Journal Club

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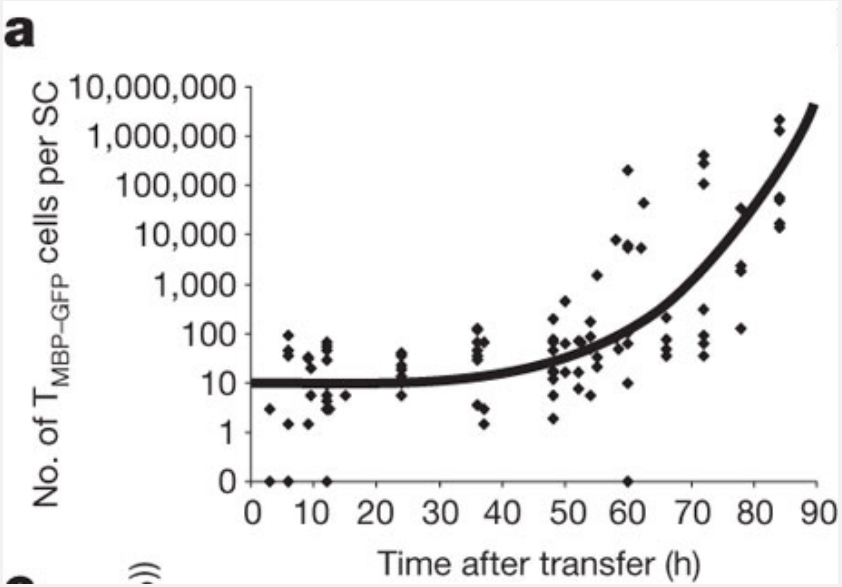
20-11-12

Invasion of the CNS by activated T cells

How do T cells invade the CNS?

- They monitored the invasion of the CNS by GFP-expressing MBP-reactive T cells ($T_{MBP\text{-}GFP}$ cells) over the preclinical phase of EAE in Lewis rats.
- Only a few early invading pioneer T cells were detected in the spinal cord during the first 60 h after intravenous transfer of activated $T_{MBP\text{-}GFP}$ cells.
- The majority of the $T_{MBP\text{-}GFP}$ cells started to invade the spinal cord 60-84 h after T cell transfer, shortly before the onset of clinical symptoms.

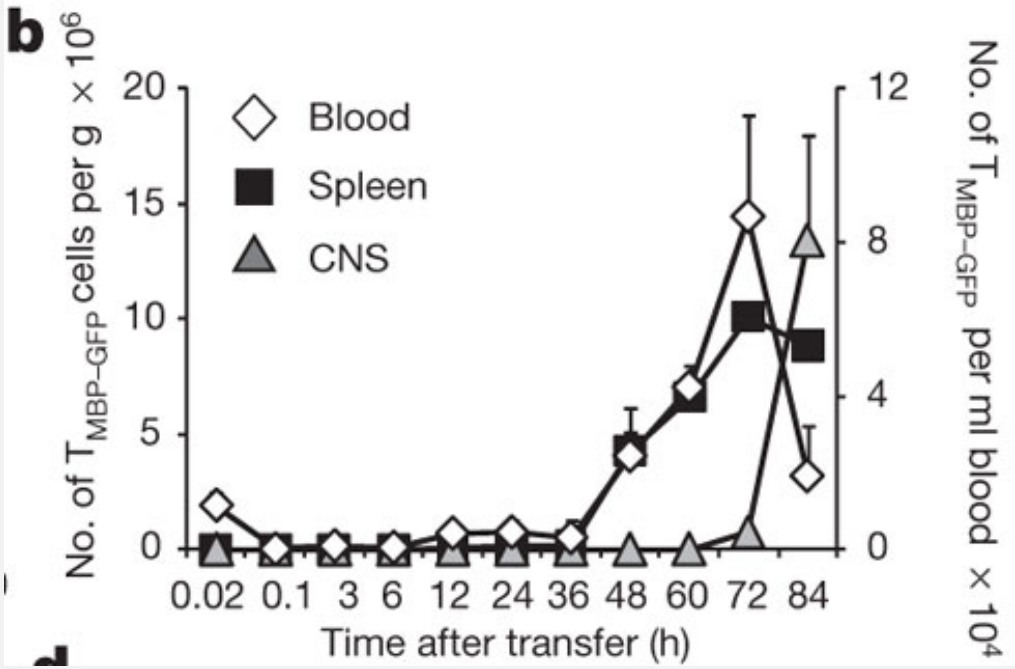


The $T_{MBP\text{-}GFP}$ cells do not reside in the blood or spleen.

Where do the activated T cells reside before they enter the CNS?

- The $T_{MBP\text{-}GFP}$ cells immediately disappeared from the blood after intravenous infusion.
- $T_{MBP\text{-}GFP}$ cells show up 48 h later in the blood and spleen.
- Shortly after the invasion of the T cells into the CNS starts.

Conclusion: The $T_{MBP\text{-}GFP}$ cells do not reside in the blood or spleen.



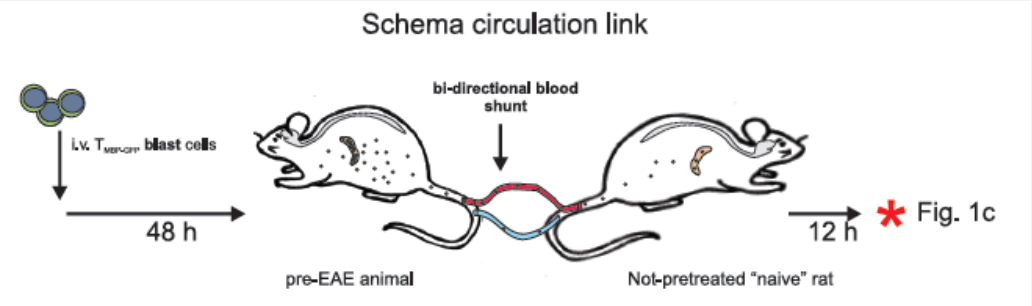
$T_{migratory}$ cells are able to invade the CNS of a naïve rat.

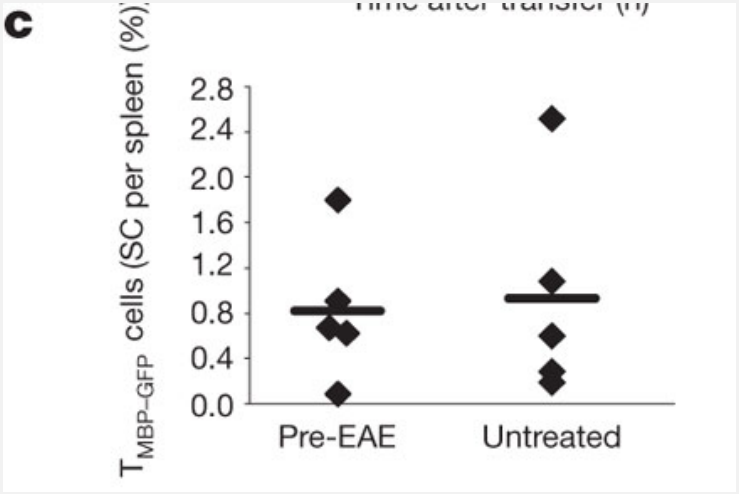
What happed to the disappeared cells?

Can the re-emerged cells (termed T migratory cells) enter the CNS more efficiently than the initially transferred $T_{MBP\text{-}GFP}$ cells?

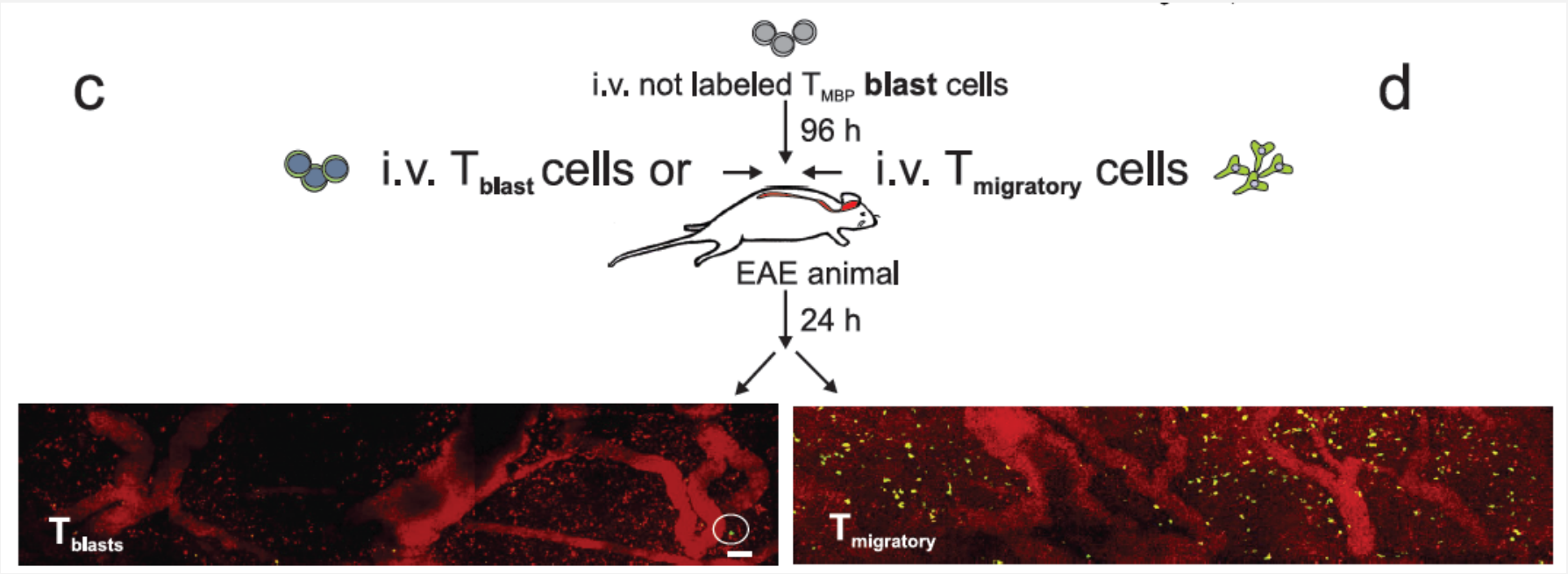
Method: Parabiosis. Tail arteries of two rats were made. One rat received T blast cells 48 h before, the other rat was naïve.

Result: 12 h after anastomosis CNS invasion of $T_{MBP\text{-}GFP}$ cells was the same in both animals.

