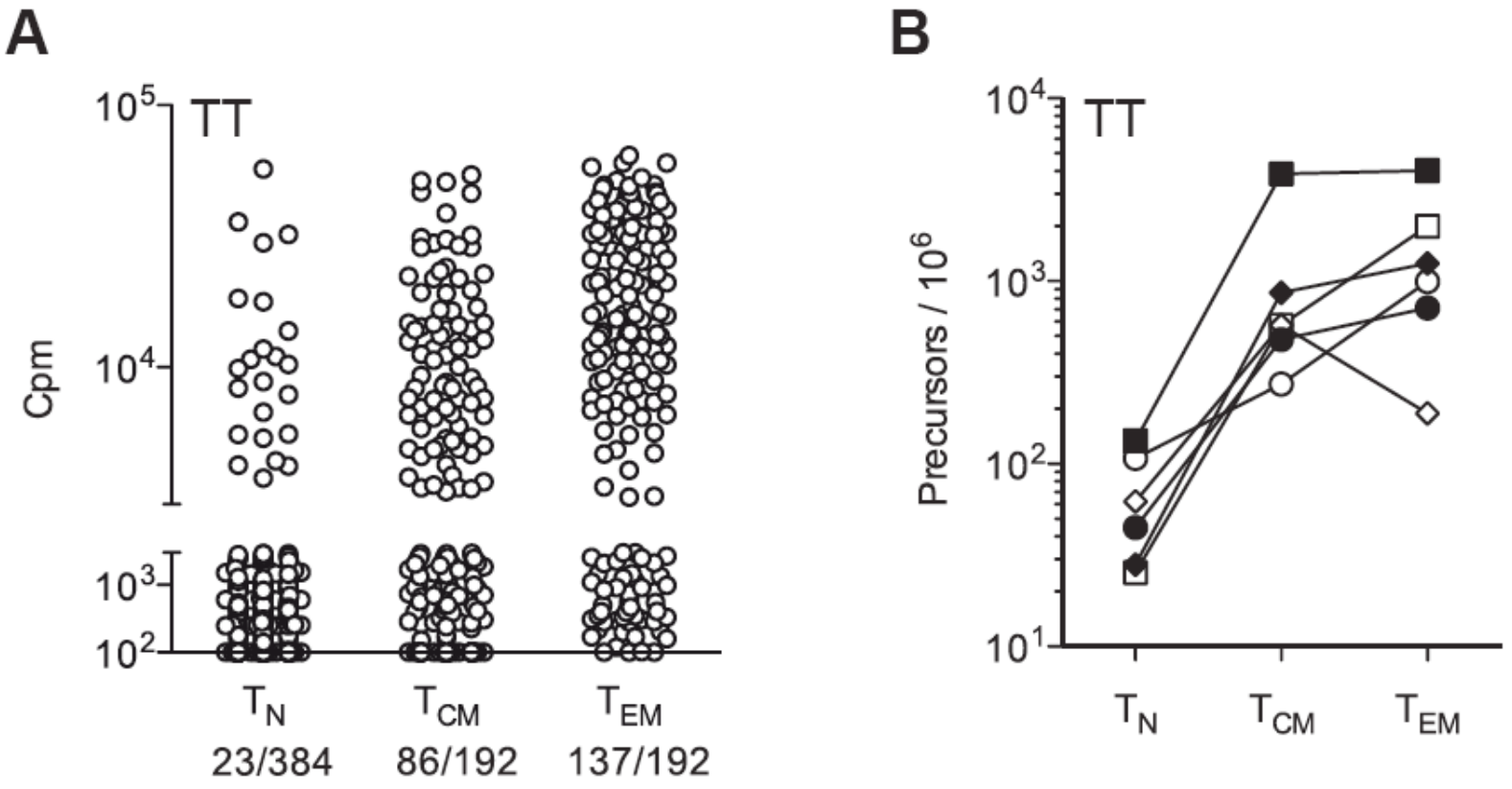




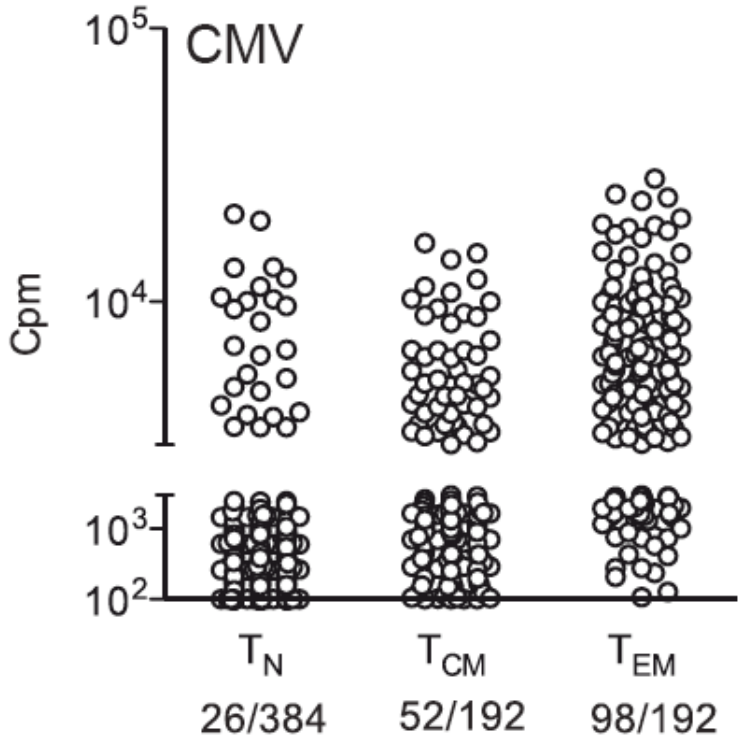
Analysis of memory repertoires

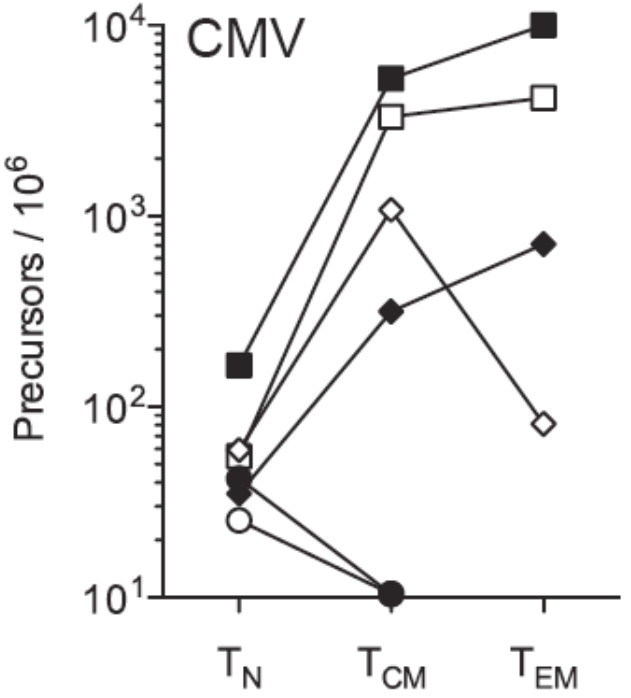


Increased frequencies in the memory compartment (Tet Tox)

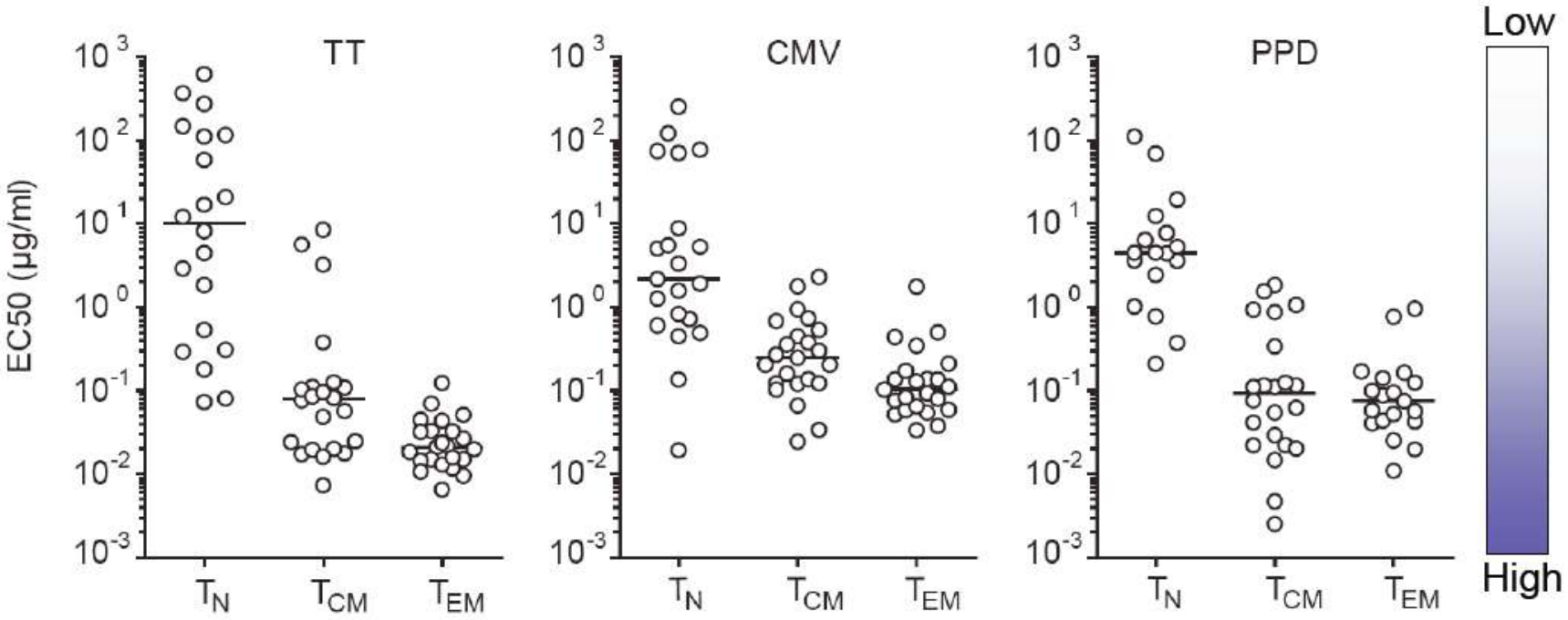


Increased frequencies in the memory compartment (CMV)





Selection of high avidity T cells in the memory pool



Applications

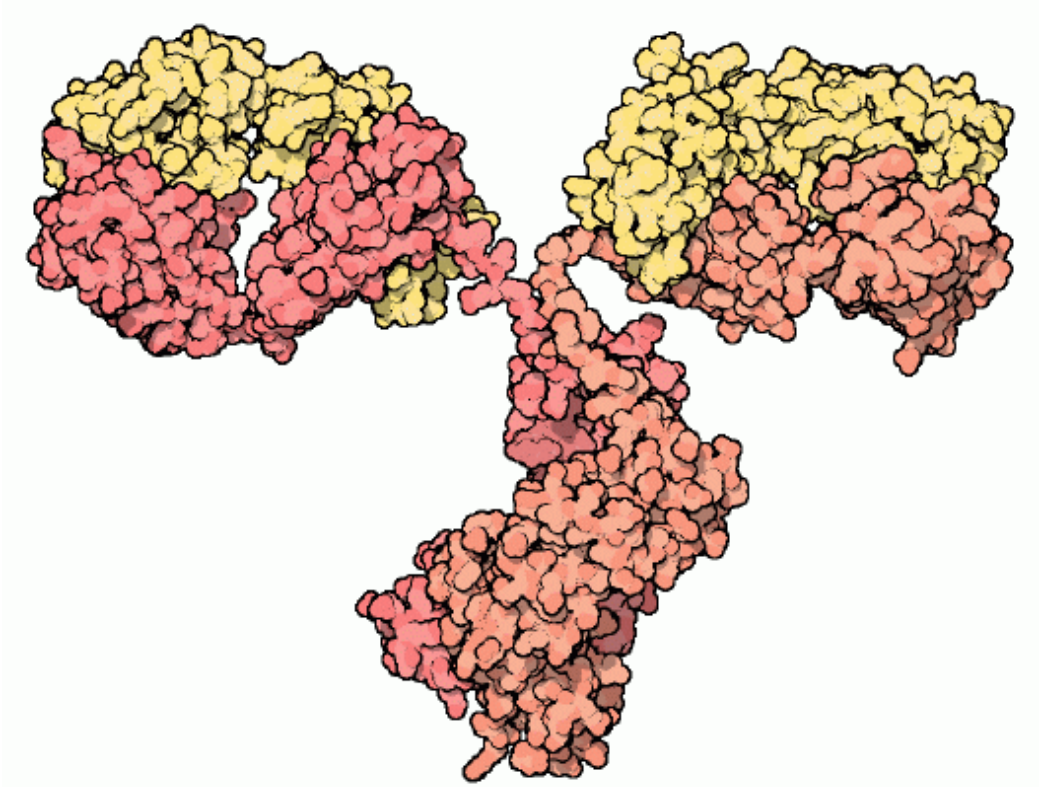
- Predict antigenicity of complex molecules (even whole pathogens)
- Assess immunocompetence (elderly, HIV)
- Assess memory in different T cell subsets
- Generate T cells for cellular immunotherapy