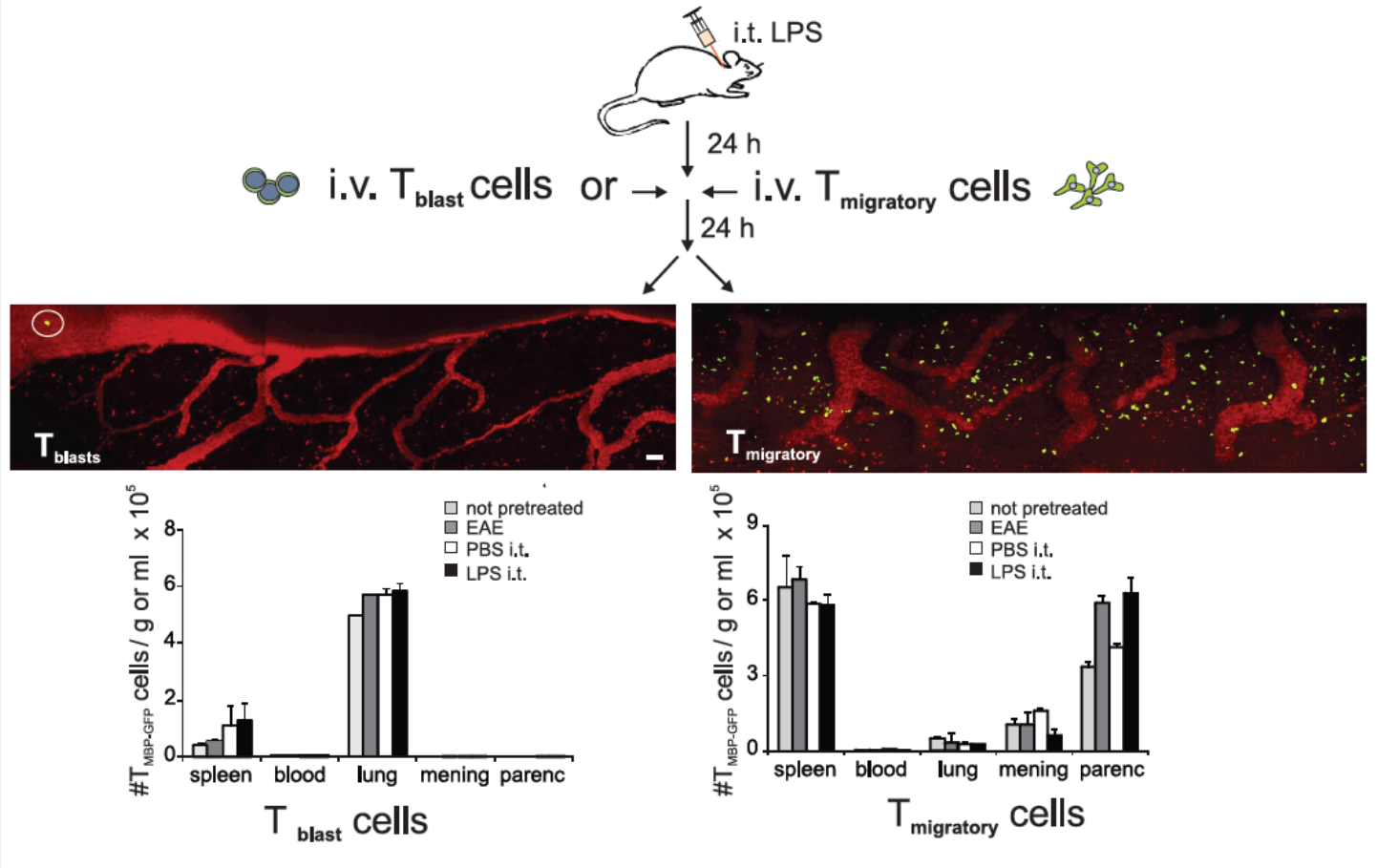




- hdhj


c
i.v. not labeled T_{MBP} **blast** cells
96 h
i.v. T_{blast} cells or
i.v. $T_{migratory}$ cells
EAE animal
24 h
T_{blasts}
$T_{migratory}$
d

- hdhj

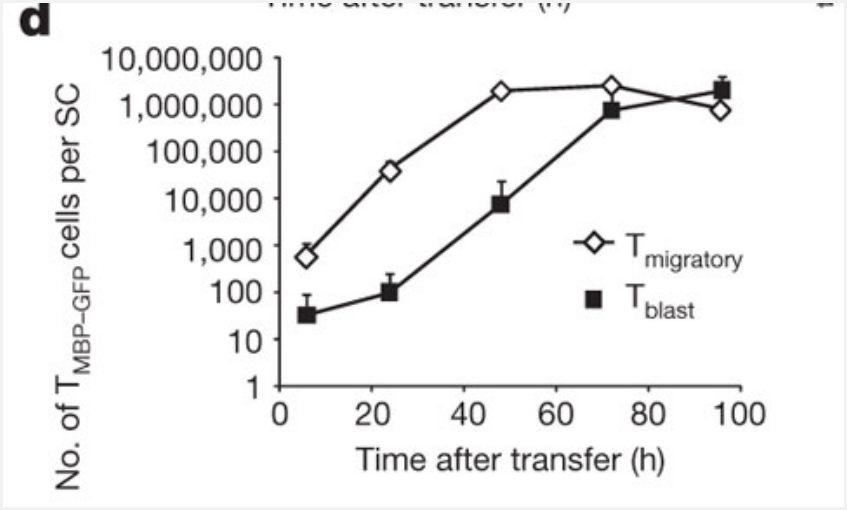

i.t. LPS
24 h
i.v. T blast cells or
i.v. T migratory cells
24 h
T blasts
T migratory
not pretreated
EAE
PBS i.t.
LPS i.t.
#T MBP-GFP cells / g or ml x 10^5
spleen
blood
lung
mening
parenc
T blast cells
T migratory cells

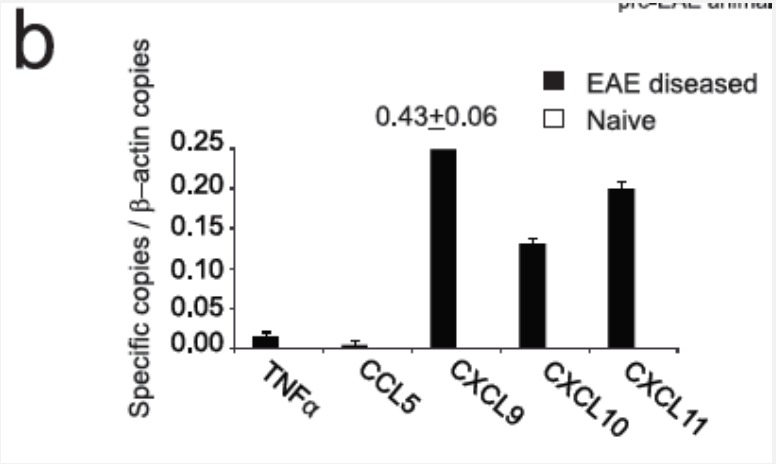
$T_{migratory}$ cells but not T_{blast} cells home efficiently into the CNS.

Method: T migratory cells were isolated form the spleen 60 h after T cell transfer.

Results:

- In contrast to T blast cells, T migratory cells readily immigrated the CNS.
- T migratory cells efficiently invade the CNS of untreated animals.





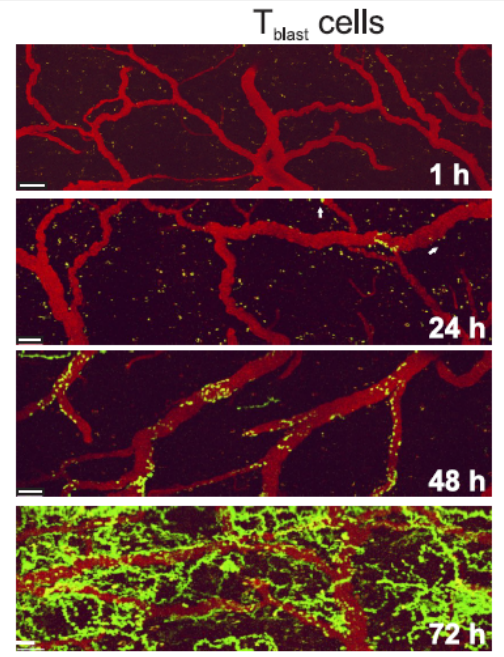
Intravital two photon microscopy

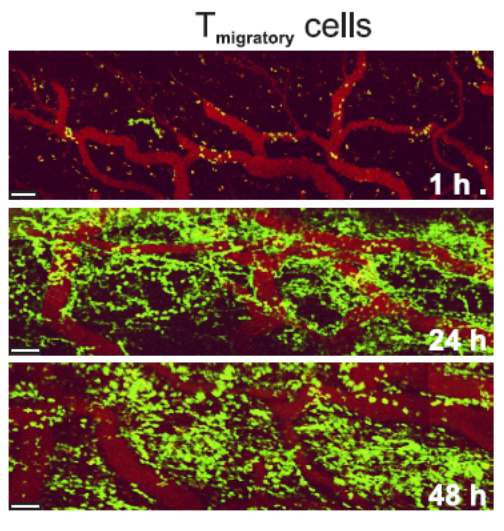
Invasion of the CNS: T_{blast} cells vs. $T_{migratory}$ cells

Method: Time-lapse Intravital two photon microscopy of leptomeninges.

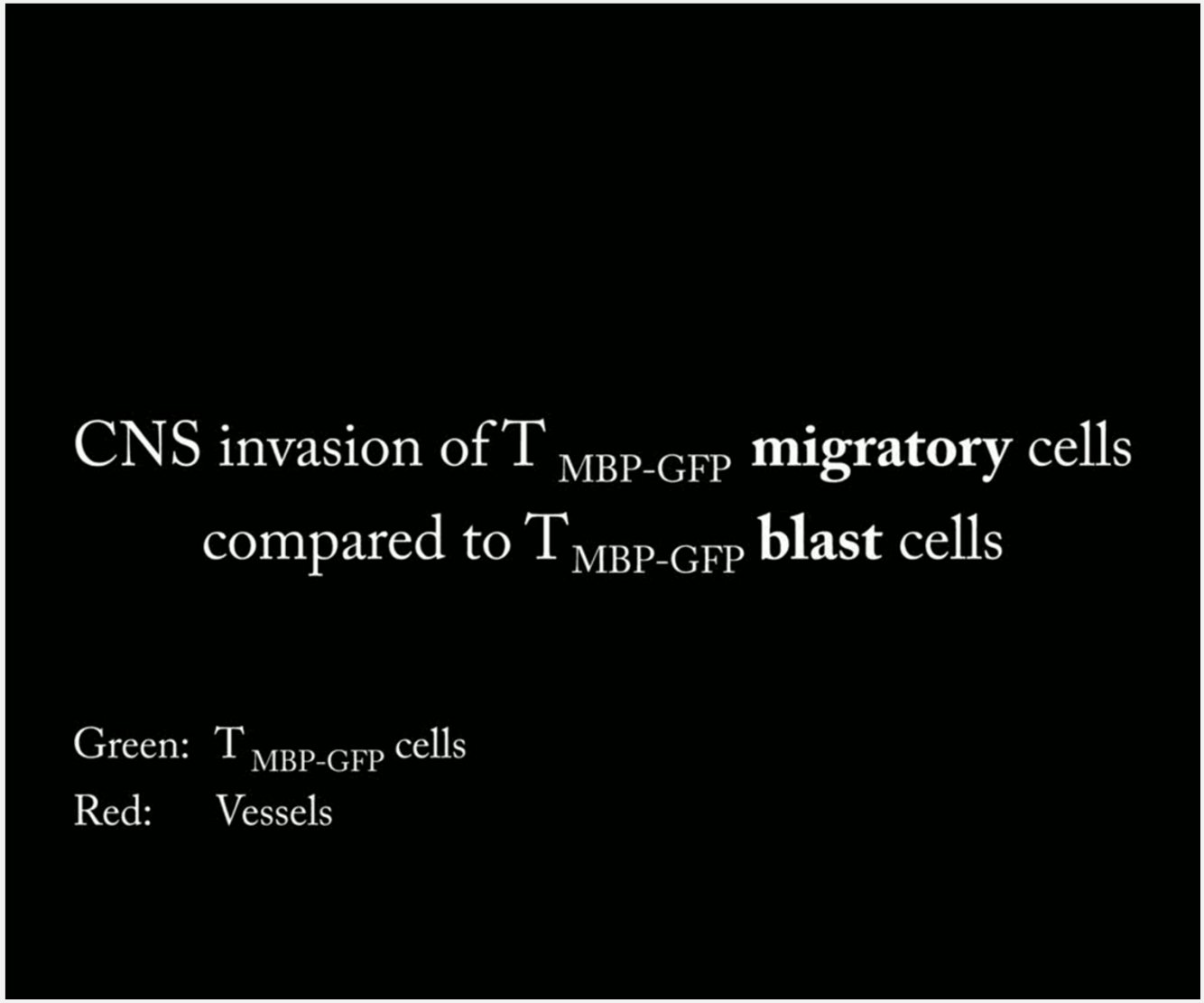
Results:

- Minutes after injection, $T_{migratory}$ cells are crawling on the meningeal vessels.
- At 24 h $T_{migratory}$ cells had transgressed the vessel walls and entered CNS parenchyma comarable with T_{blast} cells after 3 to 4 days.





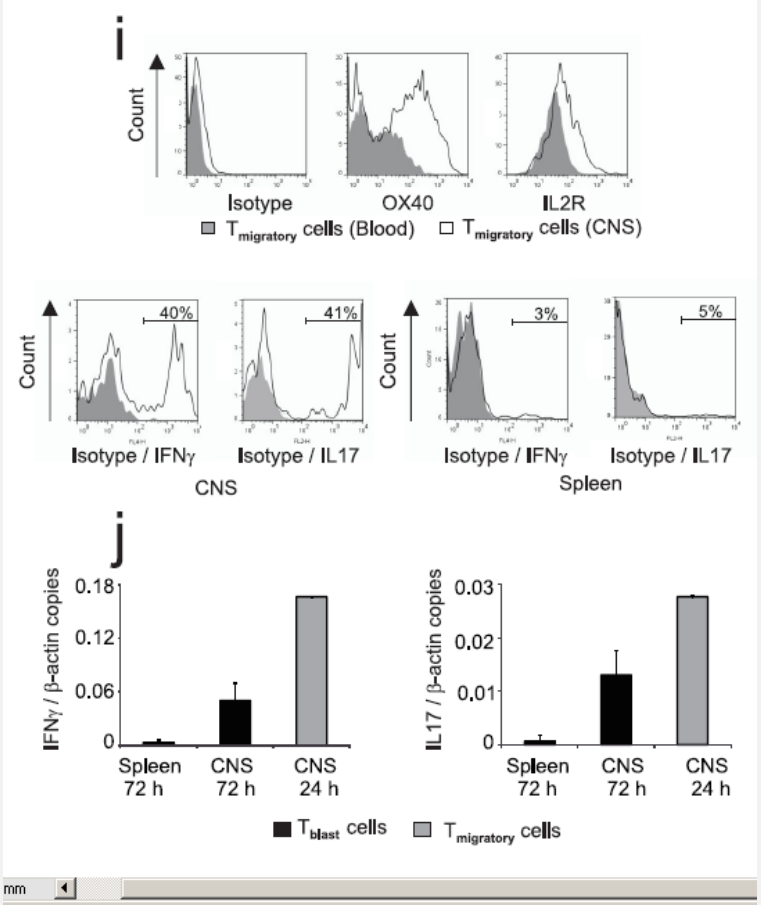
Invasion of the CNS: T_{blast} cells vs. $T_{migratory}$ cells

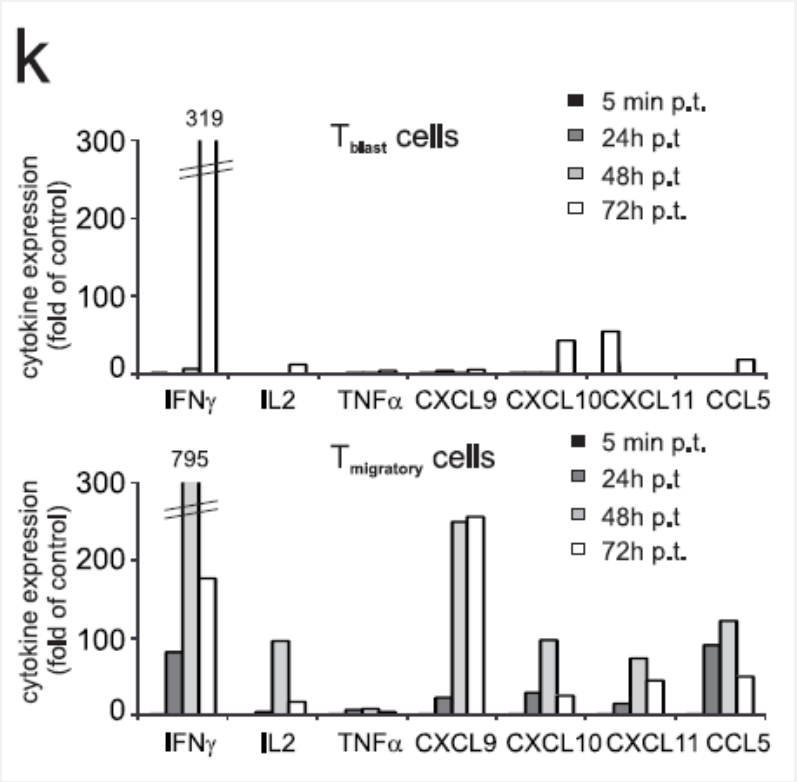


$T_{migratory}$ cells in the CNS upregulate pro-inflammatory cytokines

$T_{migratory}$ cells in the CNS upregulate cytokines indicative of a recent antigen encounter and reactivation.

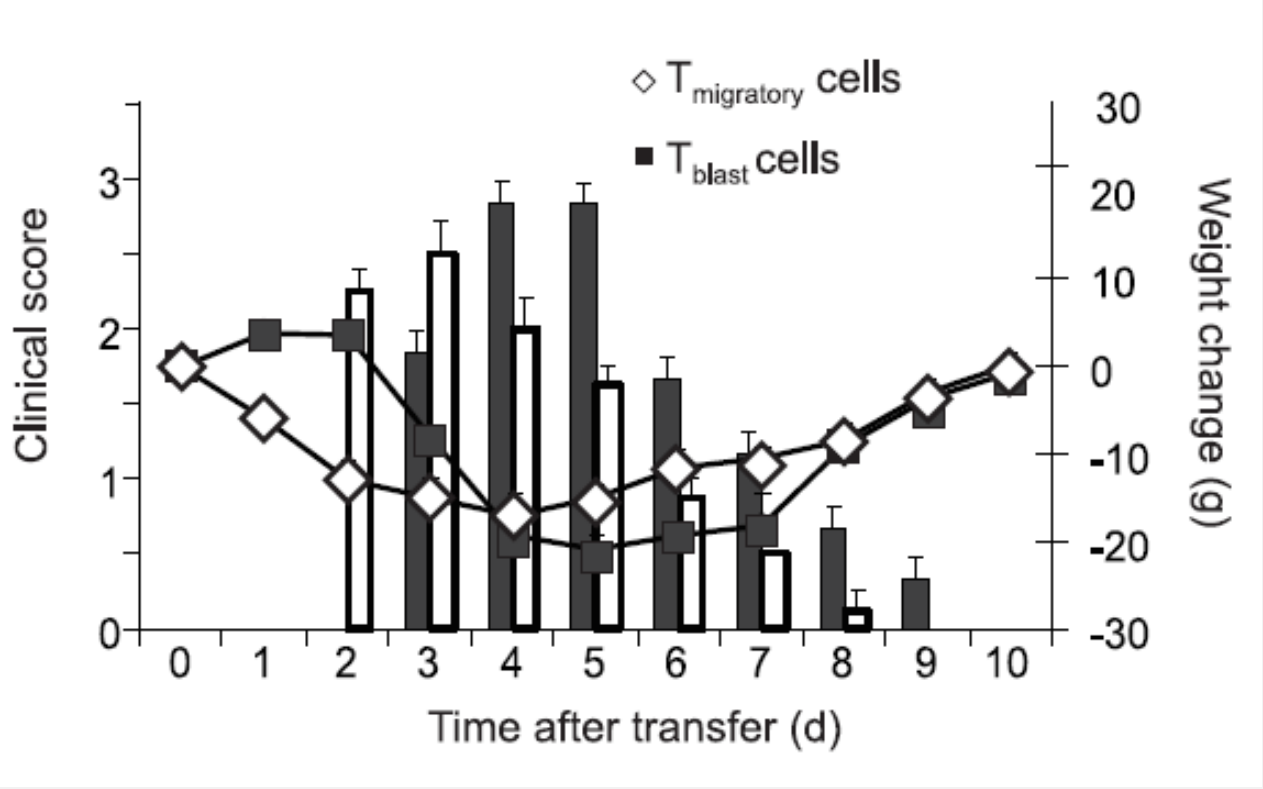
-> generalized parenchymal inflammation and disease