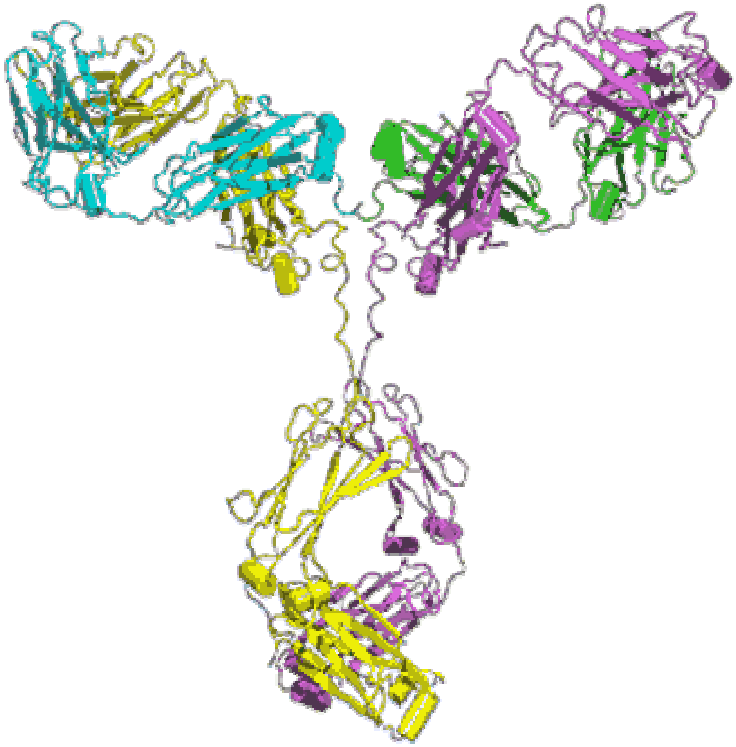
Institute for Research in Biomedicine

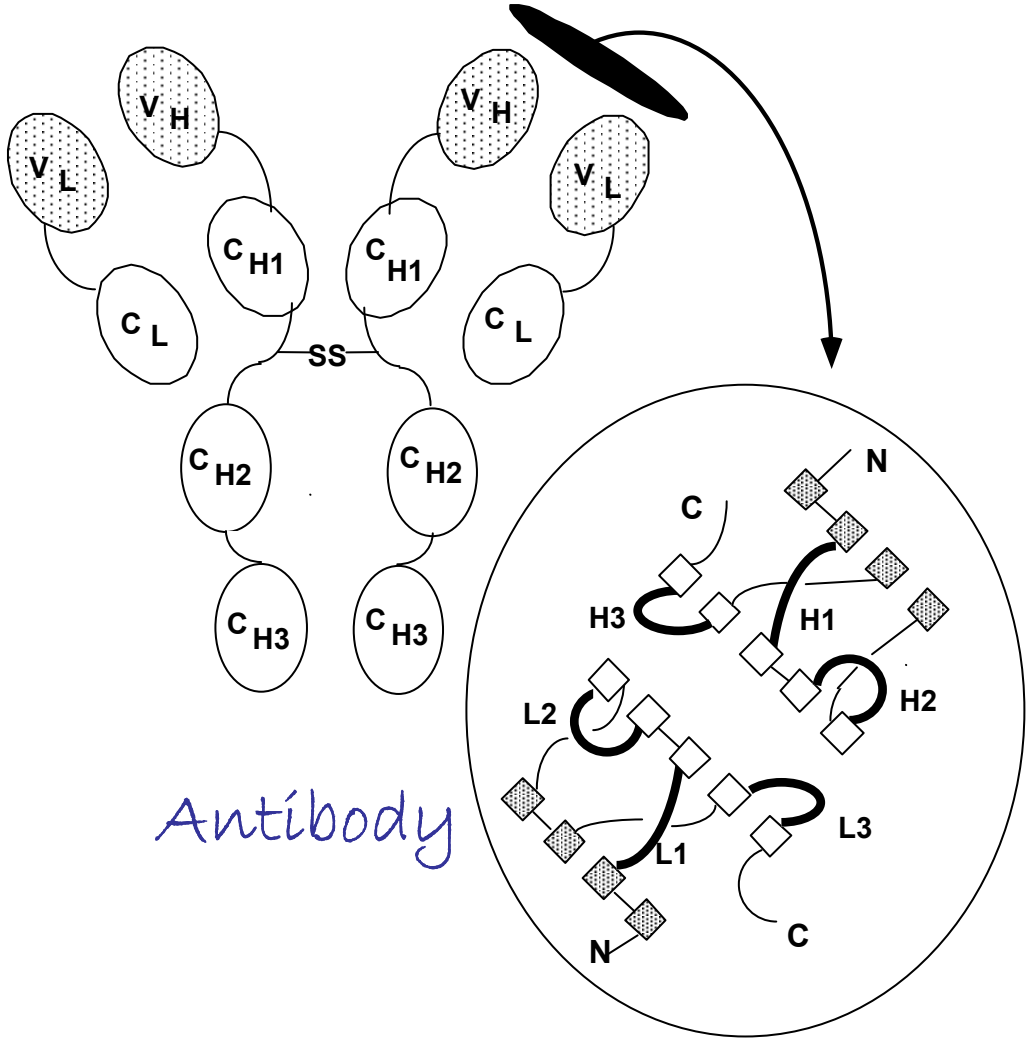
The Lanzavecchia & Sallusto Lab

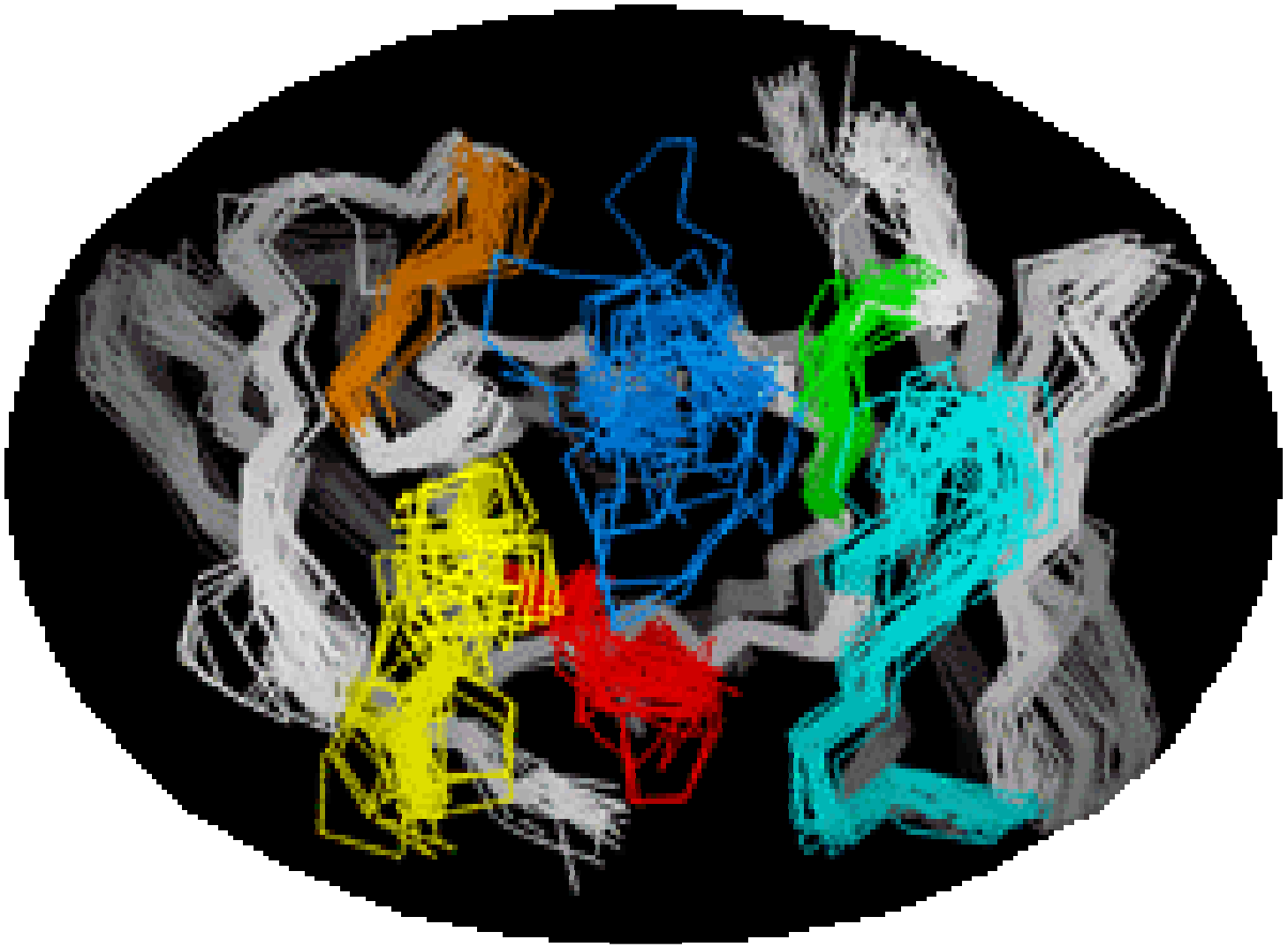
Giorgio Napolitani (Th17)
Andrea Reboldi (EAE)
Thomas Duhen (Th22)
Rebekka Geiger (T cell libraries)
Martina Beltramello (dengue)
Davide Corti (influenza)
David Jarrossay (plasma cells)