Clinical symptoms appear earlier with $T_{migratory}$ cell transfer

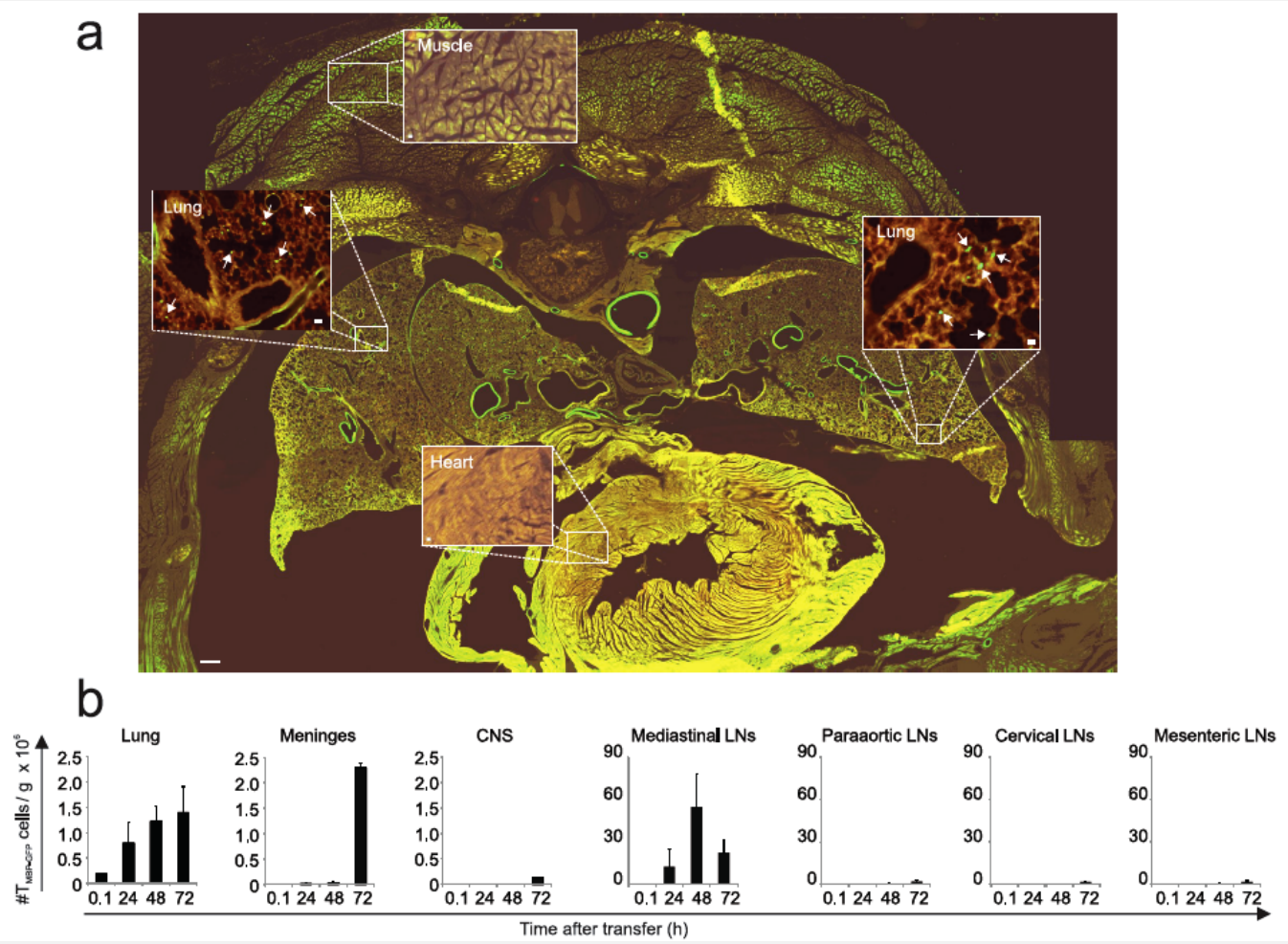
- 24 h after $T_{migratory}$ cell transfer, the animals developed weight loss
- After 48 h they suffer from paralytic disease



The lung is the homing niche for T_{blast} cells

Where is the homing niche of T_{blast} cells after transfer?

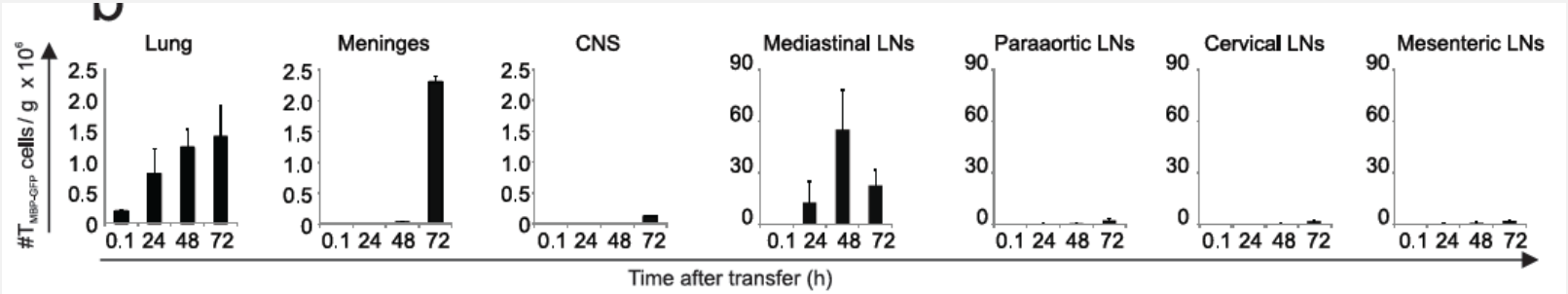
Method: Whole body sections of animals analyzed by cytofluorometric cell quantifications.

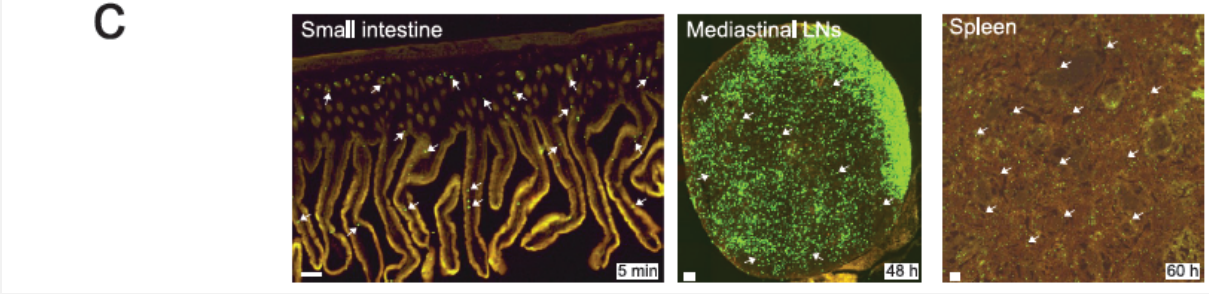


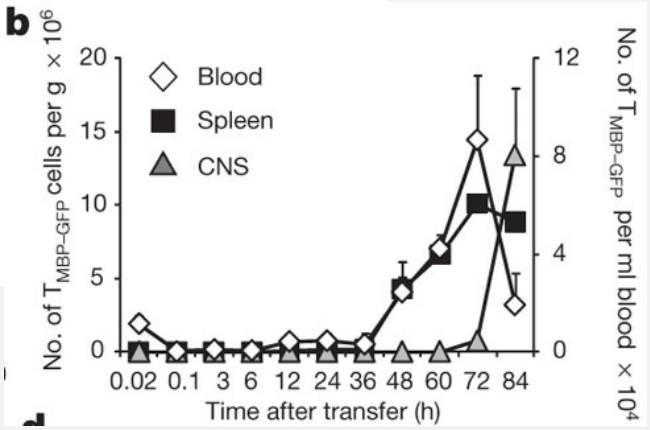
Result: T_{blast} cells first enter the lung.

Lung – mediastinal LN – Blood and Spleen

- At early time points, almost all of the T_{blast} cells were located in the lung.
- Thereafter, $T_{MBP\text{-}GFP}$ cells accumulated in lung-draining mediastinal lymph nodes.
- Then $T_{MBP\text{-}GFP}$ cells appear in blood and spleen.





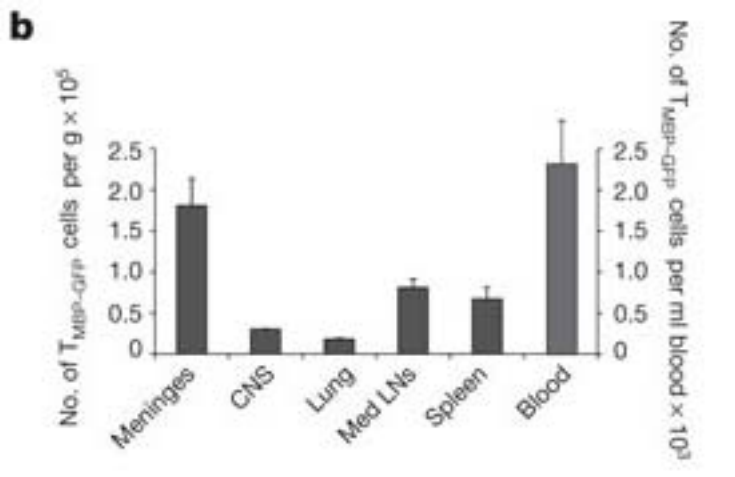


Lung derived $T_{migratory}$ cells efficiently enter and are reactivated in the CNS

Can T cells isolated from lung enter the CNS?

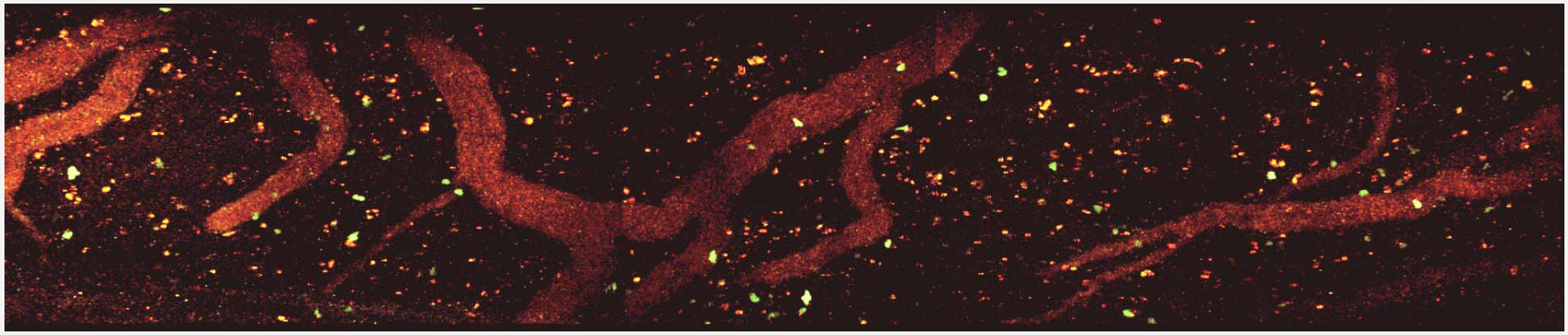
Method: $1x10^6$ $T_{migratory}$ cells isolated from lung 48 h p.t. were i.v. injected into naïve animals. 24h later, $T_{MBP\text{-}GFP}$ cells were quantified by cytofluorometry.

Result: $T_{migratory}$ cells from lung accumulate in meninges.



Conclusion: T cells became competent in the lung to enter and to get reactivated within the CNS.

Intravital two photon microscopy of leptomeninges:

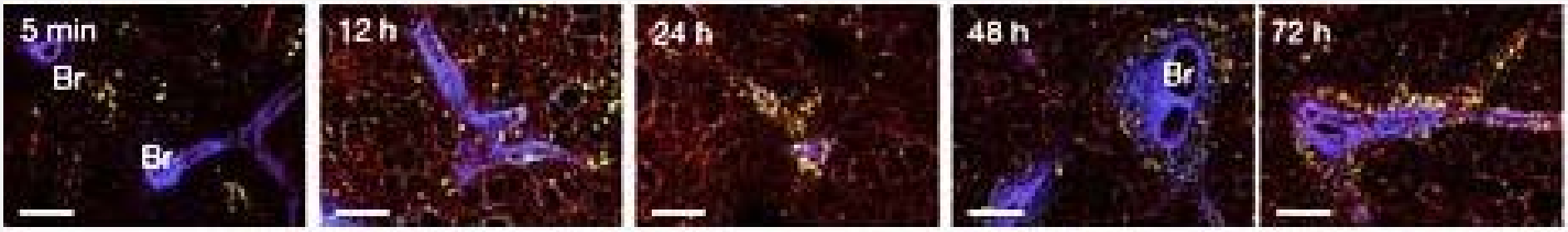


T cell homing within the lung

What happens in the lung?

- Within 12 h after transfer, T cells are distributed in the peripheral lung parenchyma.
- 24-48 h p.t., they move along bronchial structures and accumulate in the bronchus-associated lymphoid tissue BALT.

Two photon microscopy of lung slices, time points after T-cell transfer:

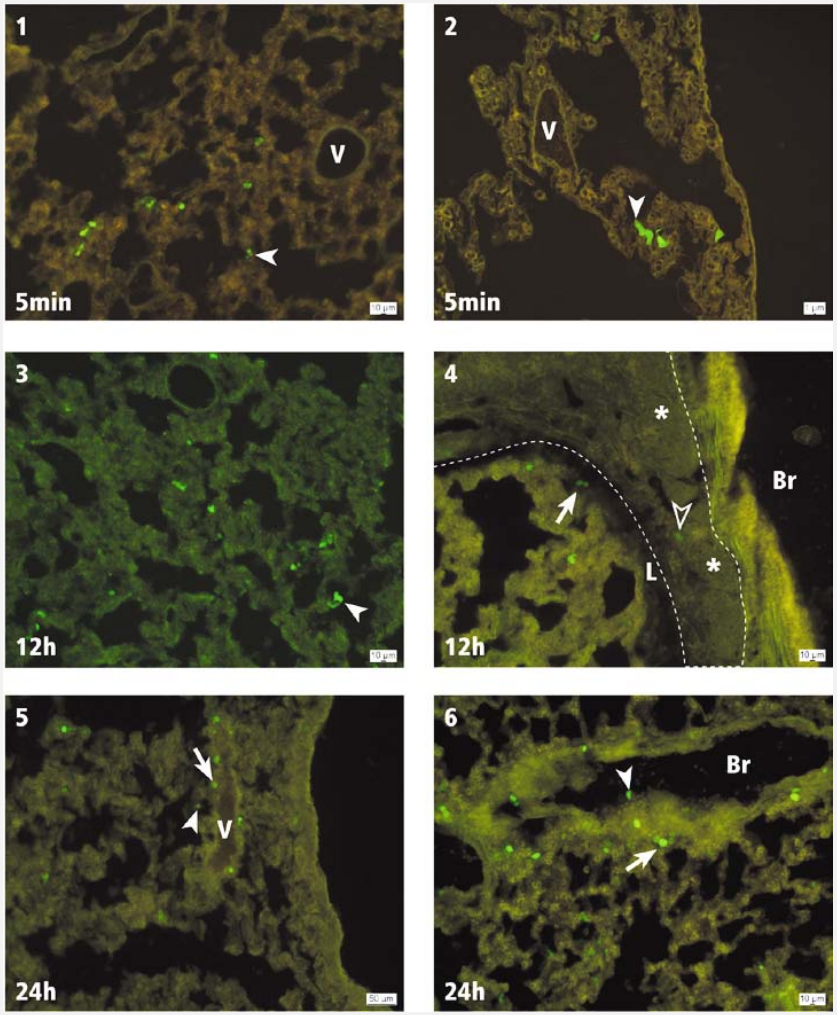


Green: $T_{MBP\text{-}GFP}$ cells
Blue: collagen fibres

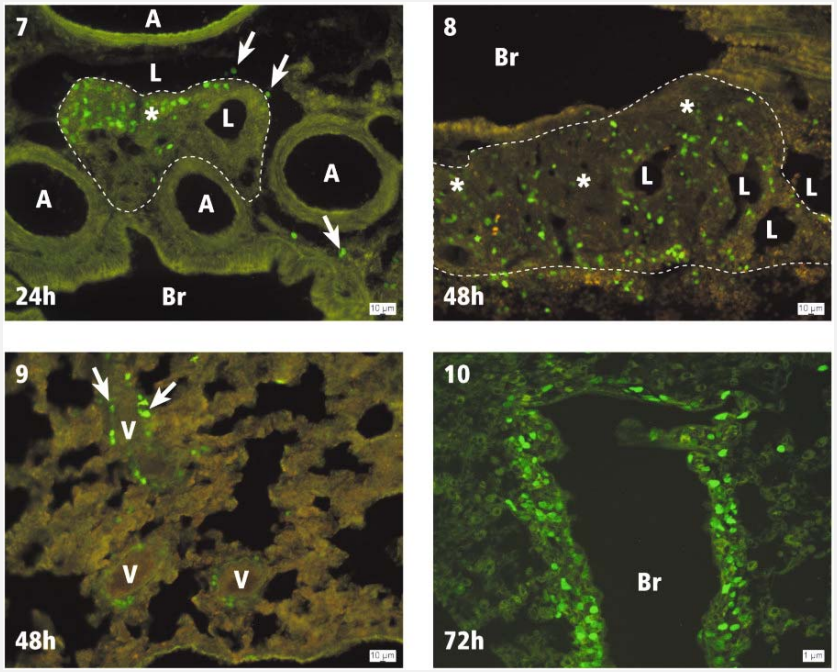
Red: autofluorescence and erythrocytes
Br: bronchial structures

T_{blast} cells invading the lung

Distribution of $T_{MBP\text{-}GFP}$ cells in frozen lung sections

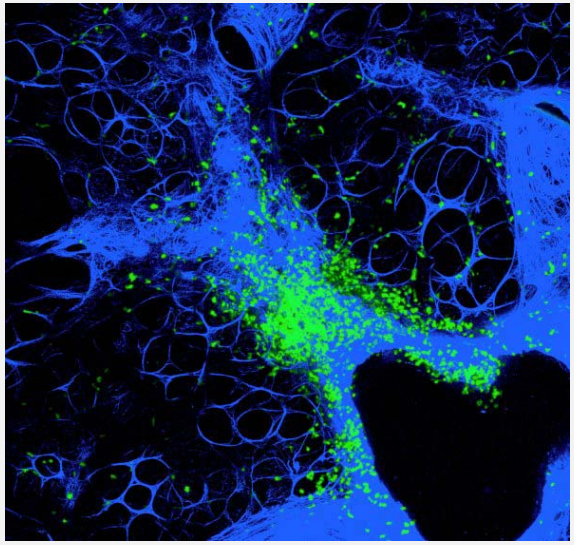


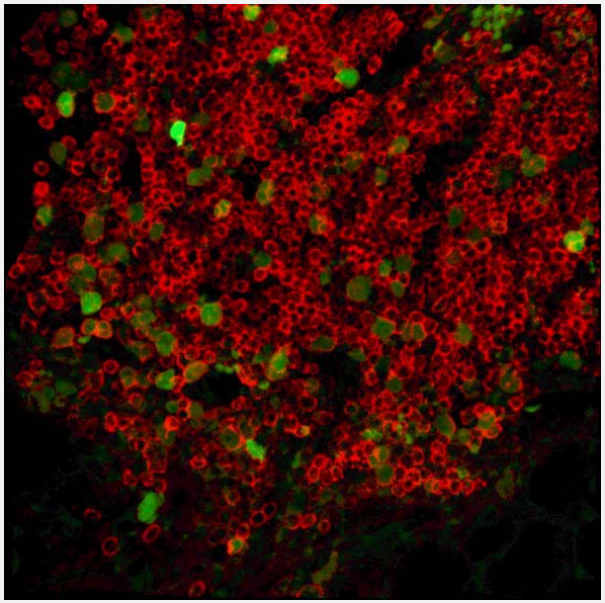
5 min p.t.: $T_{MBP\text{-}GFP}$ cells in alveolar walls
12 h p.t.: in lymphatic vessels
24 hp.t.: in lymphatic vessels around veins and arteries
48-72 h p.t.: accumulation around bronchi.
At all time points, $T_{MBP\text{-}GFP}$ cells are found in the airways



T_{blast} cells invading the lung

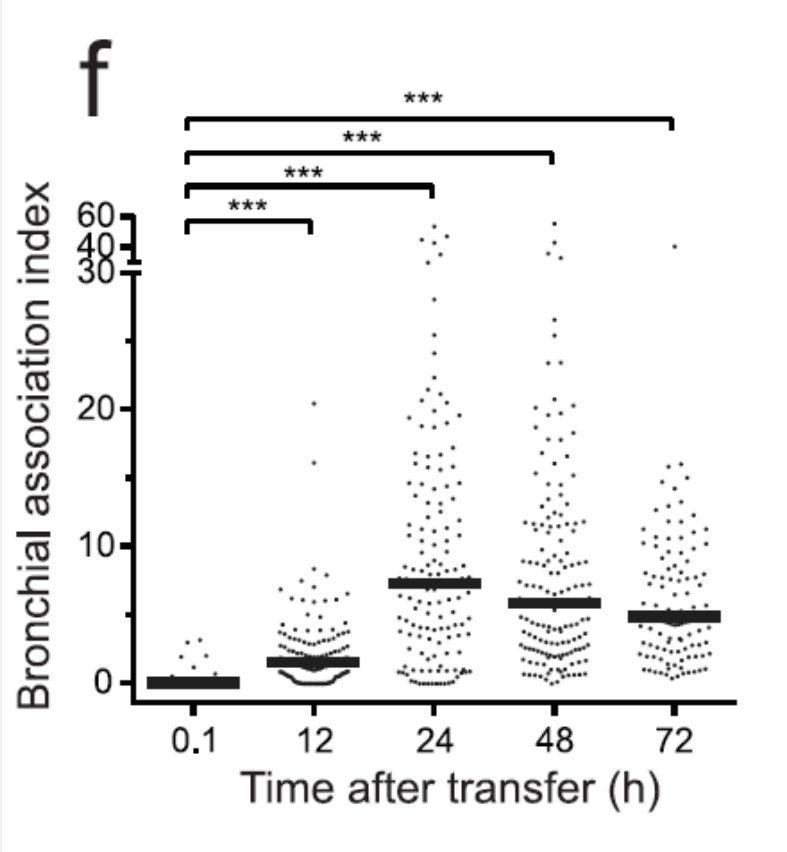
$T_{MBP\text{-}GFP}$ cells accumulate in bronchial areas during preclinical EAE





The bronchial association index

$T_{MBP\text{-}GFP}$ cells accumulate in bronchial areas during preclinical EAE



Location of $T_{MBP\text{-}GFP}$ cells was assessed by densitometric analyses based on 2-PM images of lung slices.