Dirk Baumjohann
Annalisa Macagno
Janine Stubbs
Debora Pinna
Blanca Fernandez
Chiara Silacci
Fabrizia Vanzetta

Eva Acosta-Rodriguez, Cordoba - Jens Geginat, Berlin - Mara Messi, Basel - Amanda Gett, Basel - Laura Rivino, Berlin

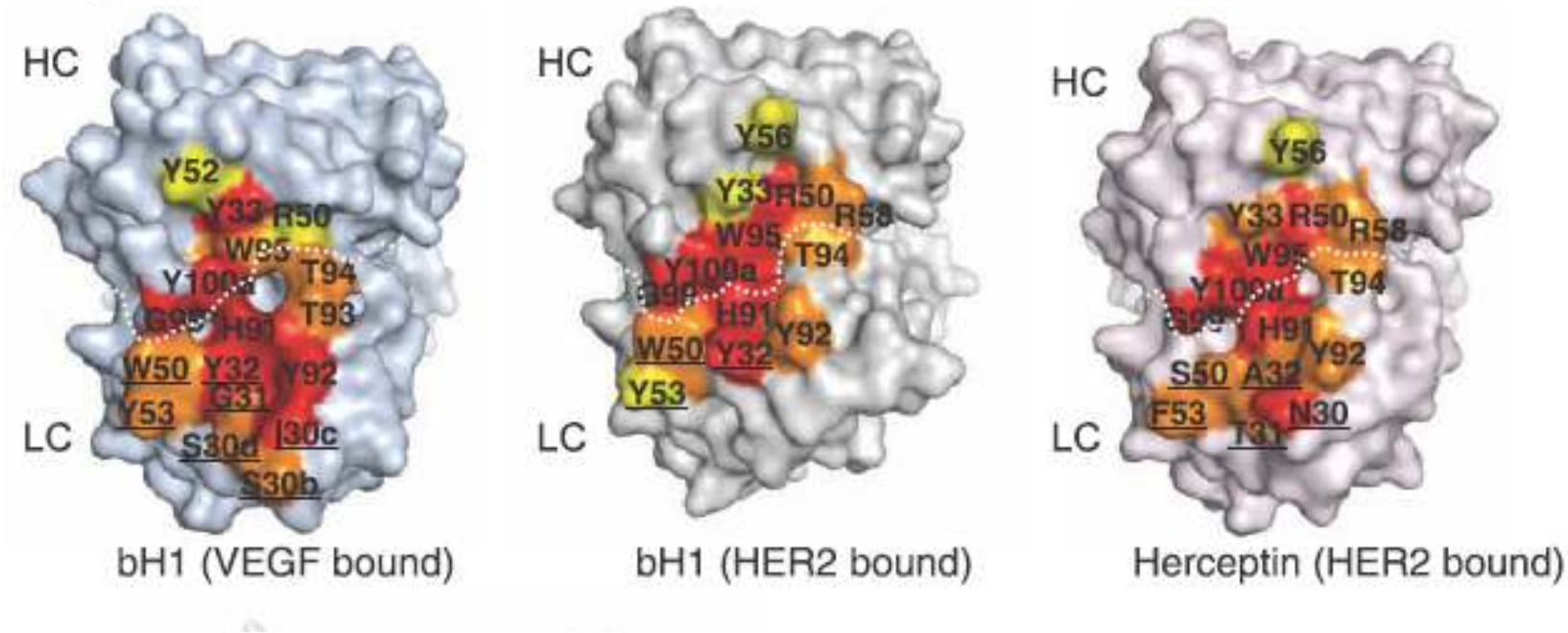





V H
V L
C H1
C L
SS
C H2
C H3
N
C
H3
H1
H2
L2
L1
L3
C
N
Antibody
Antigen binding site