The bronchial association index indicates the ratio of peribronchial towards adjacent-parenchymal mean GFP pixel densities for single bronchi.

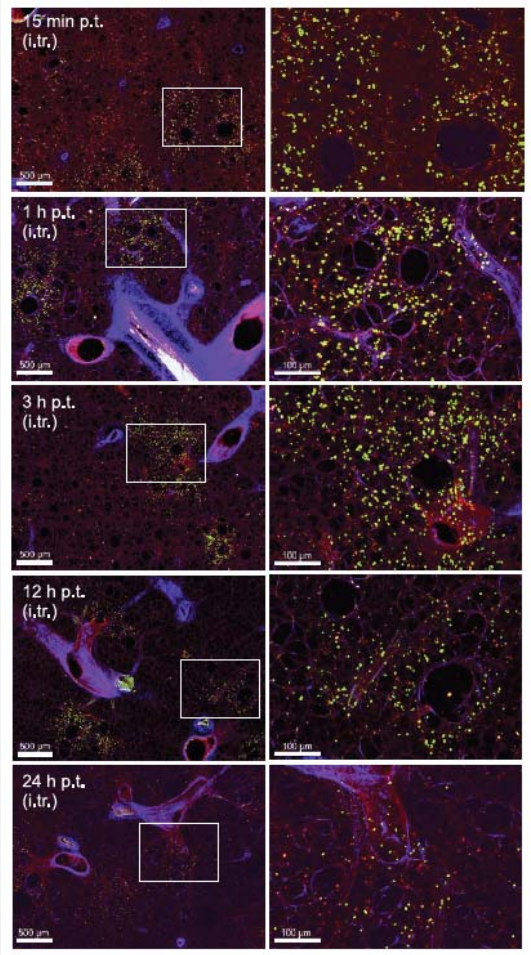
T cell motility within the BALT

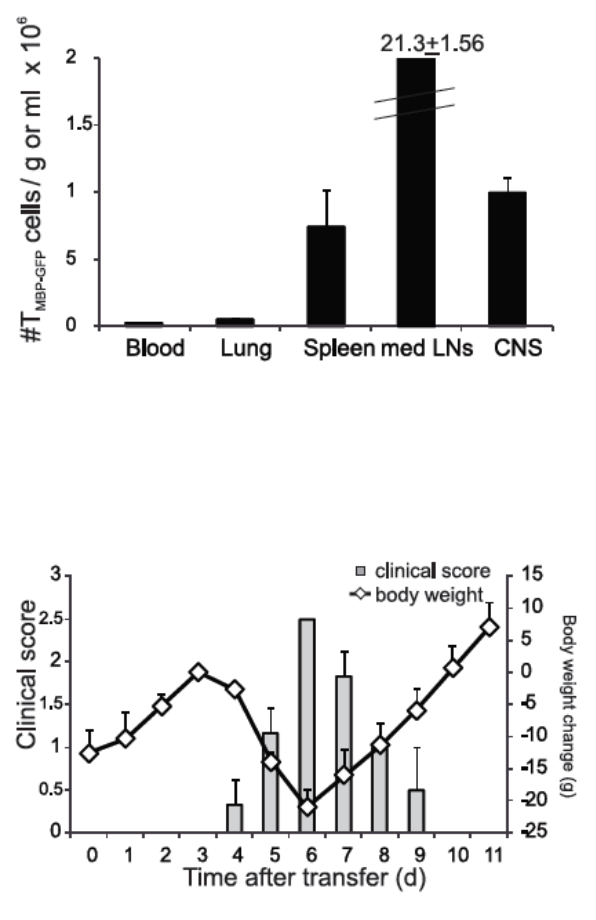
$T_{MBP\text{-}GFP}$ cells use airways as roads

Can T_{blast} cells injected into trachea induce EAE?

Background: T cells are known to accumulate in the lung and airways during lung infections and allergic reactions. The transepithelial migratory path into the airways has been considered to be a «dead end» for the cells.

Question: Can T_{blast} cells injected into trachea induce EAE?



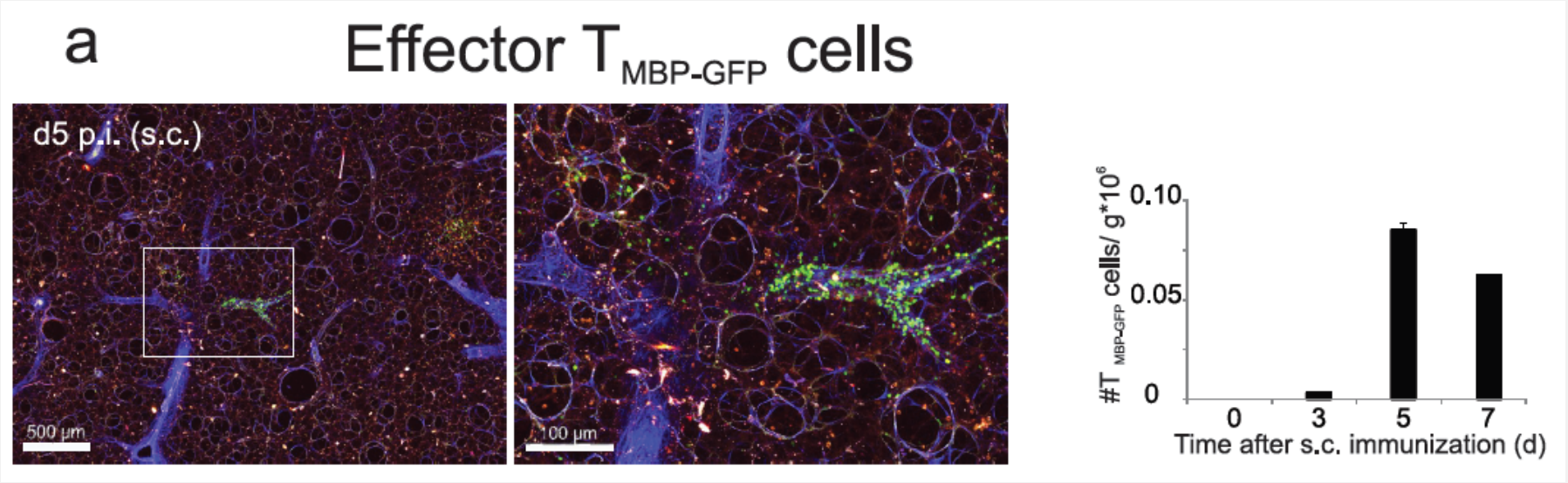


Result: Similarli to i.v. transfer, intratracheally transferred T cells migrated along the airways and induced EAE.

Accumulation of T cells in the lung in classical stimulation

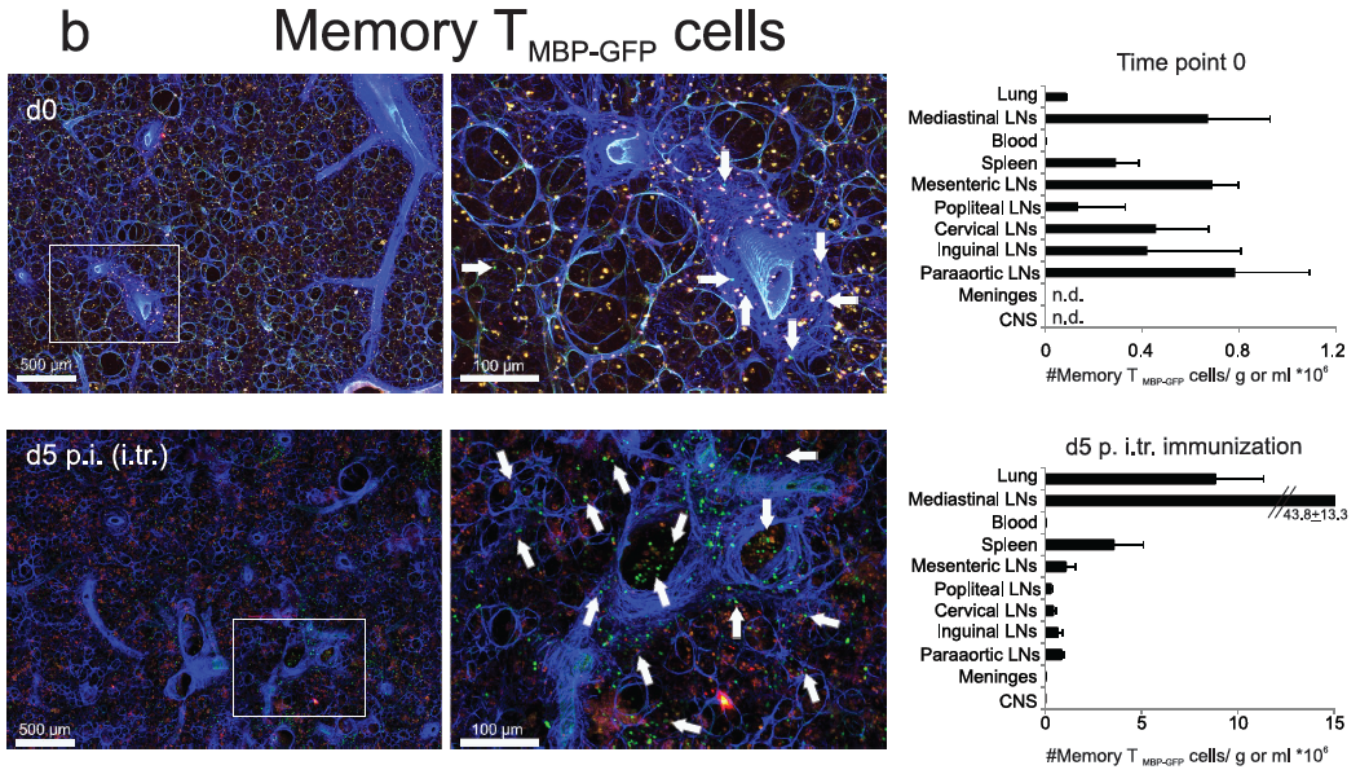
Is the homing of effector T cells to the lung an artifact?

$T_{MBP\text{-}GFP}$ cells also accumulated in the lung after classical antigenic stimulation in peripheral lymph nodes after subcutaneous immunization.

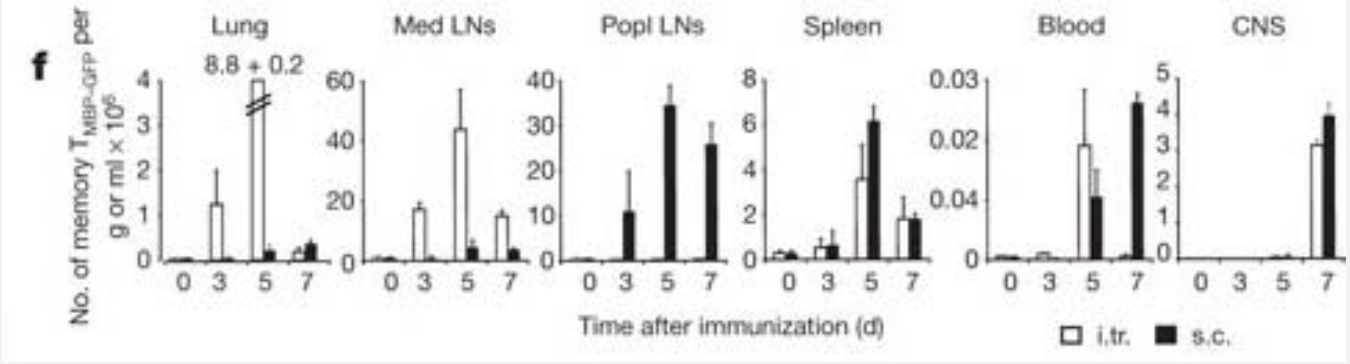


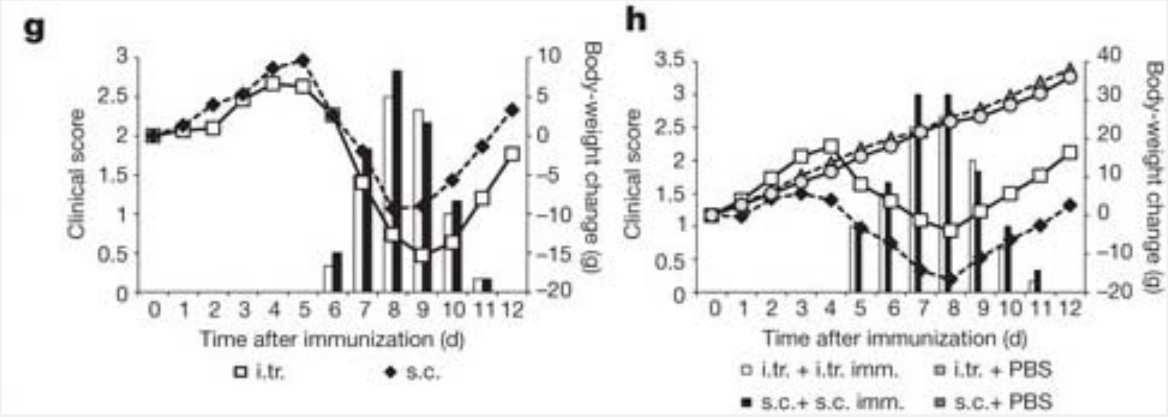
Memory $T_{MBP\text{-}GFP}$ cells persist in the lung

Memory T cells persist in the lung and can readily be activated to become pathogenic after intratracheal immunization with MBP. After amplification within the lung and ist draining lymph nodes, they moved into the blood and spleen and finally the CNS, where they triggered a paralytic disease.



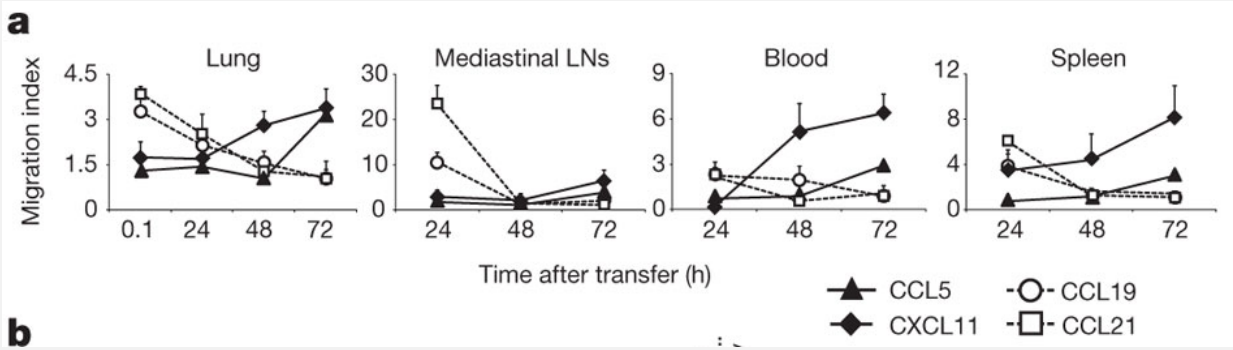
Comparison of i.tr. vs. s.c. immunization



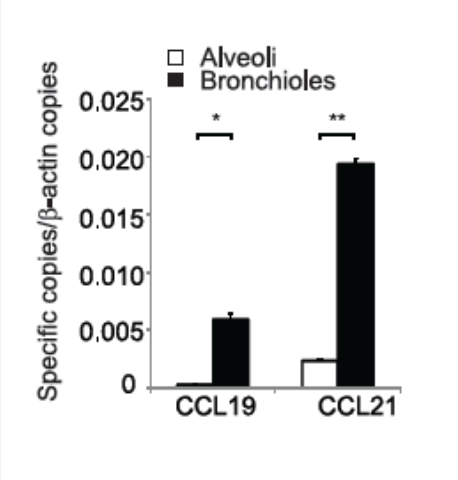


Switch of T_{blast} cells into a migratory mode.

- $T_{MBP\text{-}GFP}$ cells predominantly migrated towards the homeostatic chemokines CCL19 and CCL21
- From 48 h after transfer onwards $T_{MBP\text{-}GFP}$ cells responded mainly to gradients of the inflammatory chemokines CXCL11 and CCL5



This migration pattern fits to the expression of bronchial structures and BALT.



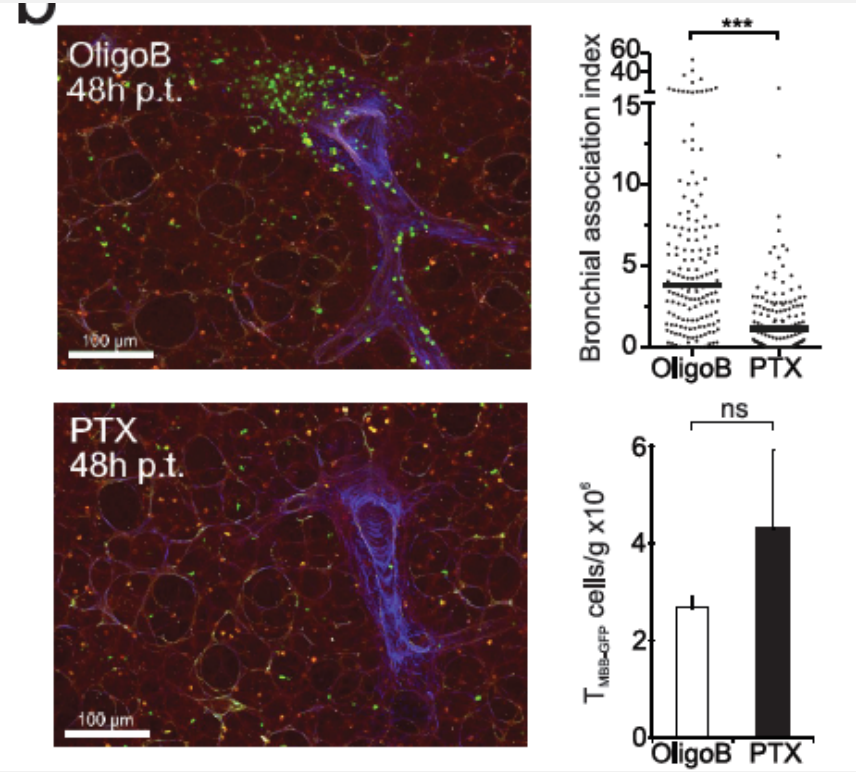
Effect of PTX on localization of $T_{MBP\text{-}GFP}$ cells

Method: Pertussis toxin (PTX) irreversibly blocks chemokine signaling.

T_{blast} cells were pretreated with PTX or Oligomer B of PTX.

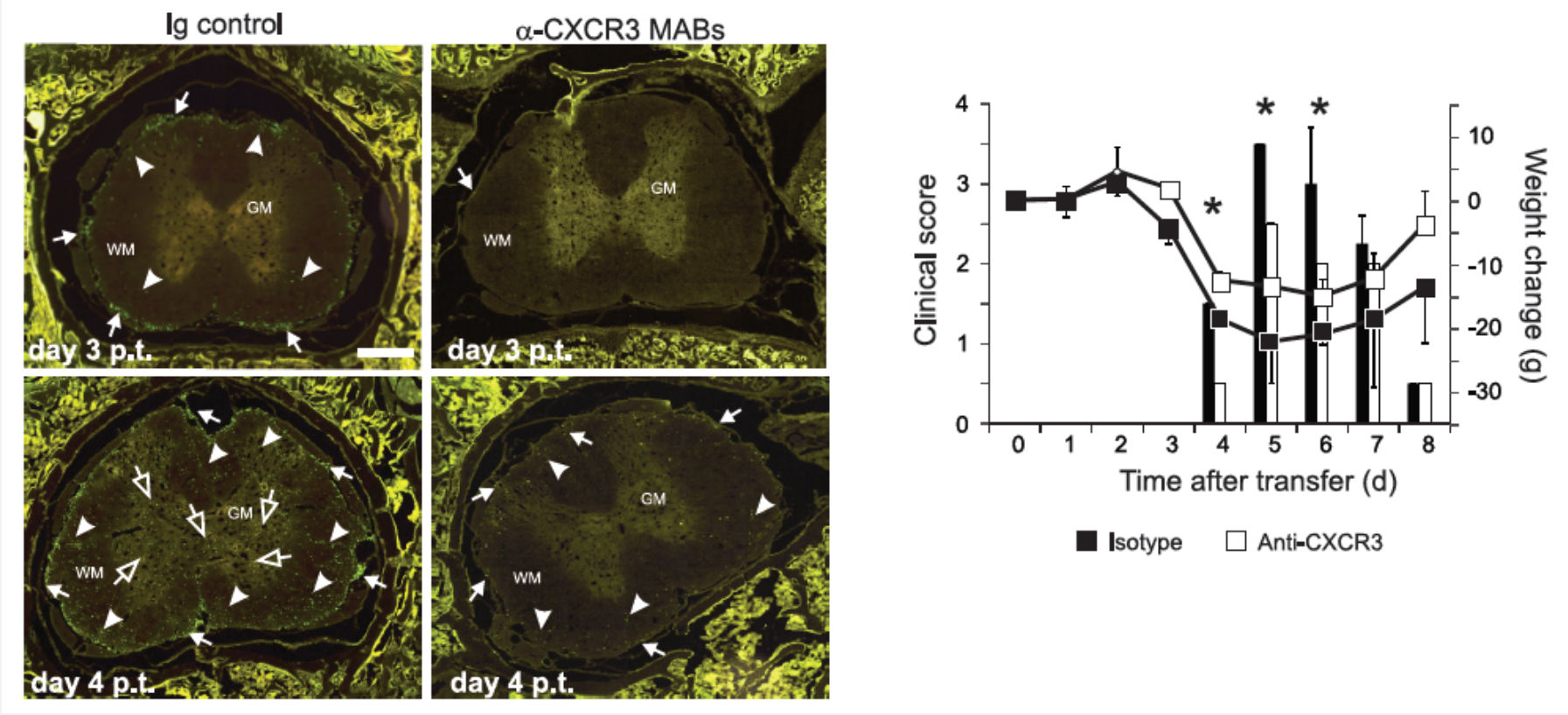
Result: PTX can reduce bronchial accumulation of $T_{MBP\text{-}GFP}$ cells. But PTX did not interfere with the homing of T cells to the lung.