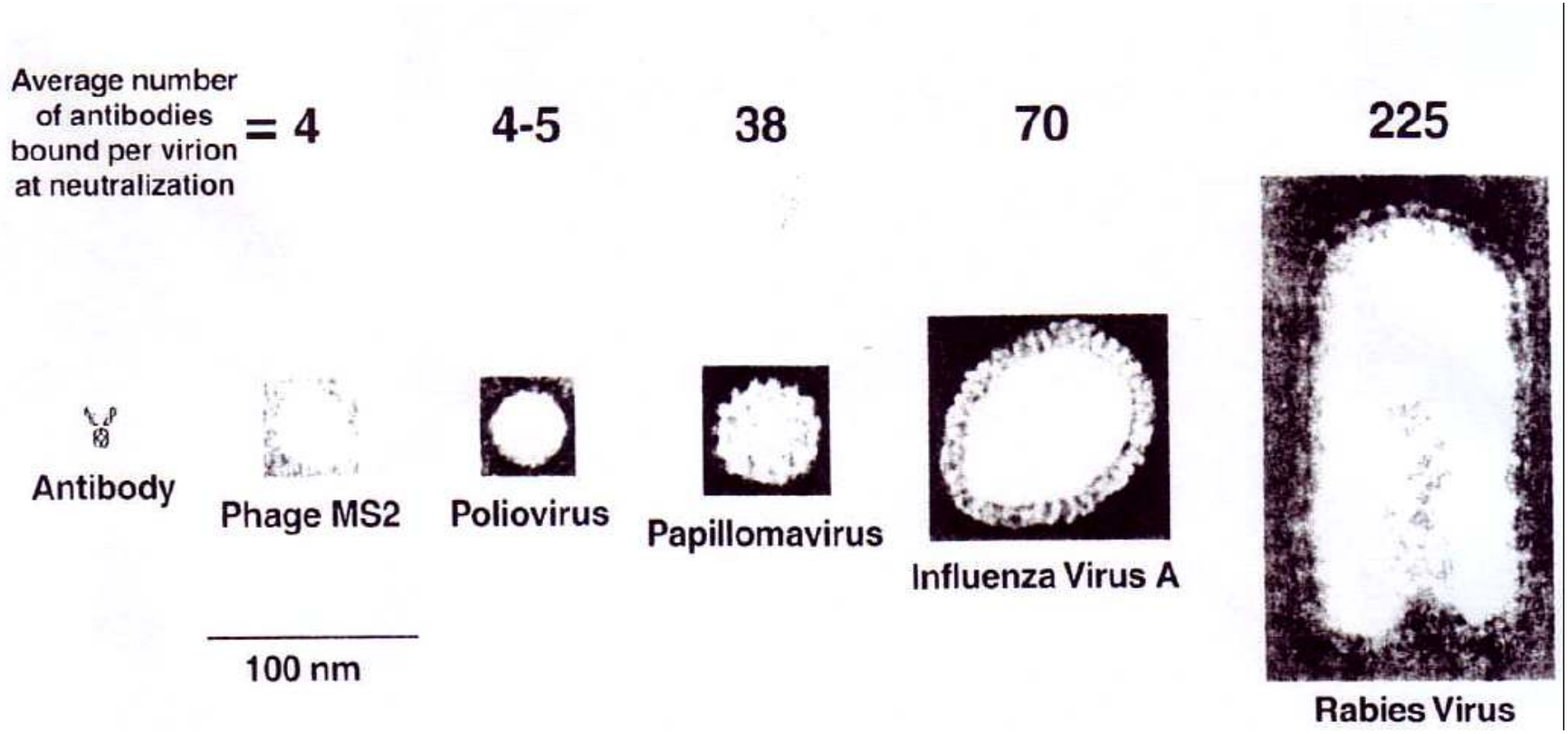


Multispecificity / Crossreactivity / Promiscuity / Degeneracy



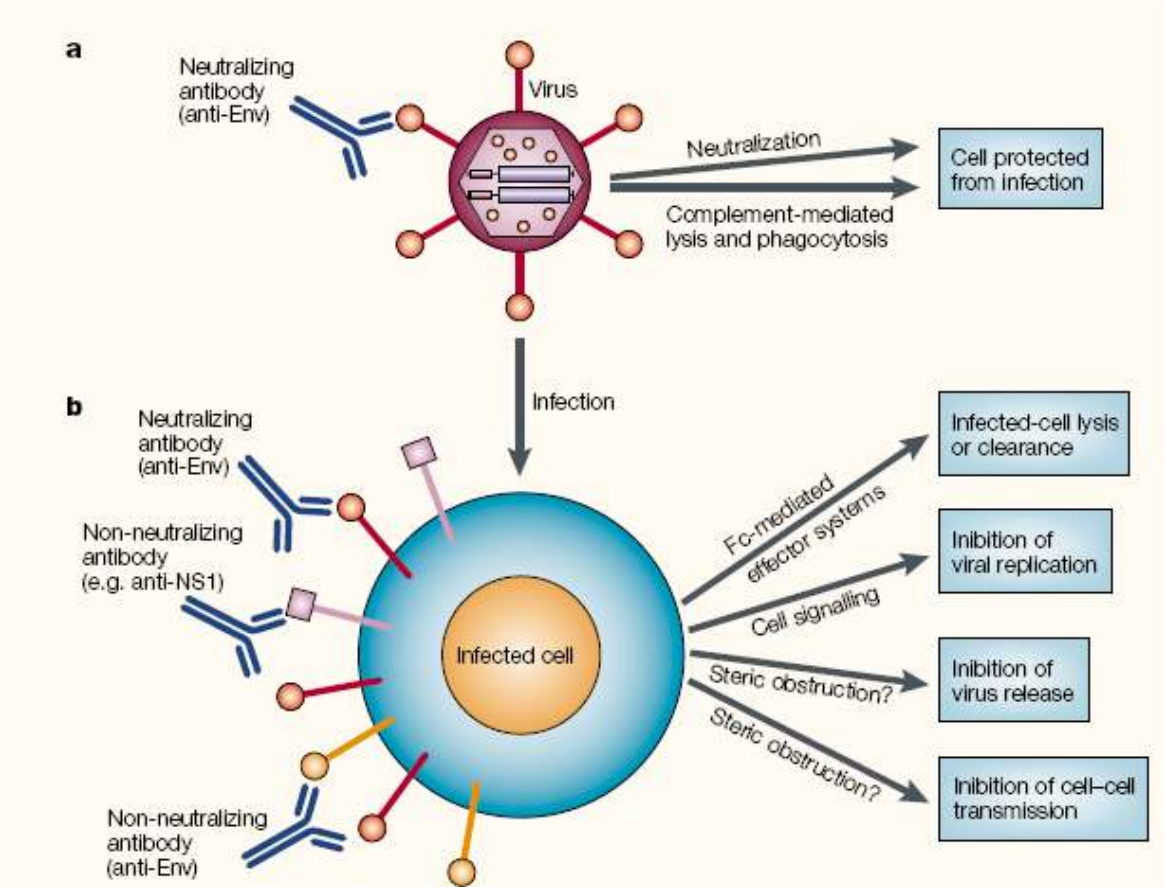
Bostrom et al Science 2009

Antiviral activities of antibodies



affinity / avidity / accessibility

Antiviral activities of antibodies



The cellular basis of immunological memory

“Effector memory”

Plasma cells (Ab)

Effector memory T cells

- Fully differentiated
- In peripheral tissues
- Immediate protection

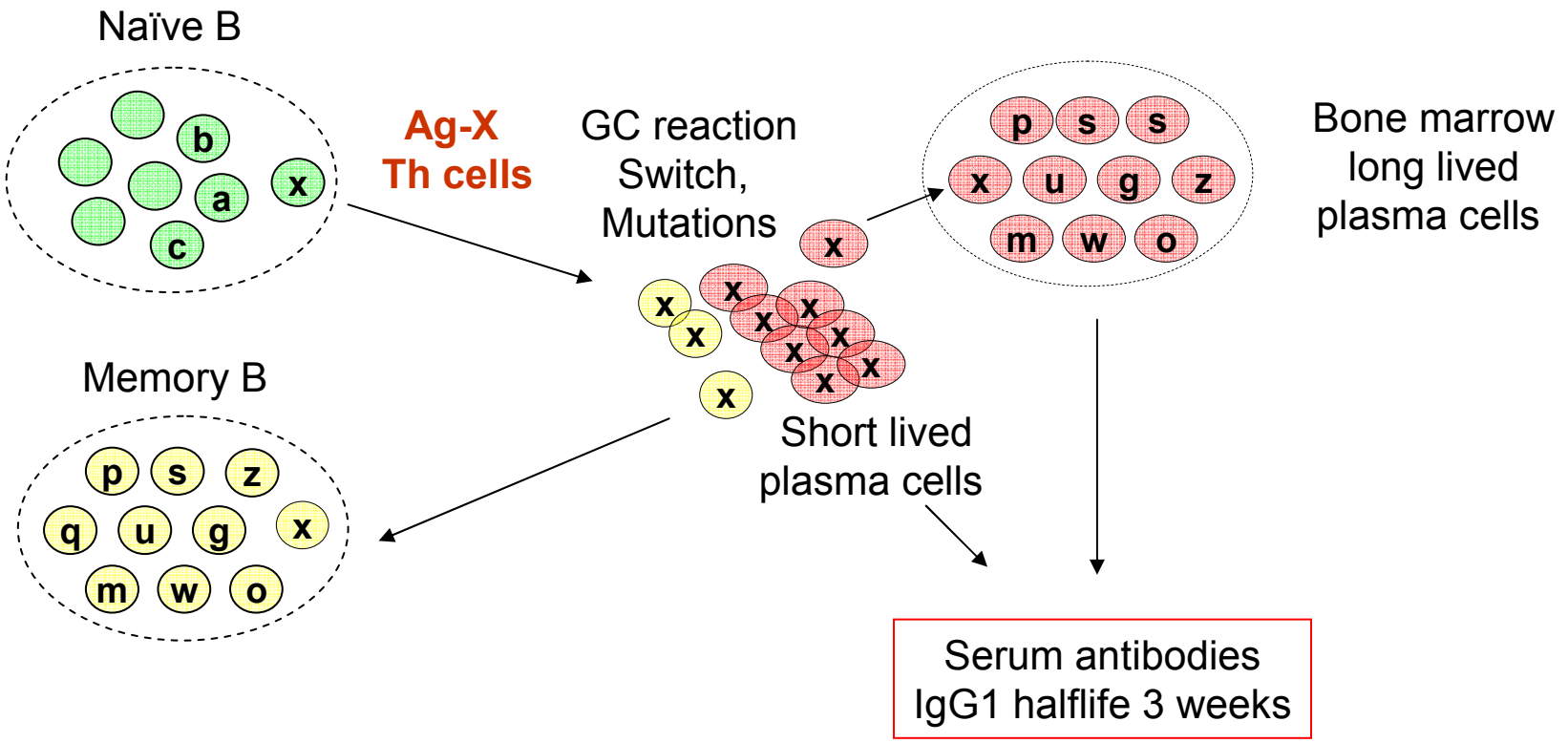
“Central memory”

Memory B cells

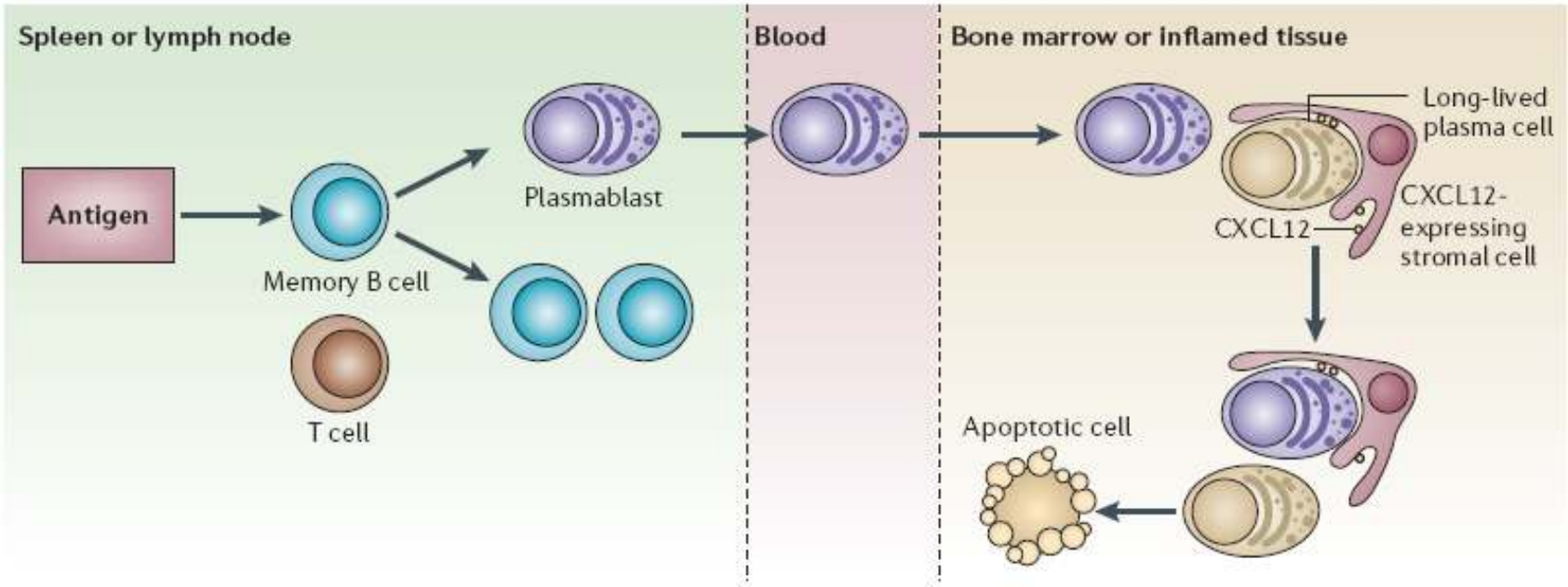
Central memory T cells

- Differentiation intermediates
- In secondary lymphoid organs
- Recall responses

B cell clonal dynamics: primary responses

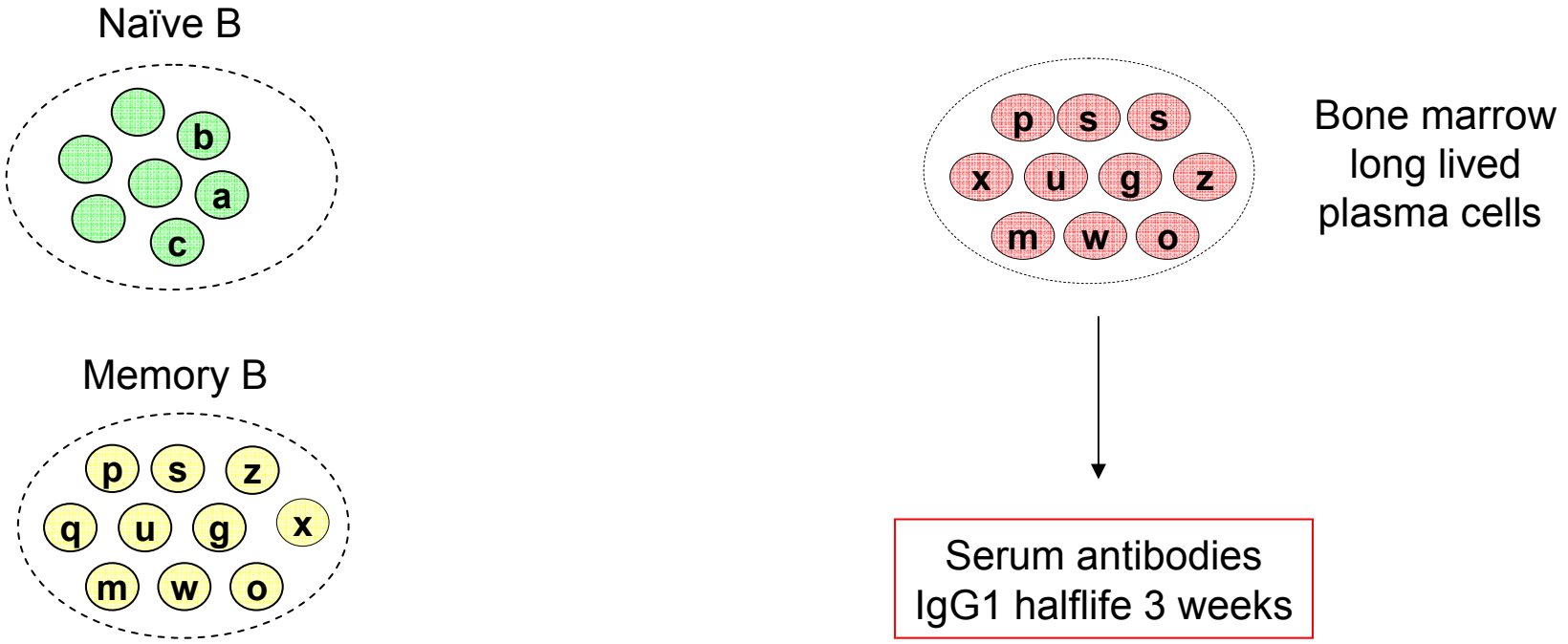


Bone marrow niches for long lived plasma cells



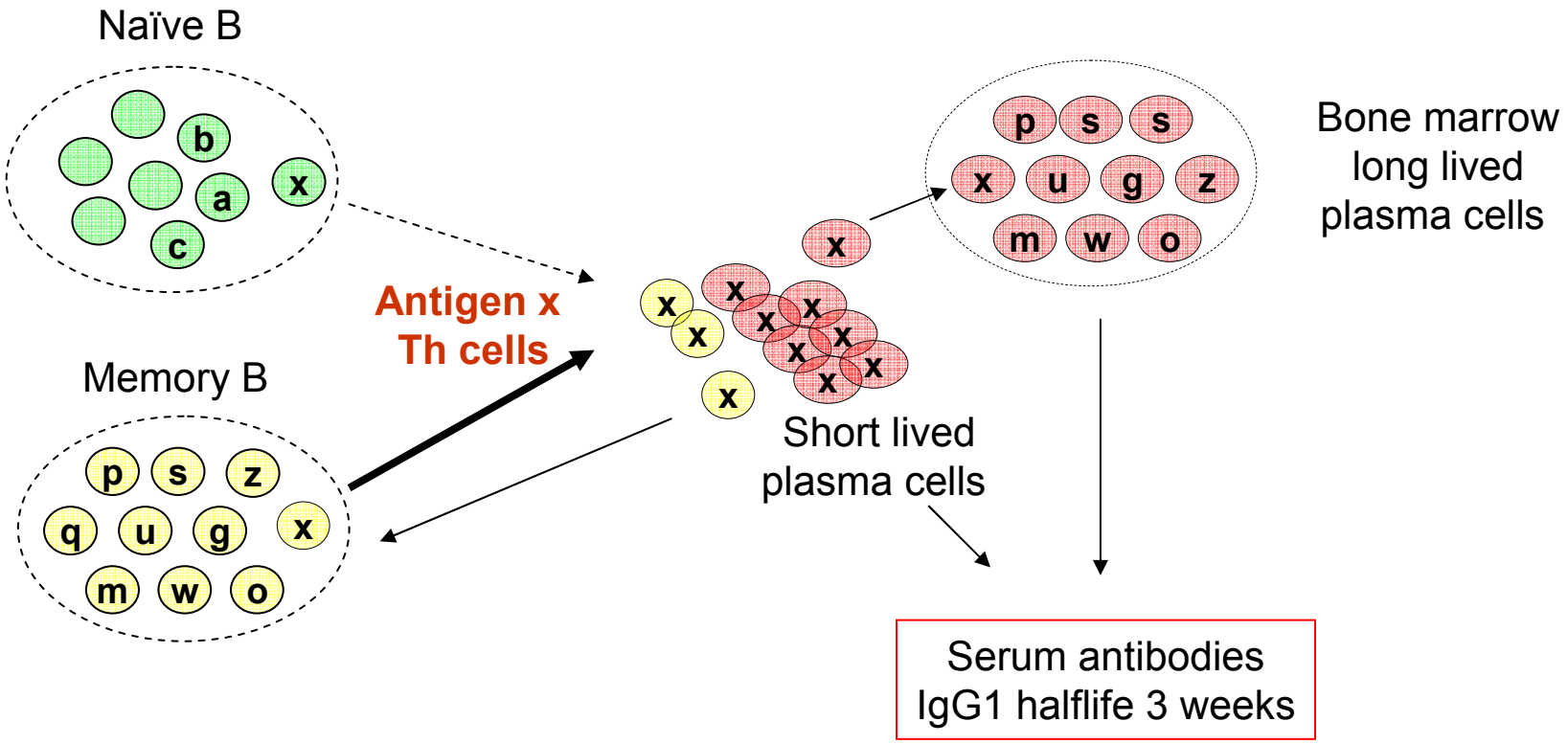
Radbruch et al Nat Rev Immunol, 2006

B cell clonal dynamics: sustained serum antibodies



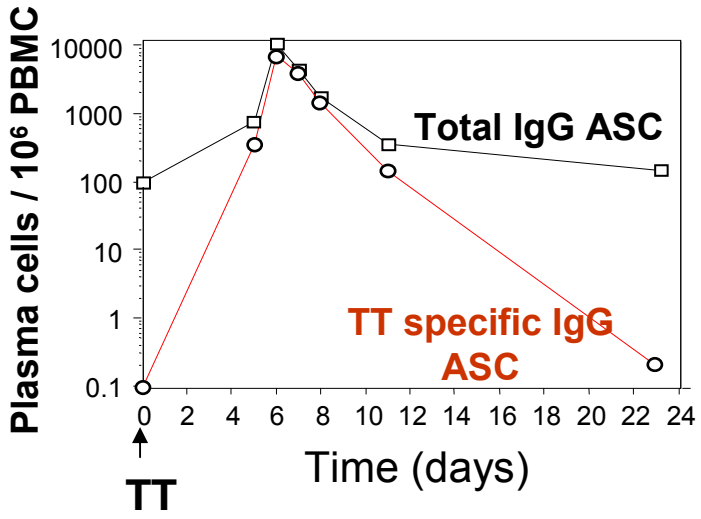
Serum antibodies to vaccinia virus are maintained constant for a lifetime

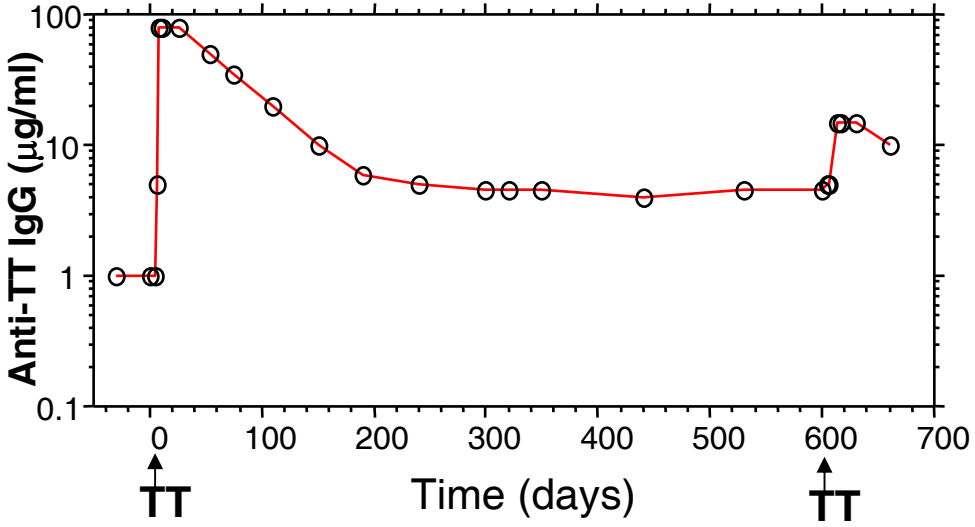
B cell clonal dynamics: secondary responses

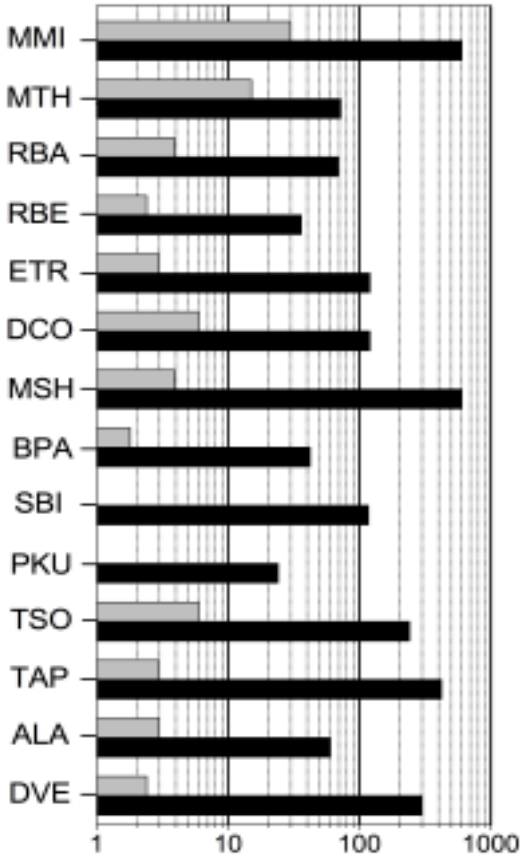


"Original antigenic sin"

Kinetics of circulating plasma cells, memory B cells and serum antibodies







Ag-specific IgG⁺ B cells (‰) on day 14

Human memory B cell subsets