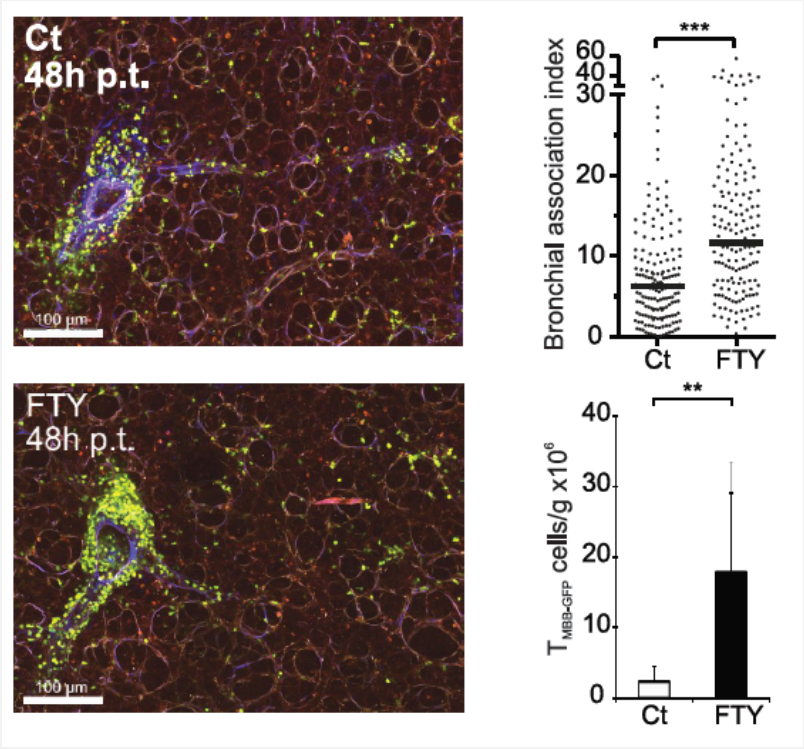
Conclusion: T-cell migration to bronchi is driven by CCL19 and CCL21.



Blocking CXCL11 signaling

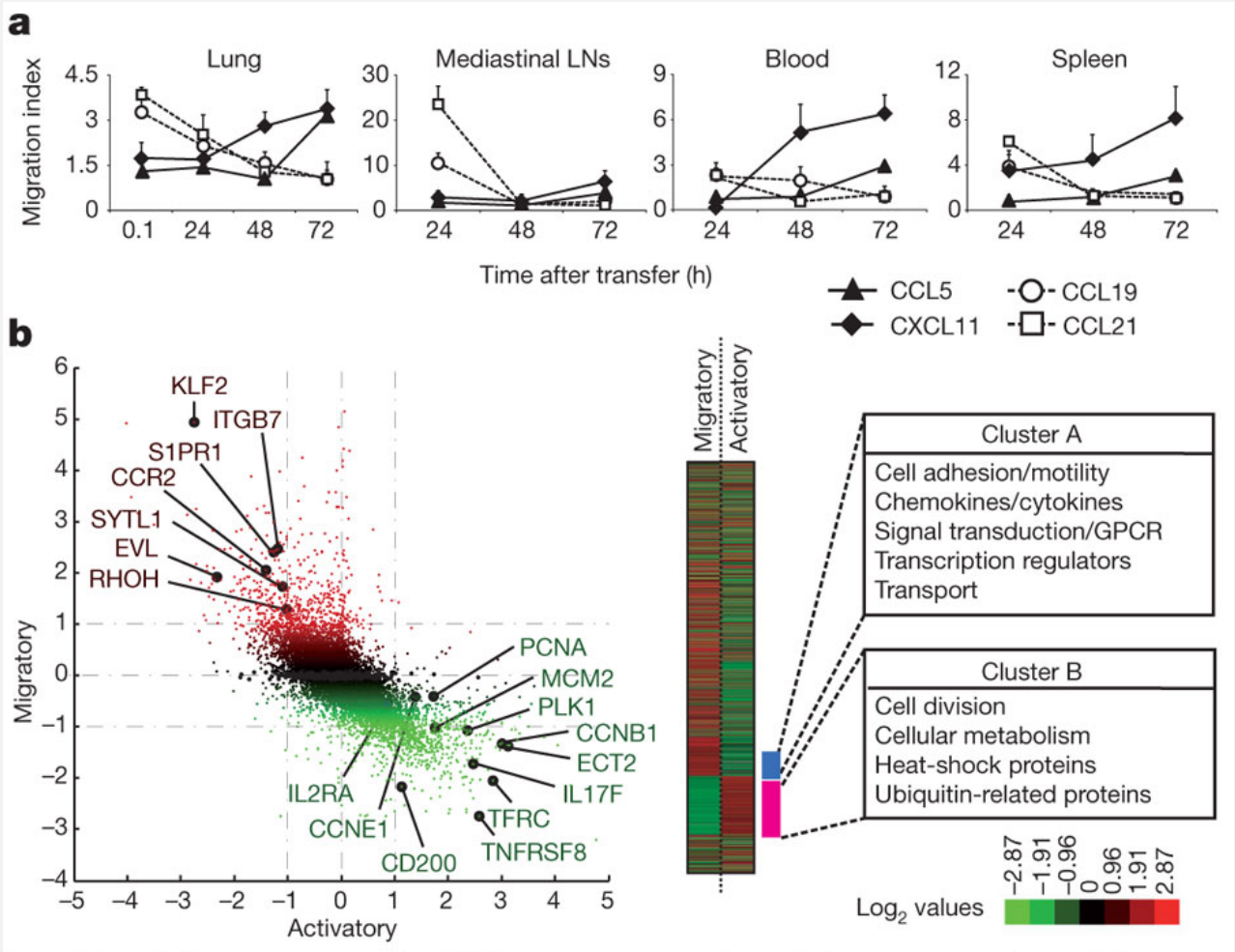
CXCR3 is the receptor of CXCL11. Anti-CXCR3 antibody treatment reduced $T_{MBP\text{-}GFP}$ cell invasion into CNS tissue and ameliorates clinical EAE.




Ct
48h p.t.
100 µm
FTY
48h p.t.
100 µm
***
Bronchial association index
60
40
30
20
10
0
Ct
FTY
**
$T_{MBB-GFP}$ cells/g x10^6
40
30
20
10
0
Ct
FTY

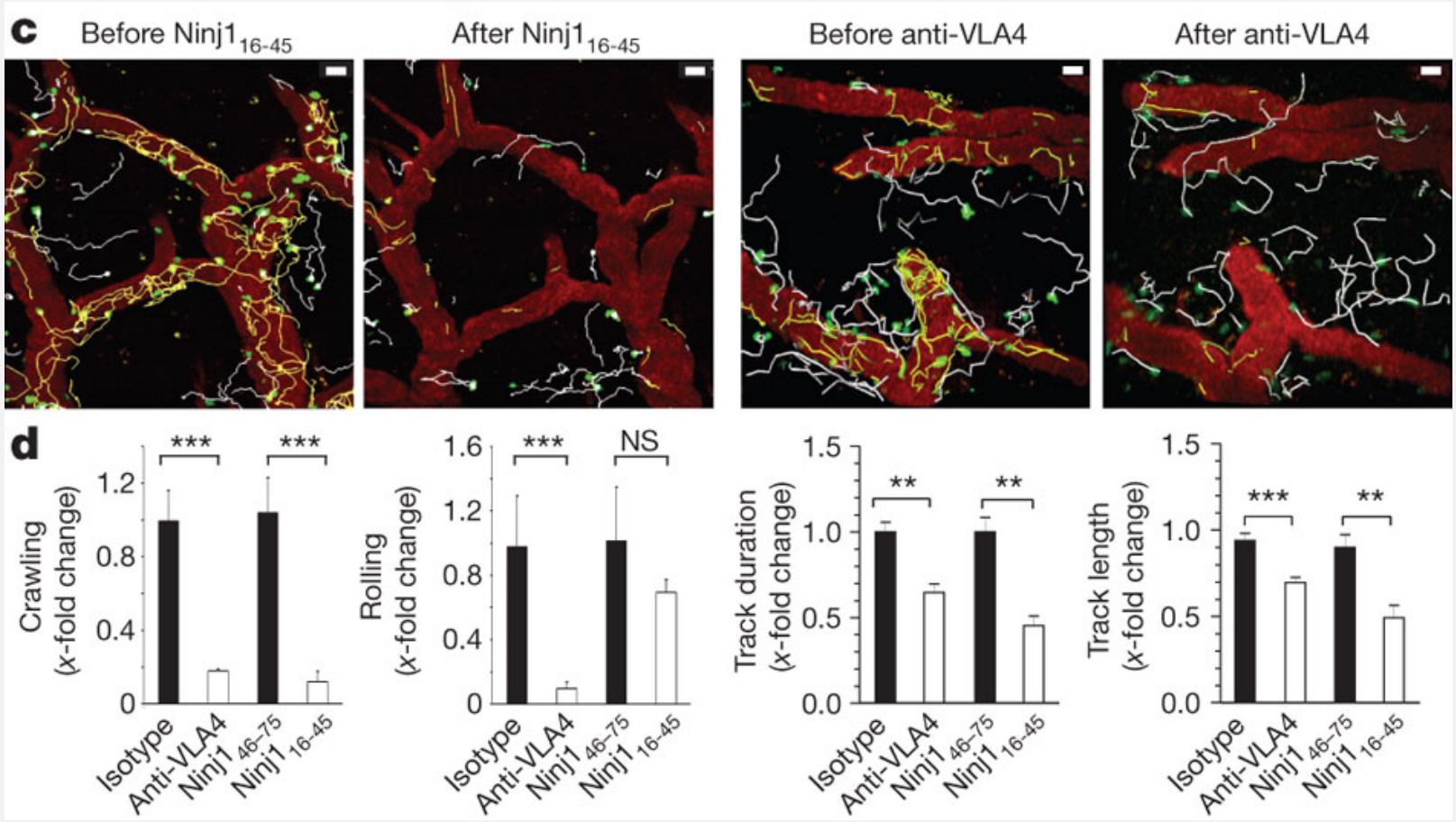
Switch of T_{blast} cells into a migratory mode.

Tmigratory cells but not Tblast cells home efficiently into the CNS:



Switch of T_{blast} cells into a migratory mode.

Tmigratory cells but not Tblast cells home efficiently into the CNS:



Homing into the lung is not limited to encephalitogenic T cells.

Tmigratory cells but not Tblast cells home efficiently into the CNS: