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# Regulation of acquired immune responses during infection with intracellular bacteria

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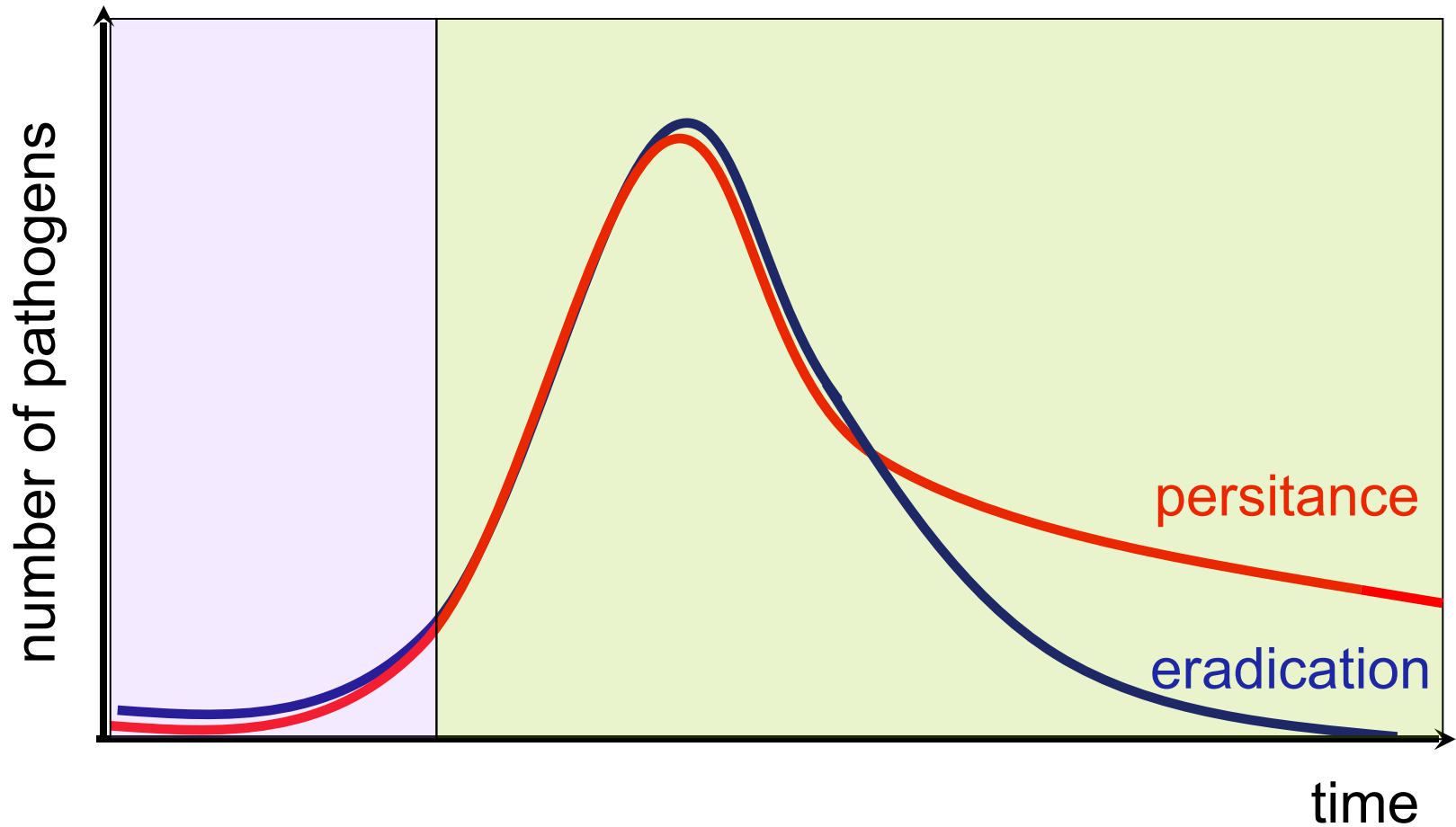
## Research areas of interest

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- Regulation of T cell responses
  - activation and differentiation of T cells
  - T cell differentiation and responses in lymphoid and non-lymphoid tissues (liver, intestine, kidney)
- Immune responses in autoimmune kidney diseases (experimental autoimmune glomerulonephritis)
- Immune responses against intracellular bacteria
  - *Listeria monocytogenes*
  - *Salmonella typhimurium* (infection model for *S. typhi*)

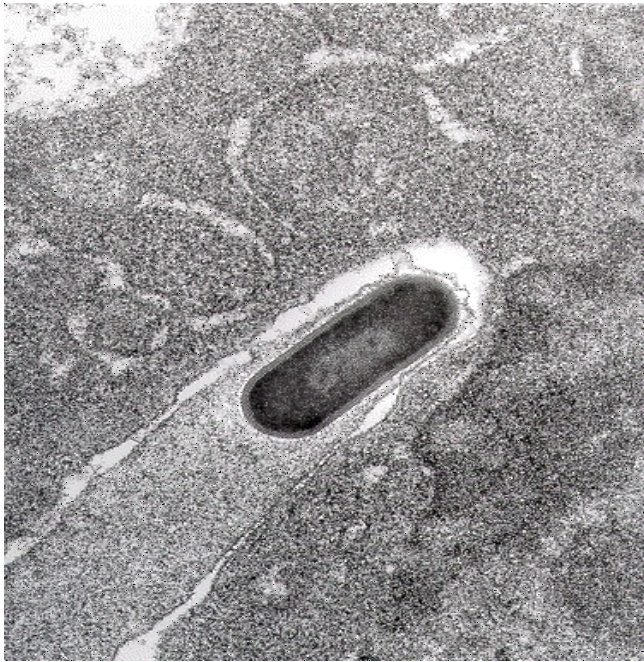


# Time course of infection



# *Listeria monocytogenes*

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- **cause of listeriosis**
- **infection usually arises from uptake of contaminated food**
- **target organs after leaving the intestine are spleen and liver**
- **facultative intracellular bacterium (macrophages and hepatocytes)**
- **induces strong CD4<sup>+</sup> Th<sub>1</sub> and CD8<sup>+</sup> T cell responses**

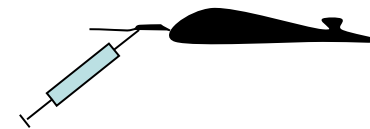
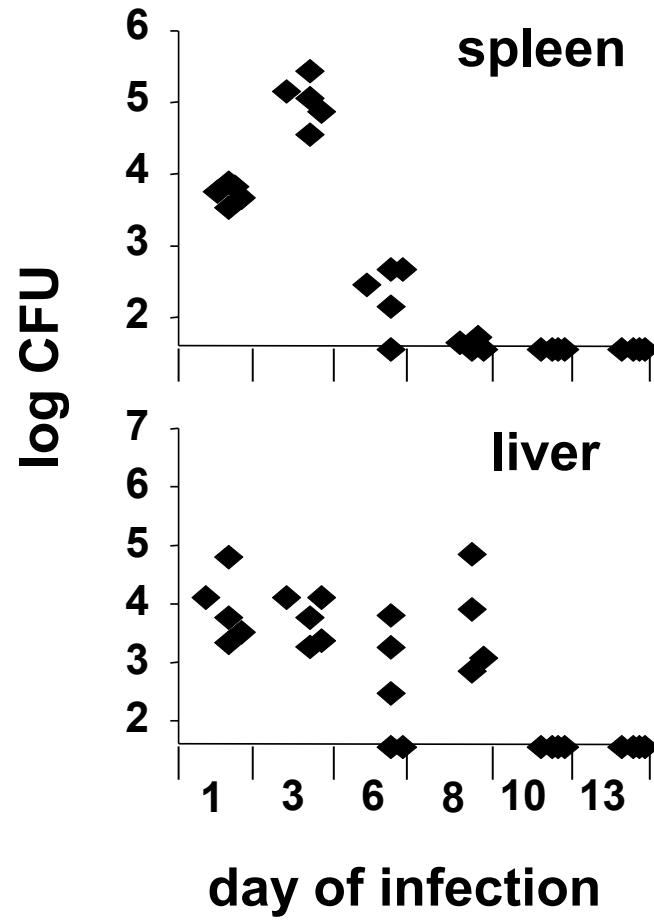


**Killer-Käse aus Österreich**

# Dungkäfer übertragen die Todes-Bakterien

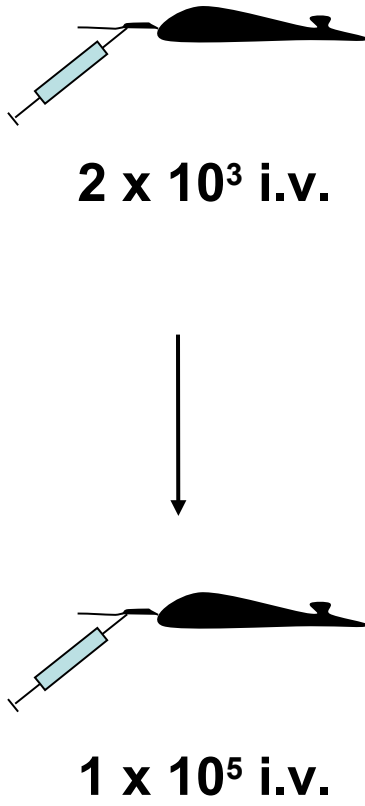
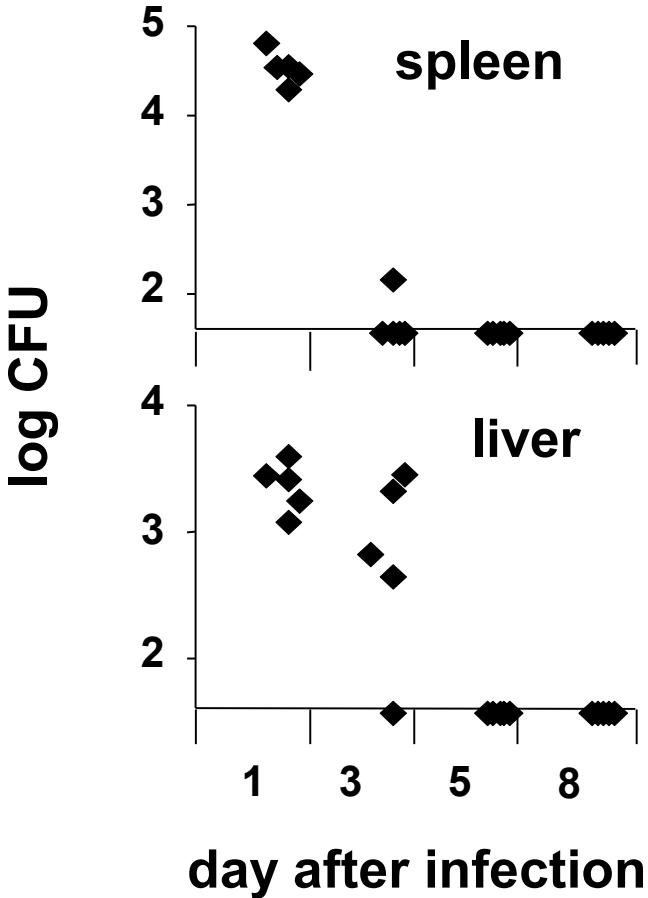


# Listeria titers after primary infection

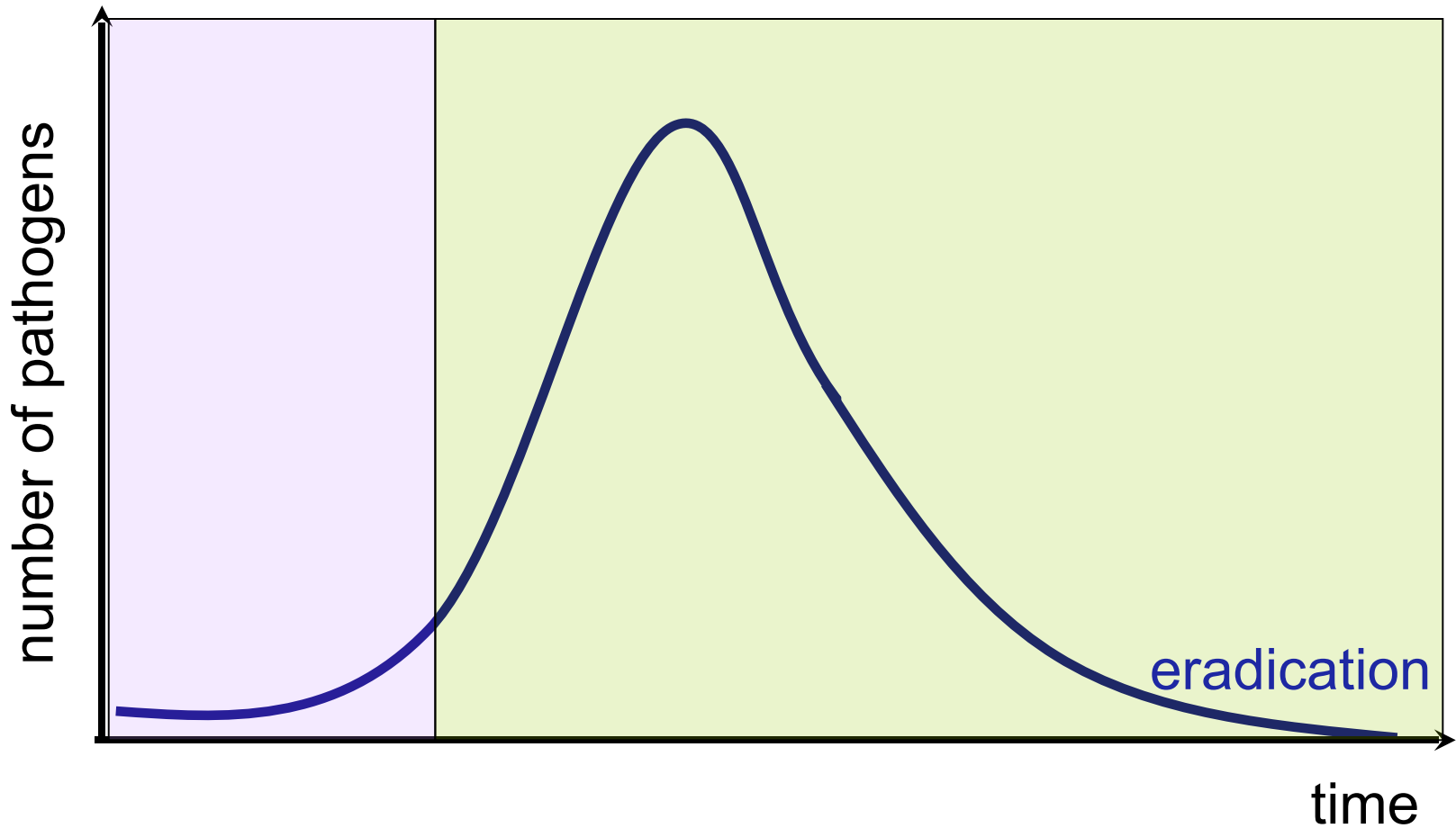


$2 \times 10^3$  i.v.

# Listeria titers after secondary infection

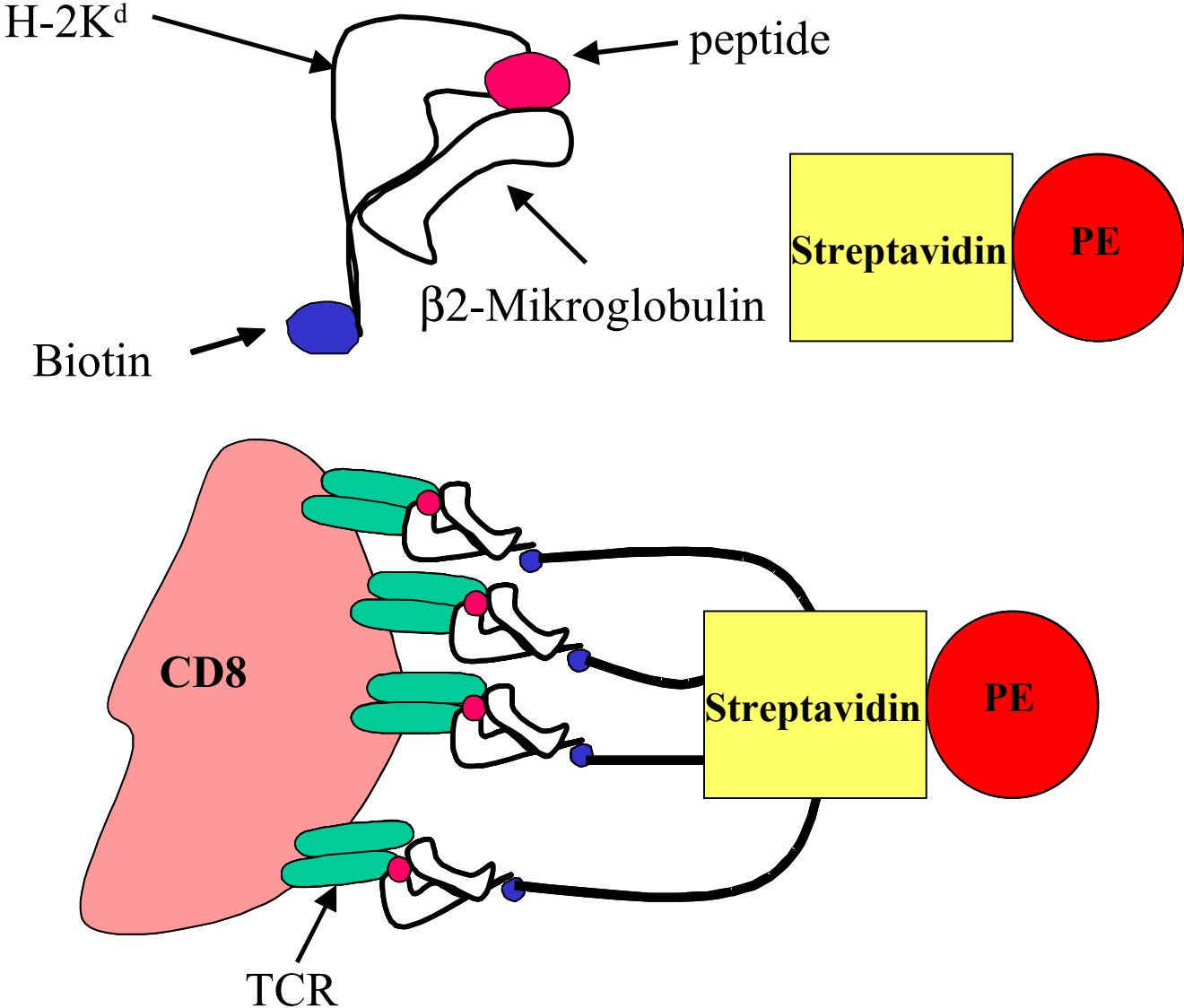


# Time course of infection

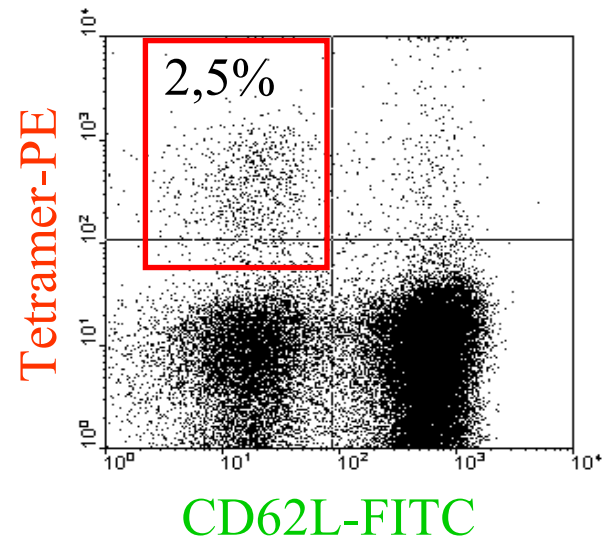
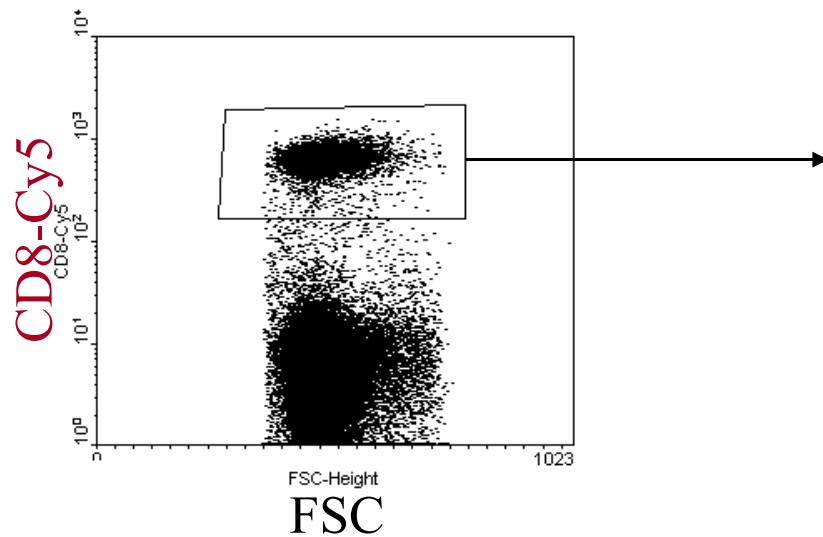


-> Magnitude and time course of T cell responses?

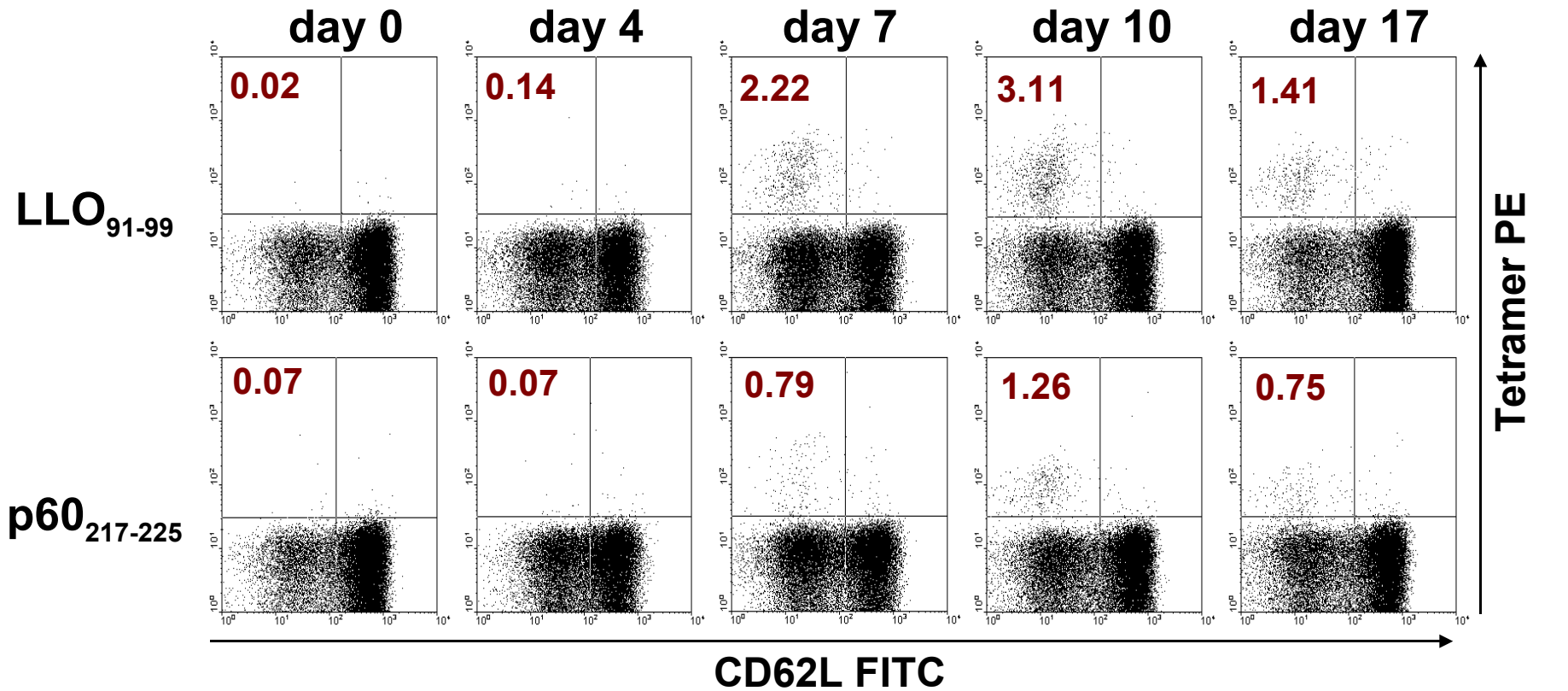
# MHC class I tetramer



# MHC class I-Tetramer example



# LLO<sub>91-99</sub> and p60<sub>217-225</sub>-specific CD8<sup>+</sup> T cell responses during a primary *L. monocytogenes*-infection

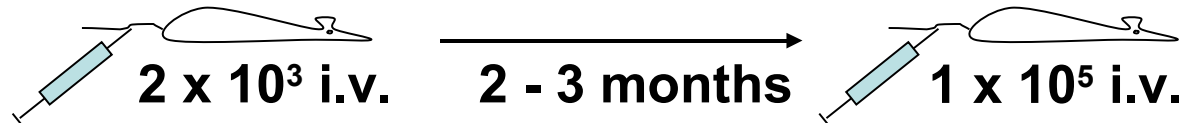
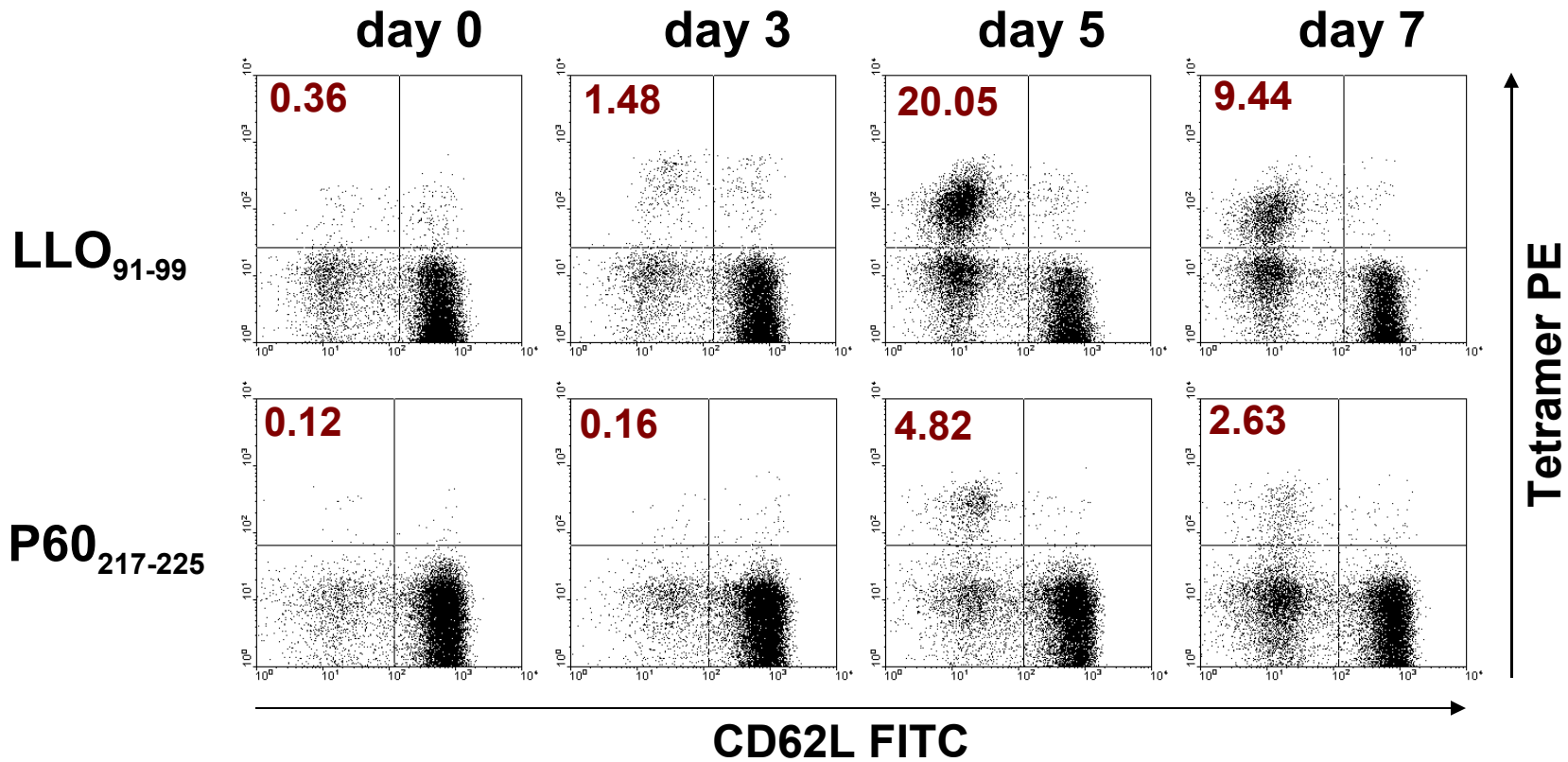


2 x 10<sup>3</sup> i.v.



CD8 gated cells

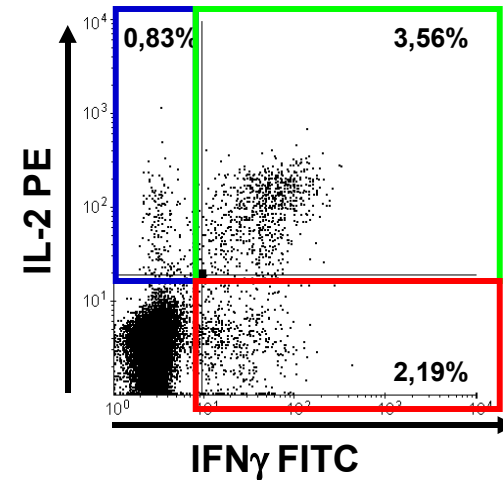
# LLO<sub>91-99</sub> and p60<sub>217-225</sub>-specific CD8<sup>+</sup> T cell responses during a secondary *L. monocytogenes*-infection



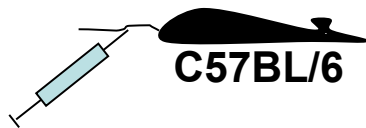
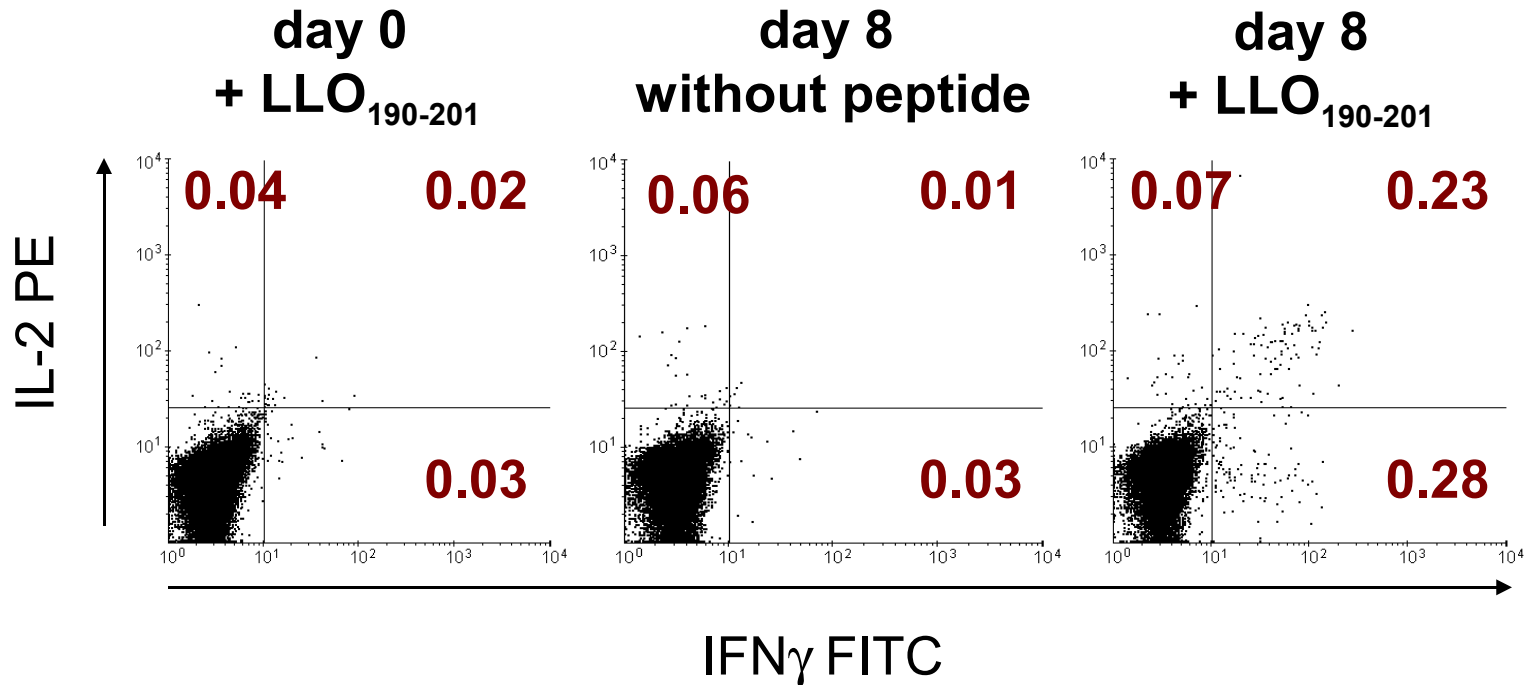
# Detection methods for listeria-specific CD4<sup>+</sup> T cells

## intracellular cytokine staining

- listeriolysin O (LLO<sub>190-201</sub>)



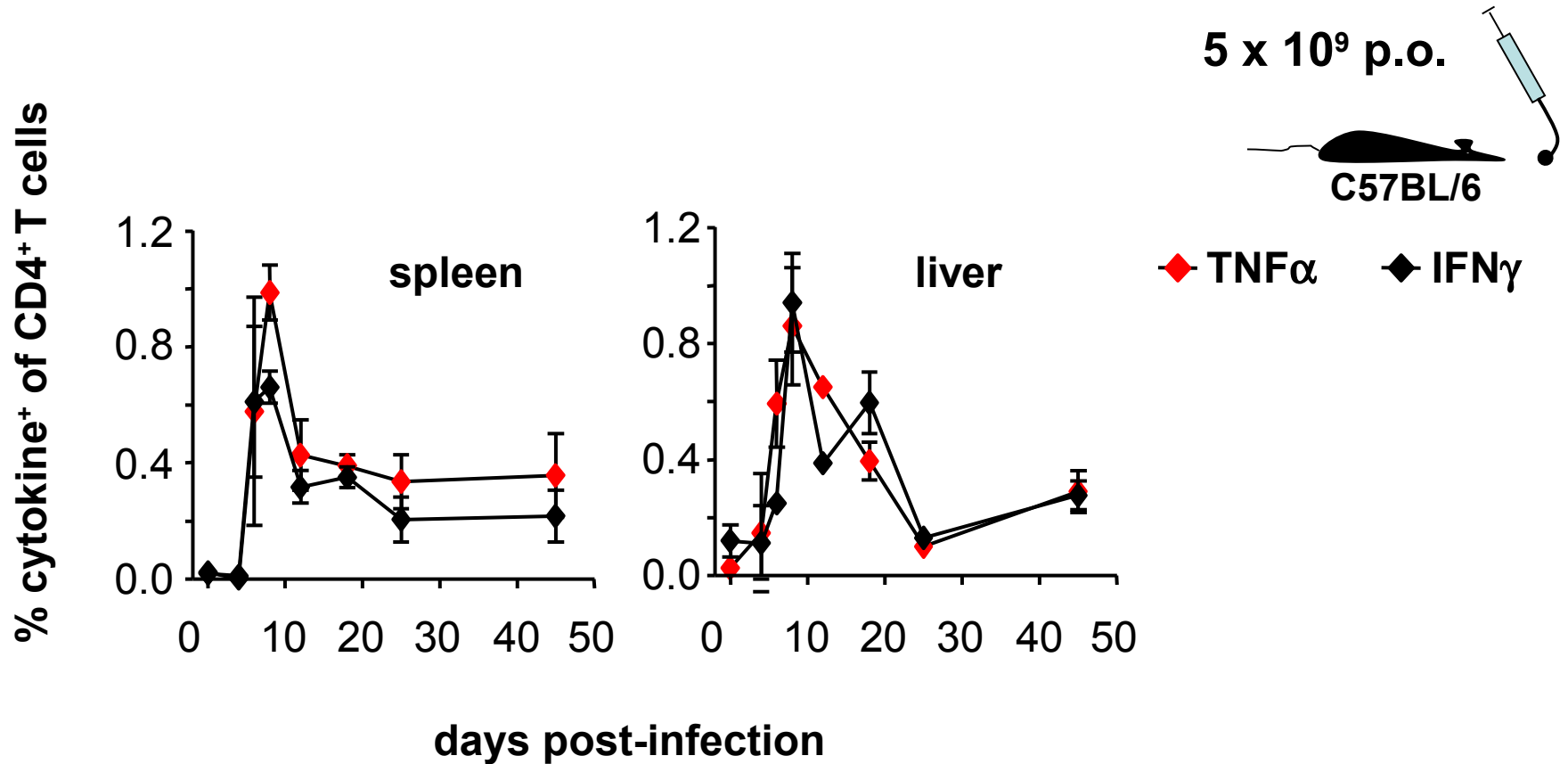
# IL-2 and IFN $\gamma$ production of CD4<sup>+</sup> T cells after peptide restimulation *in vitro*



***Lm* 5 x 10<sup>3</sup> i.v.**

gated on CD4<sup>+</sup> T cells

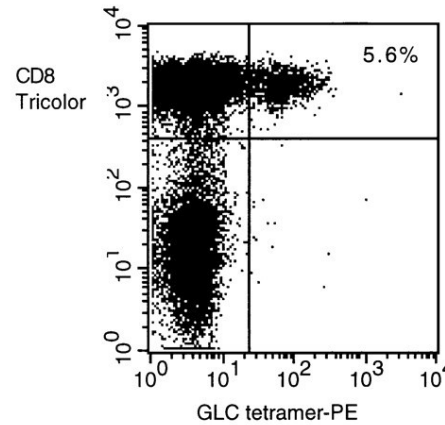
# LLO<sub>190-201</sub>-specific CD4<sup>+</sup> T cell reponses after oral *L. monocytogenes*-infection



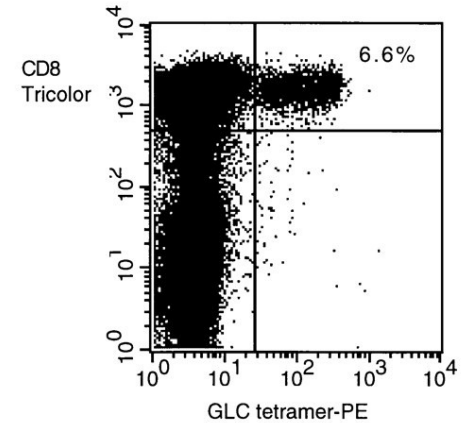
# Acute infectious mononucleosis (Epstein-Barr-Virus)

a HLA A2/GLCTLVAML tetramer staining

Donor 74

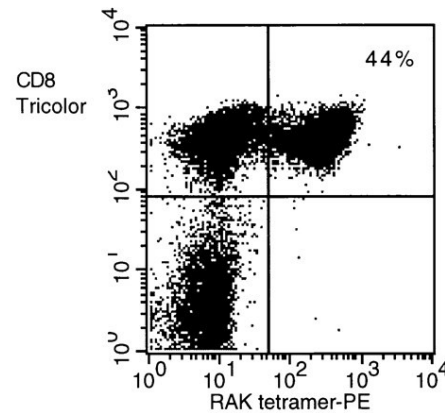


Donor 83

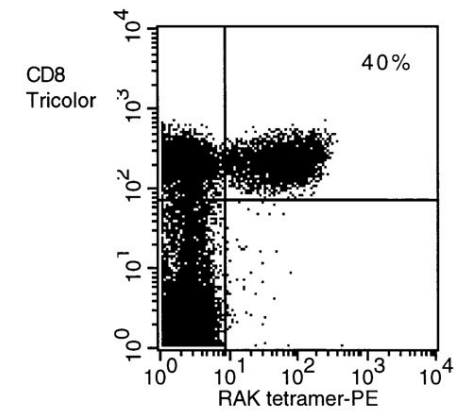


b HLA B8/RAKFKQLL tetramer staining

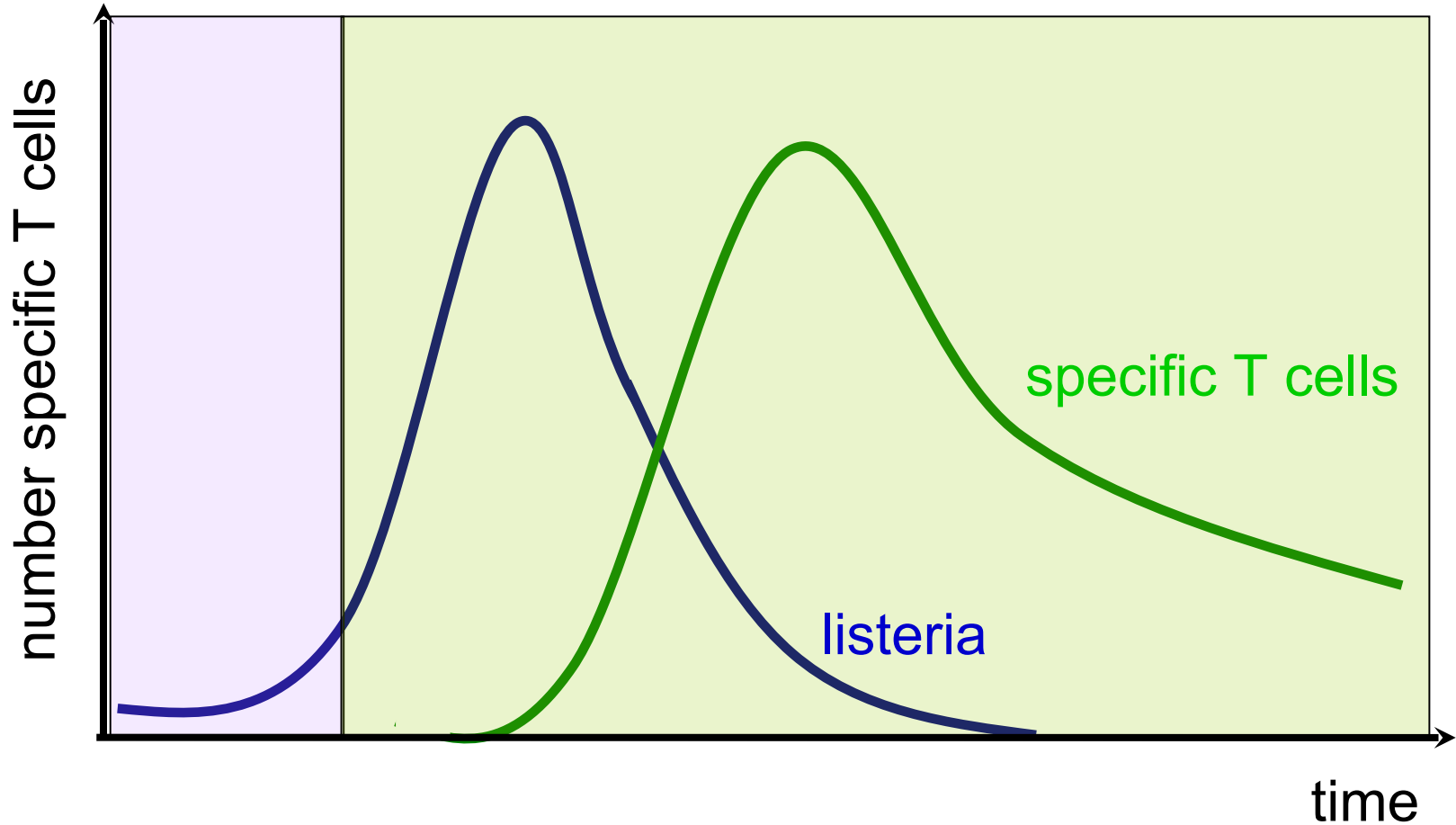
Donor 70



Donor 59

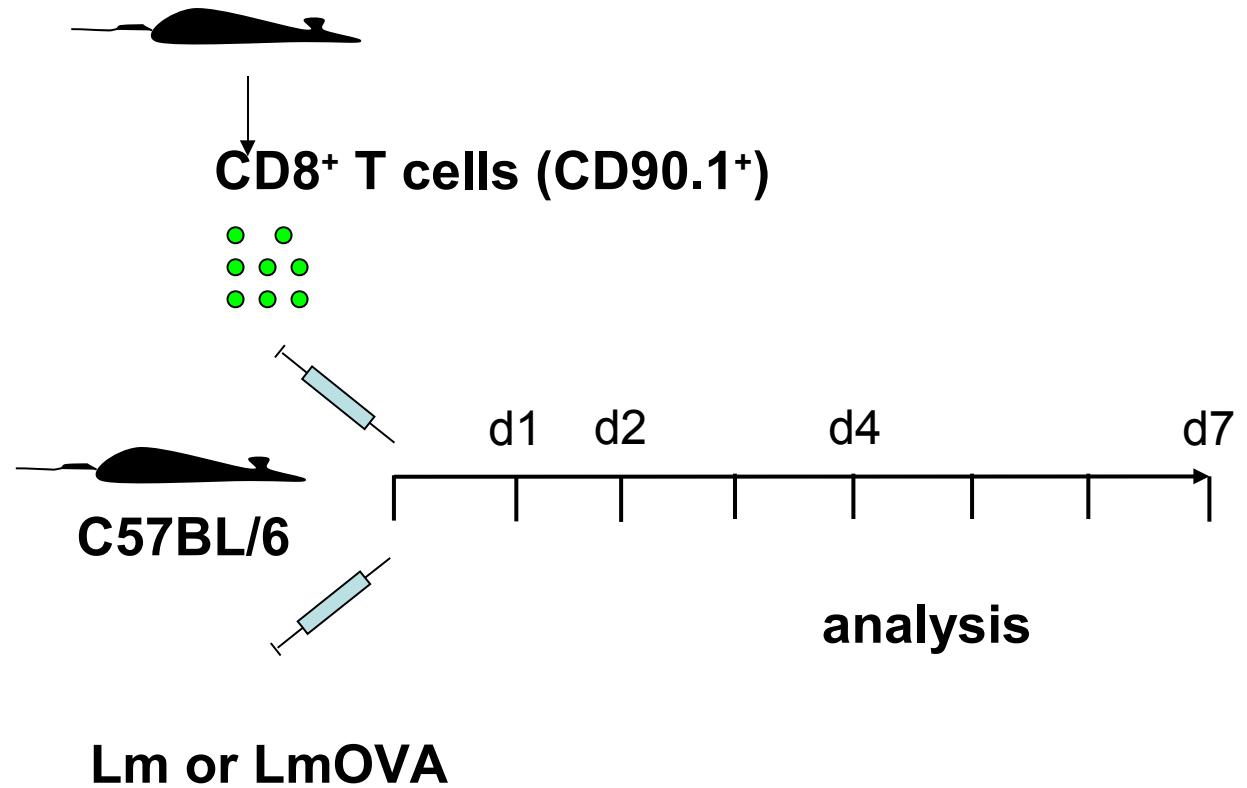


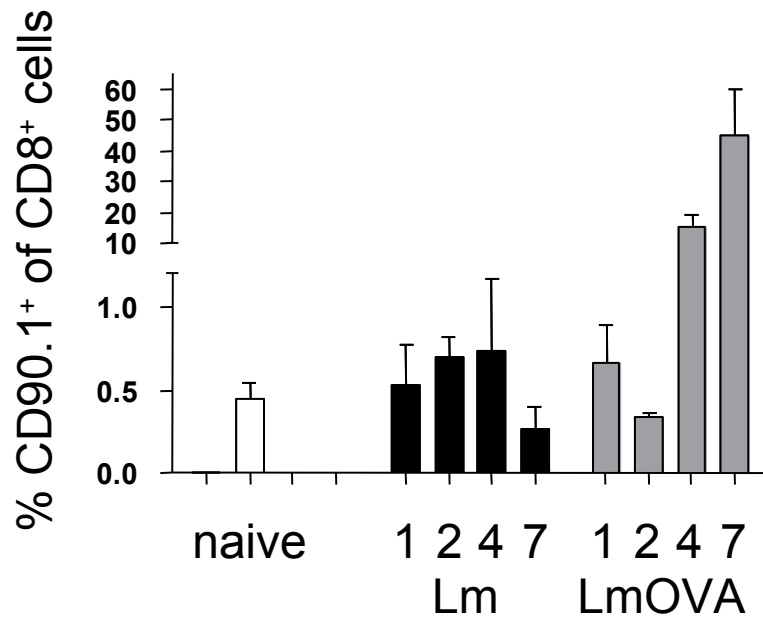
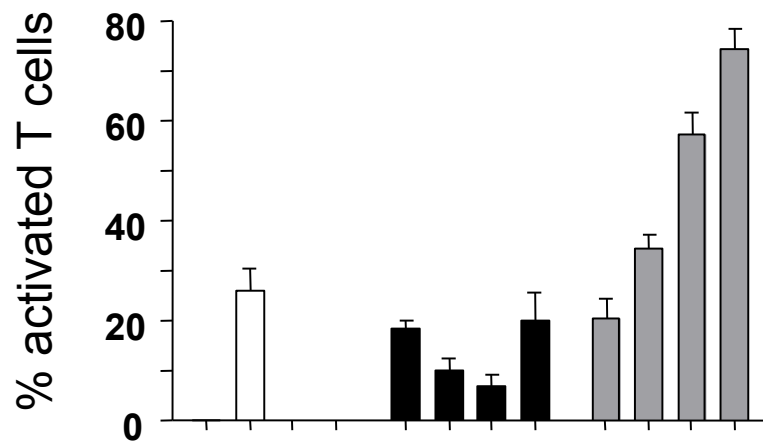
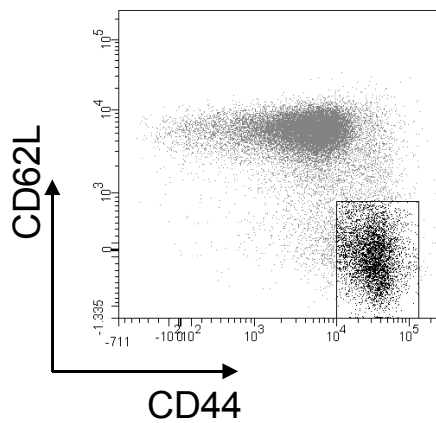
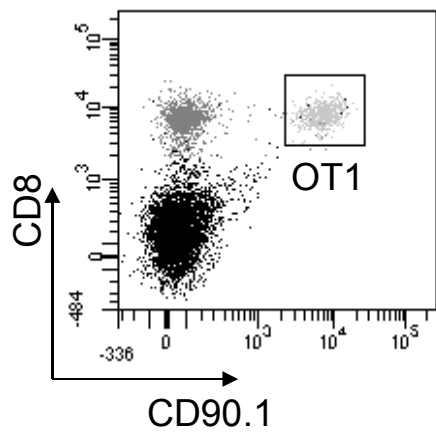
# Time course of T cell response



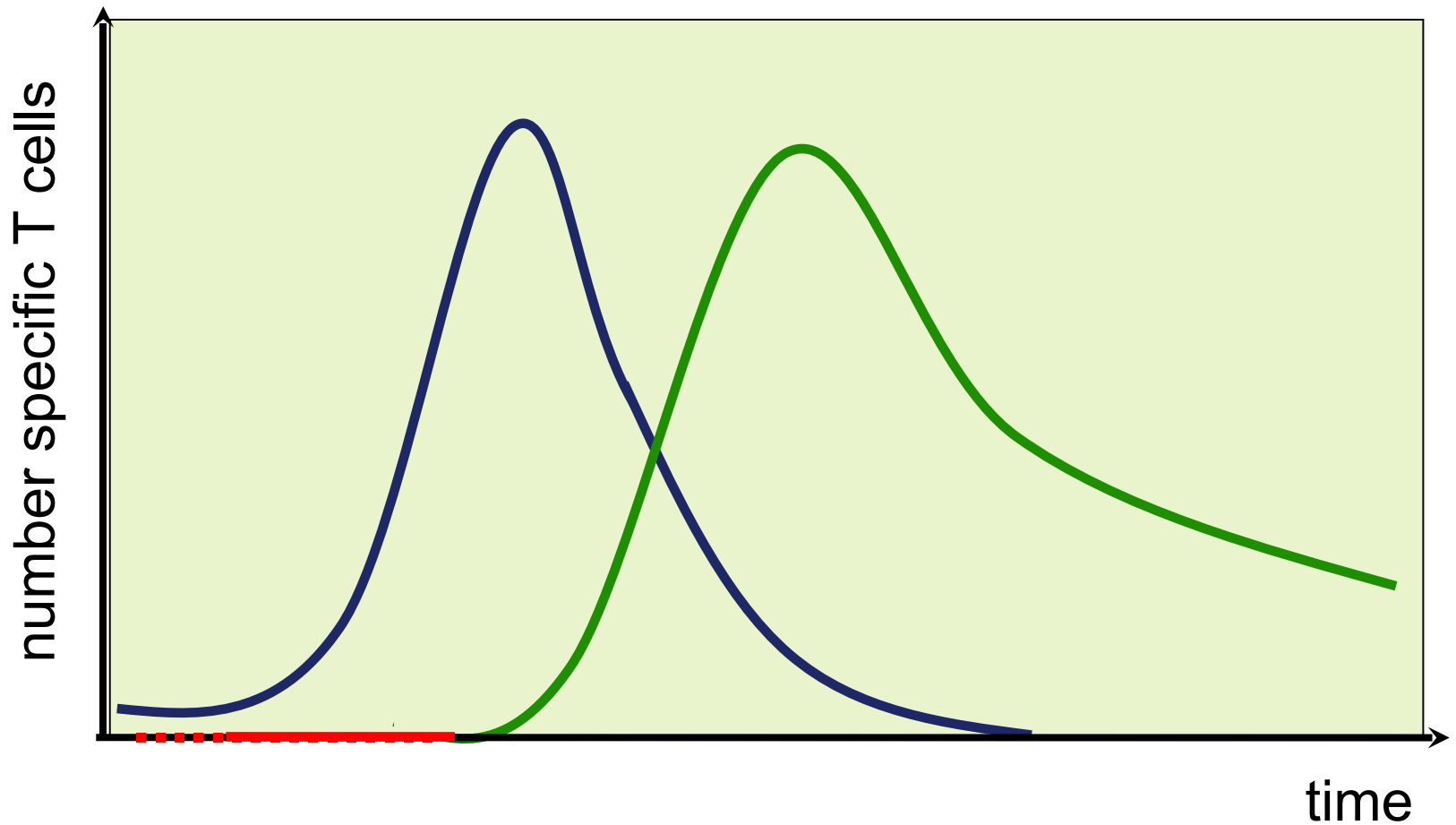
-> when does the T cell response start?

**TCR-tg mice (OT1)**  
**OVA-specific CD8<sup>+</sup> T cells**

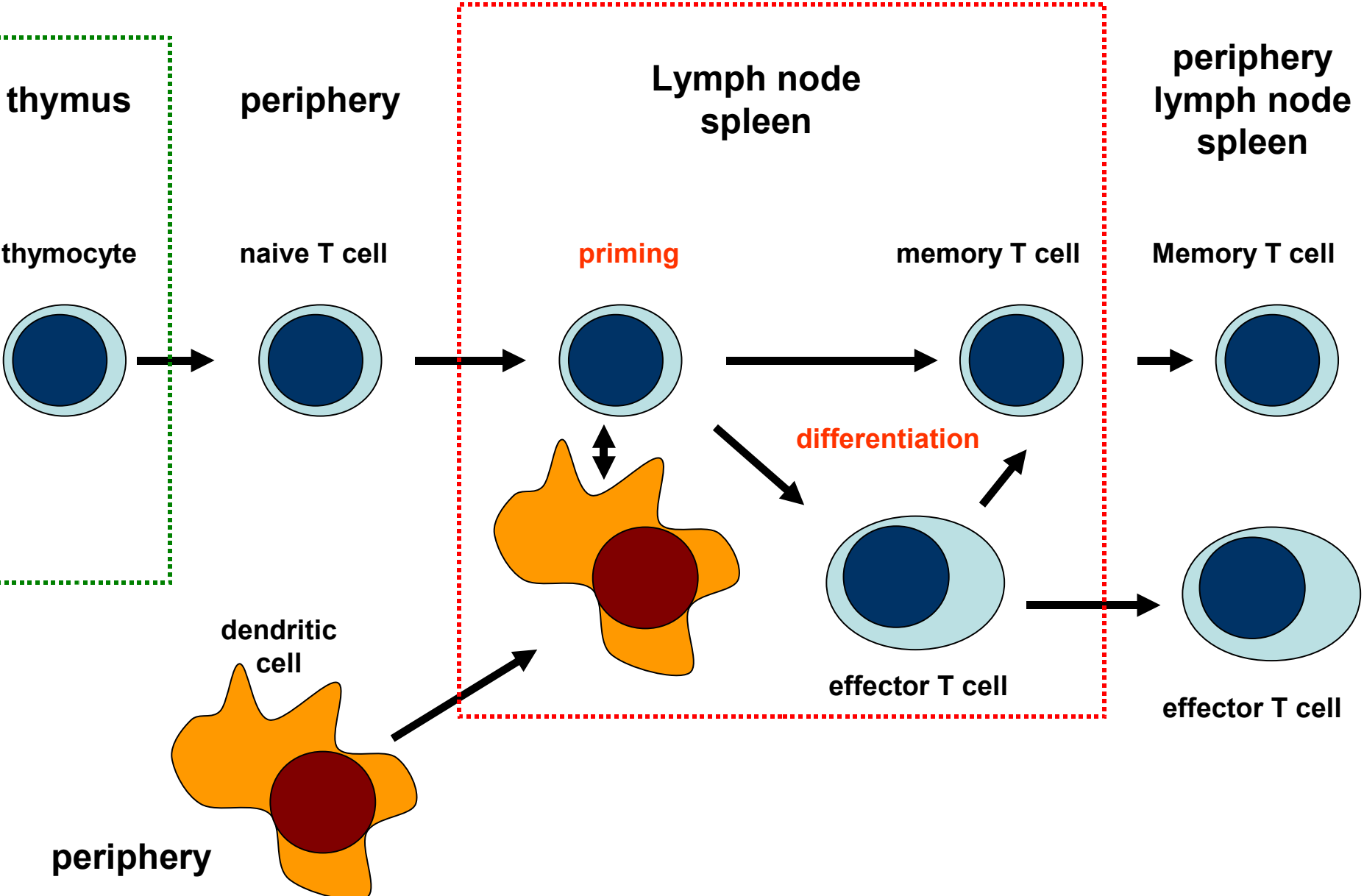




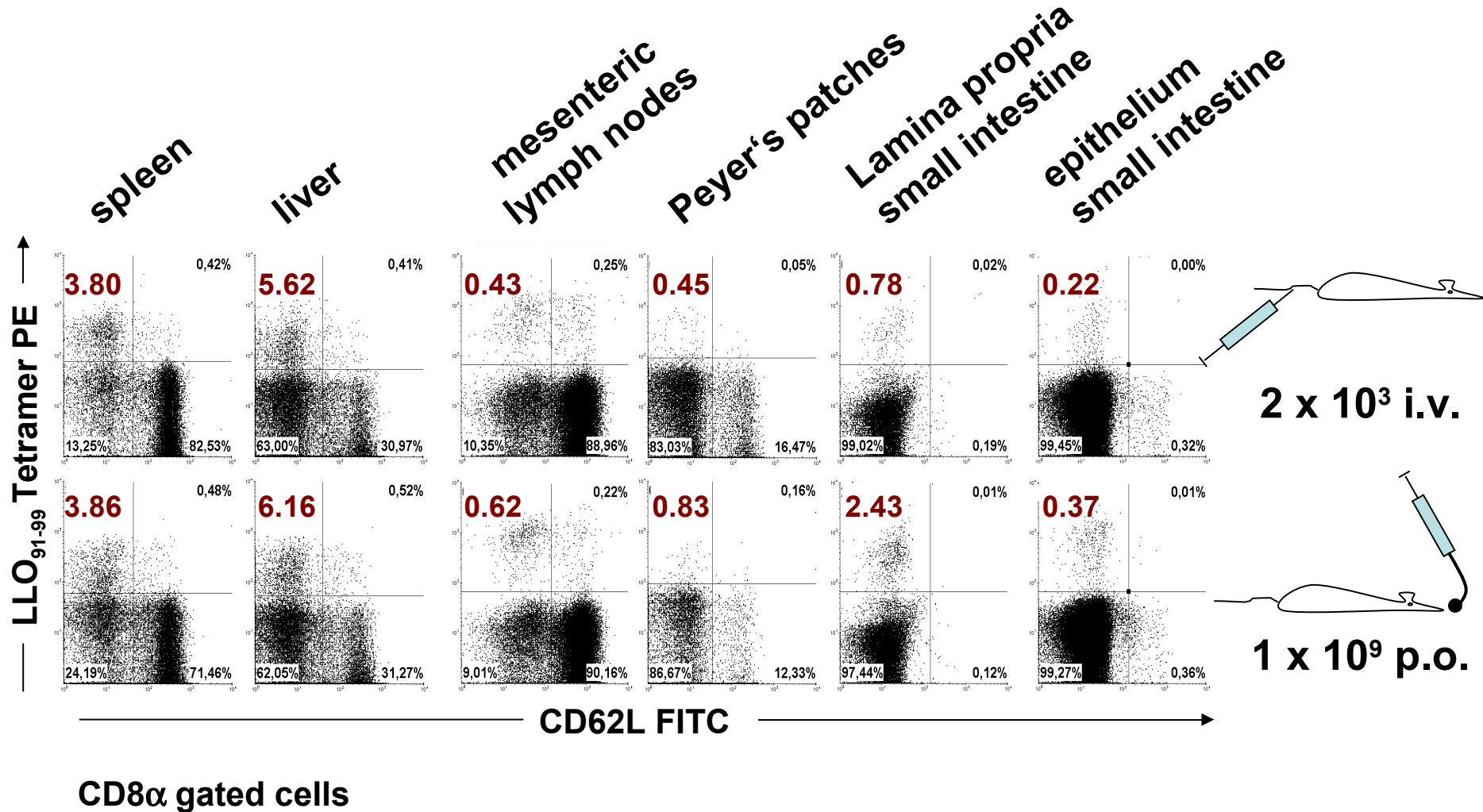
# Time course of T cell response



# Maturation and activation of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes



# Frequencies of LLO<sub>91-99</sub>-tetramer<sup>+</sup> CD8<sup>+</sup> T cells after primary i.v. or p.o. *L. monocytogenes* infection



# **How important are secondary lymphoid tissues for the function of listeria-specific T cells**

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- **Is there induction of T cells outside of secondary lymphoid tissues?**
- **How important are secondary lymphoid tissues for the induction of memory T cell responses?**

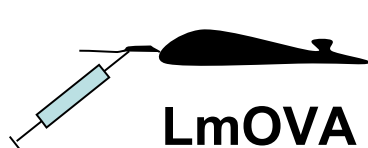
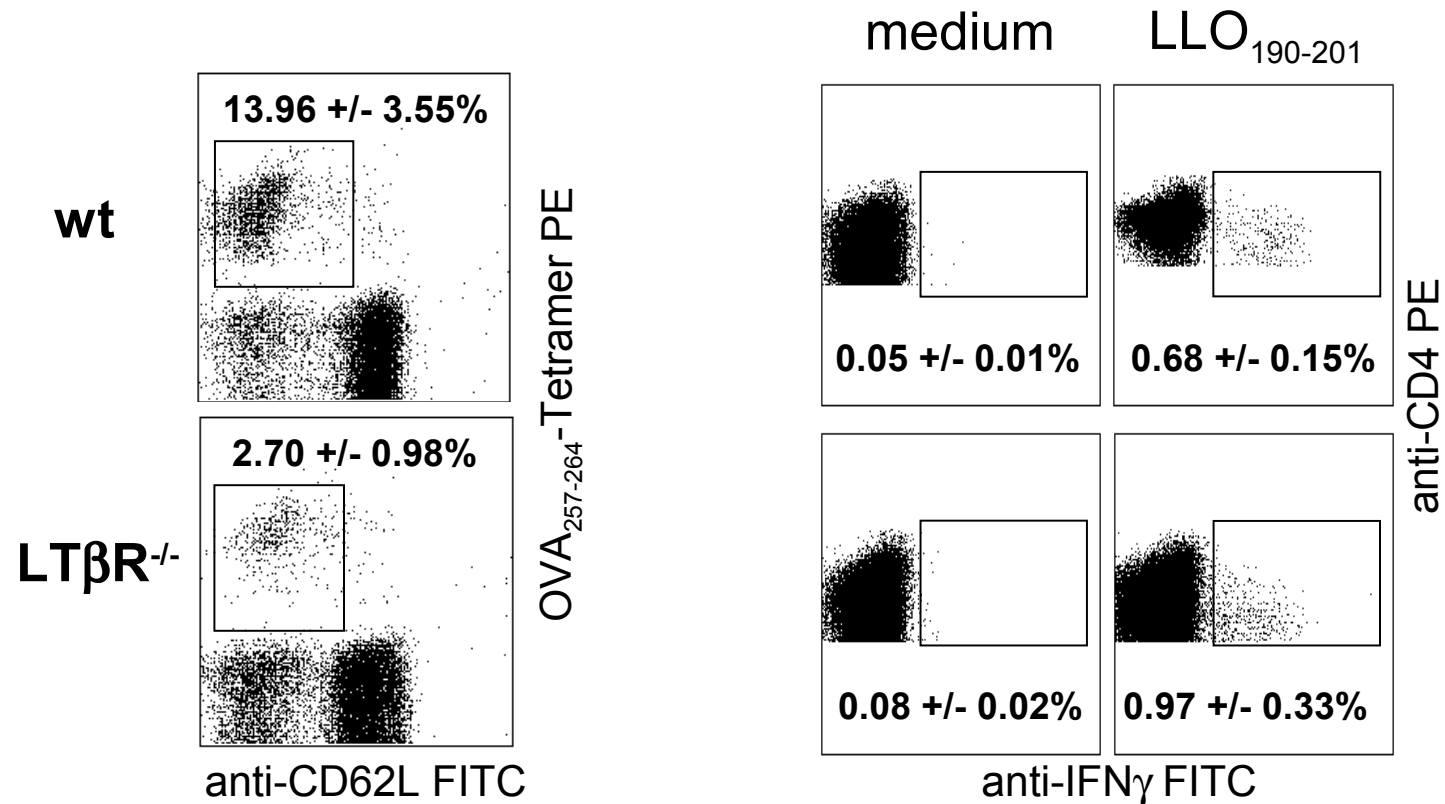
# Is there induction of CD8<sup>+</sup> T cell responses outside of secondary lymphoid tissues?

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**Lymphotoxin- $\beta$ -receptor (LT $\beta$ R) <sup>-/-</sup> mice**  
(Klaus Pfeffer, Medical Microbiology Düsseldorf)

- **LT $\beta$ R binds the LT $\alpha_1\beta_2$  heterotrimer**
- **LT $\beta$ R<sup>-/-</sup> mice have neither peripheral lymph nodes nor Peyer's patches**
- **LT $\beta$ R<sup>-/-</sup> mice show an increased susceptibility to *L. monocytogenes***

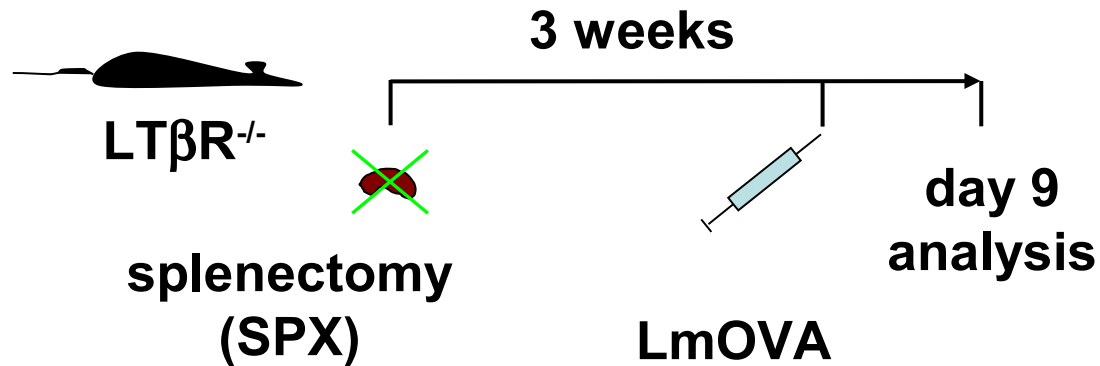
# Listeria-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in LTβR<sup>-/-</sup> mice



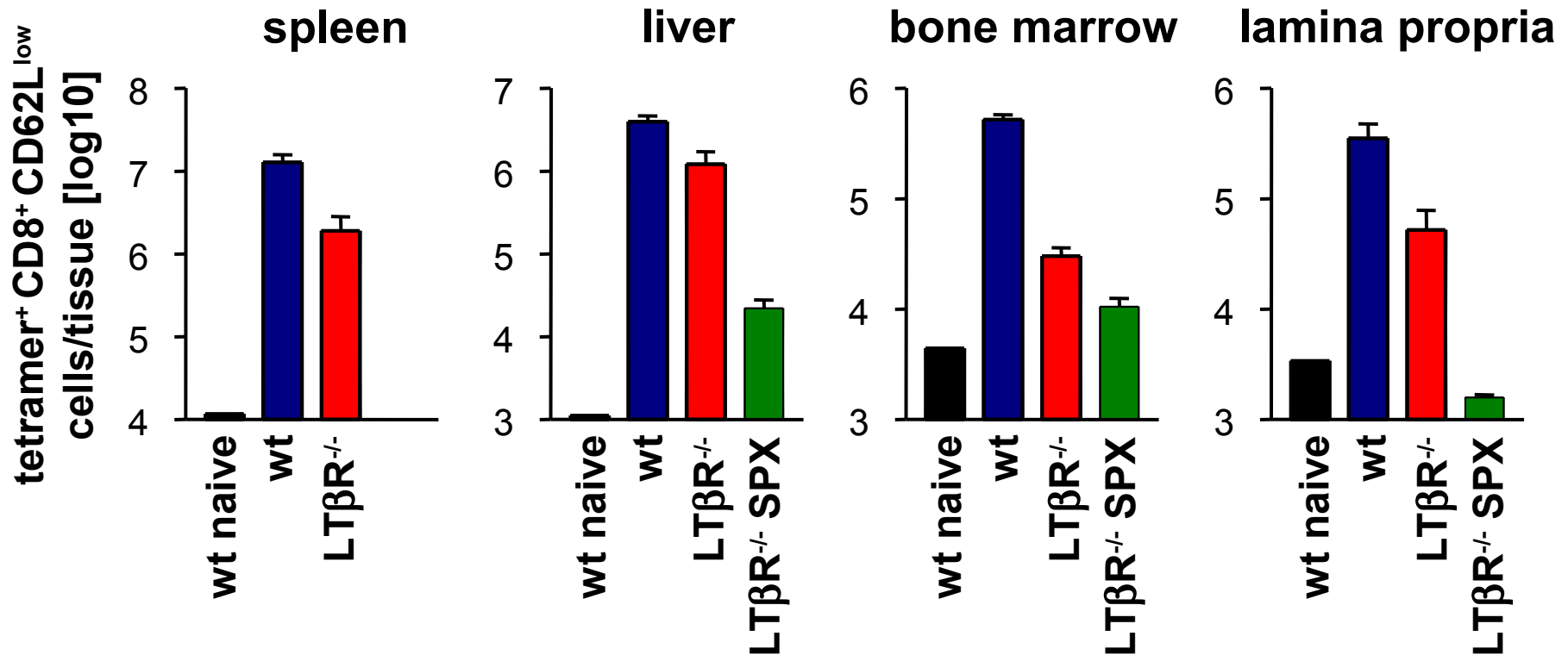
9 days

Analysis of splenocytes

# Listeria-specific T cell responses in splenectomized $LT\beta R^{-/-}$ mice



# OVA<sub>257-264</sub>-specific CD8<sup>+</sup> T cell responses in splenectomized LTβR<sup>-/-</sup> mice



## LT $\beta$ R: Summary

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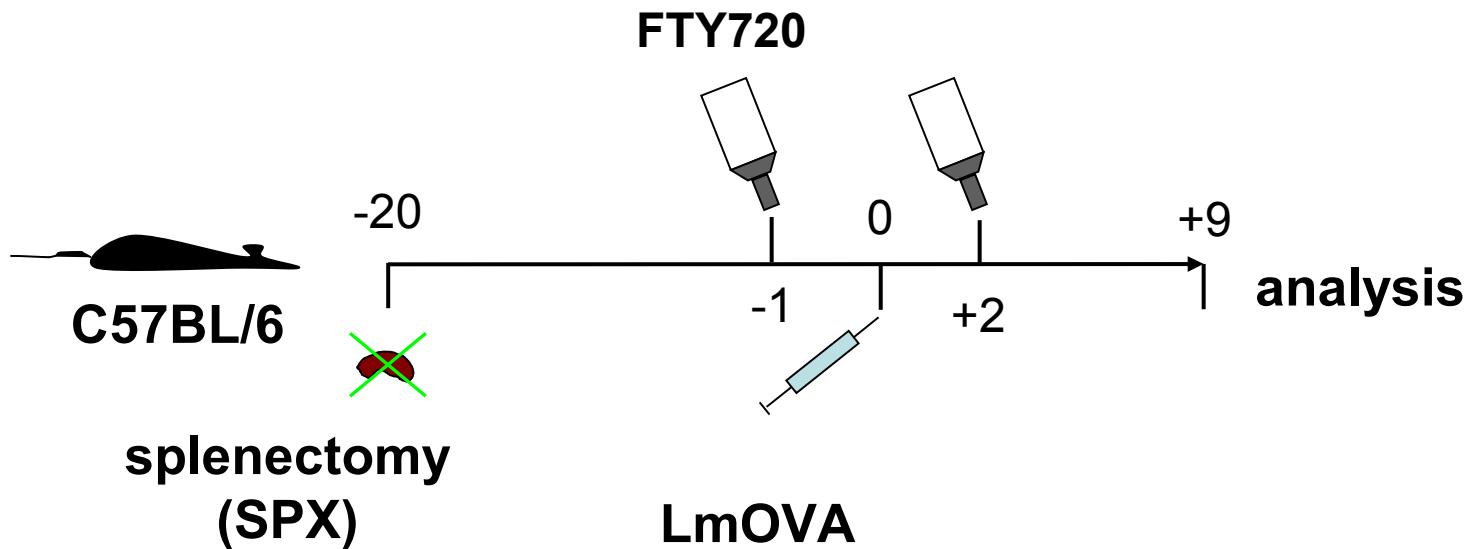
- **LT $\beta$ R<sup>-/-</sup> mice can generate listeria-specific CD4<sup>+</sup> Th1 and CD8<sup>+</sup> T cell responses.**
- **In the absence of spleen, peripheral lymph nodes and Peyer's patches, there is only very inefficient induction of T cell responses against *L. monocytogenes*.  
(in LT $\beta$ R<sup>-/-</sup> mice)**

# Is there induction of CD8<sup>+</sup> T cell responses outside of secondary lymphoid tissues: FTY720

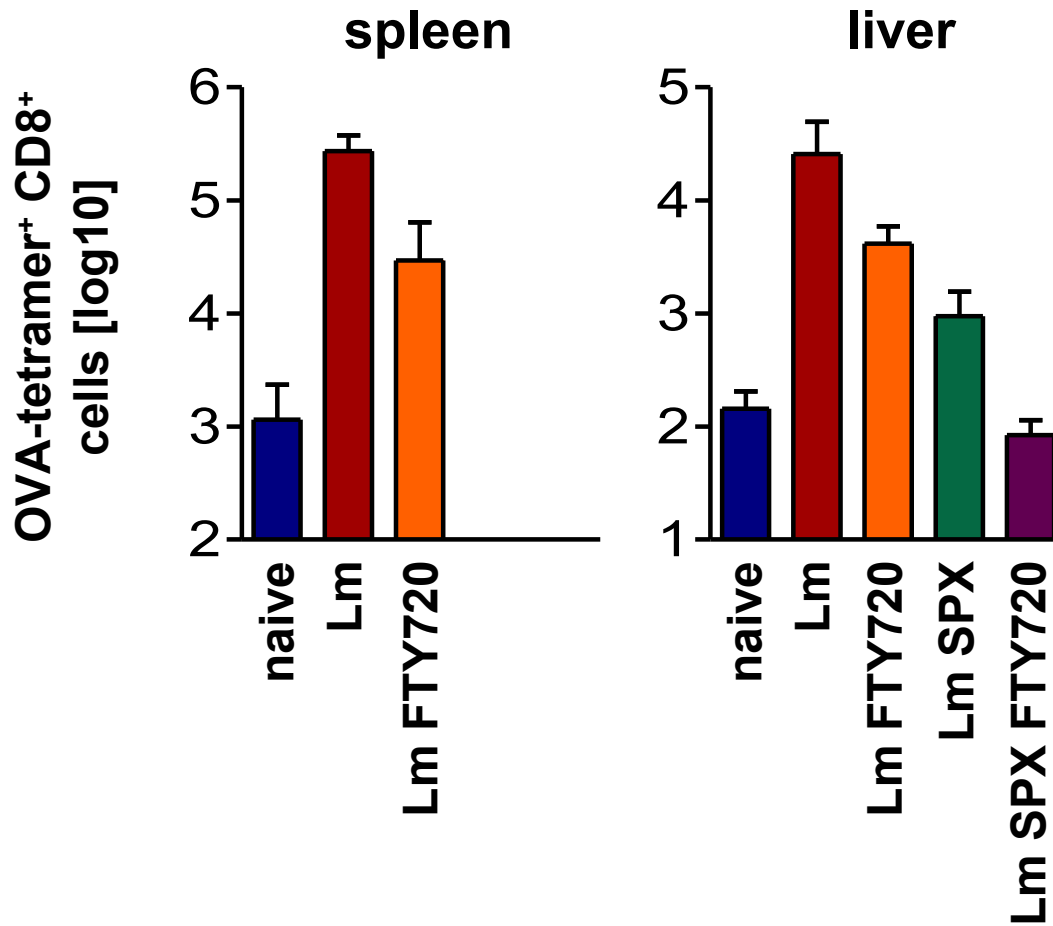
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- The interaction of sphingosine-1-phosphate (S1P) with its receptors on T cells induces egress of T cells from thymus and secondary lymphoid tissues.
- The drug FTY720 becomes rapidly phosphorylated *in vivo*, and FTY720-P binds to the S1P-receptor S1P<sub>1</sub>.
- FTY720-P exposure results in inactivation of S1P<sub>1</sub>.
- **Consequence of FTY720 treatment:**  
**Retention of naive and activated T cells in thymus and secondary lymphoid tissues.**

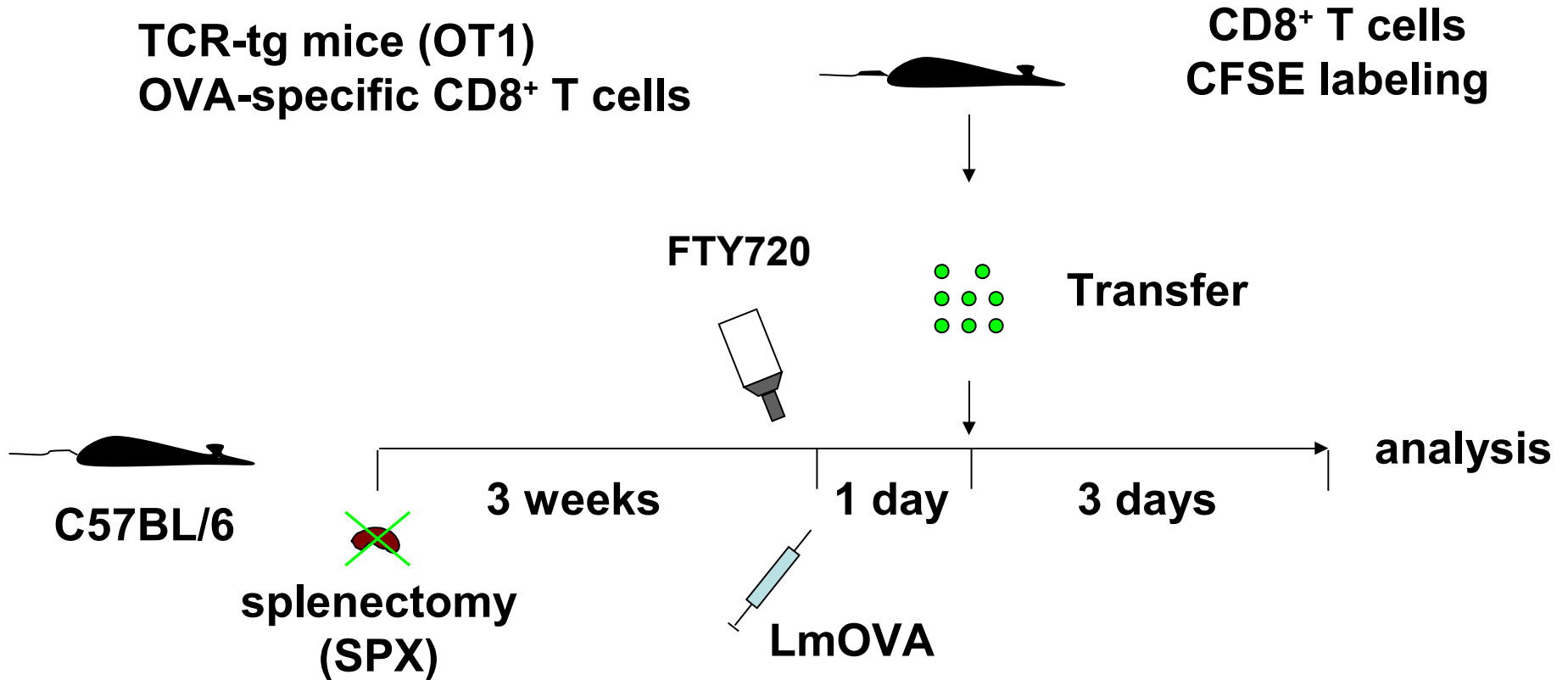
# Listeria-specific T cell responses in FTY720 treated mice



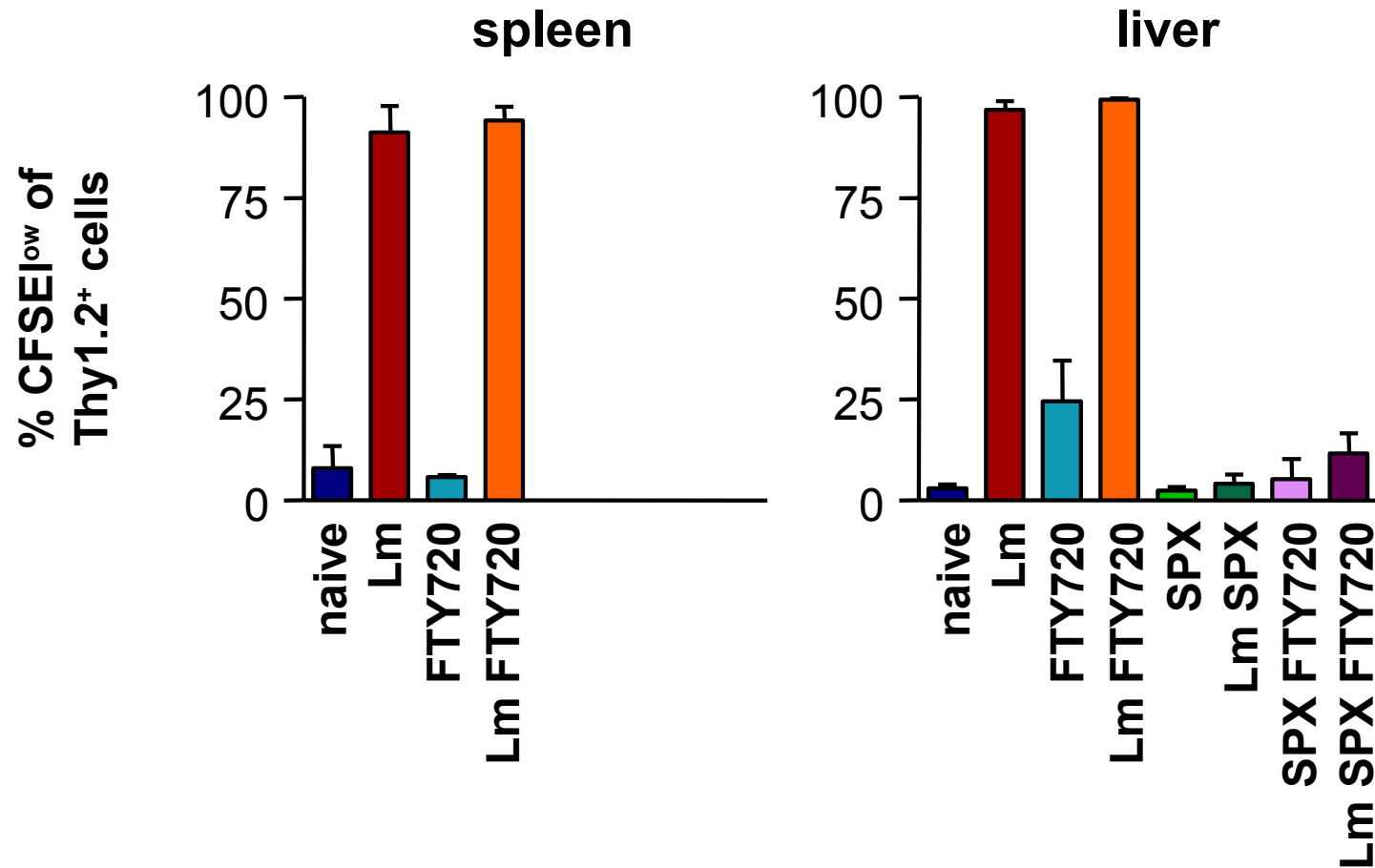
# Listeria-specific T cell responses in FTY720 treated mice



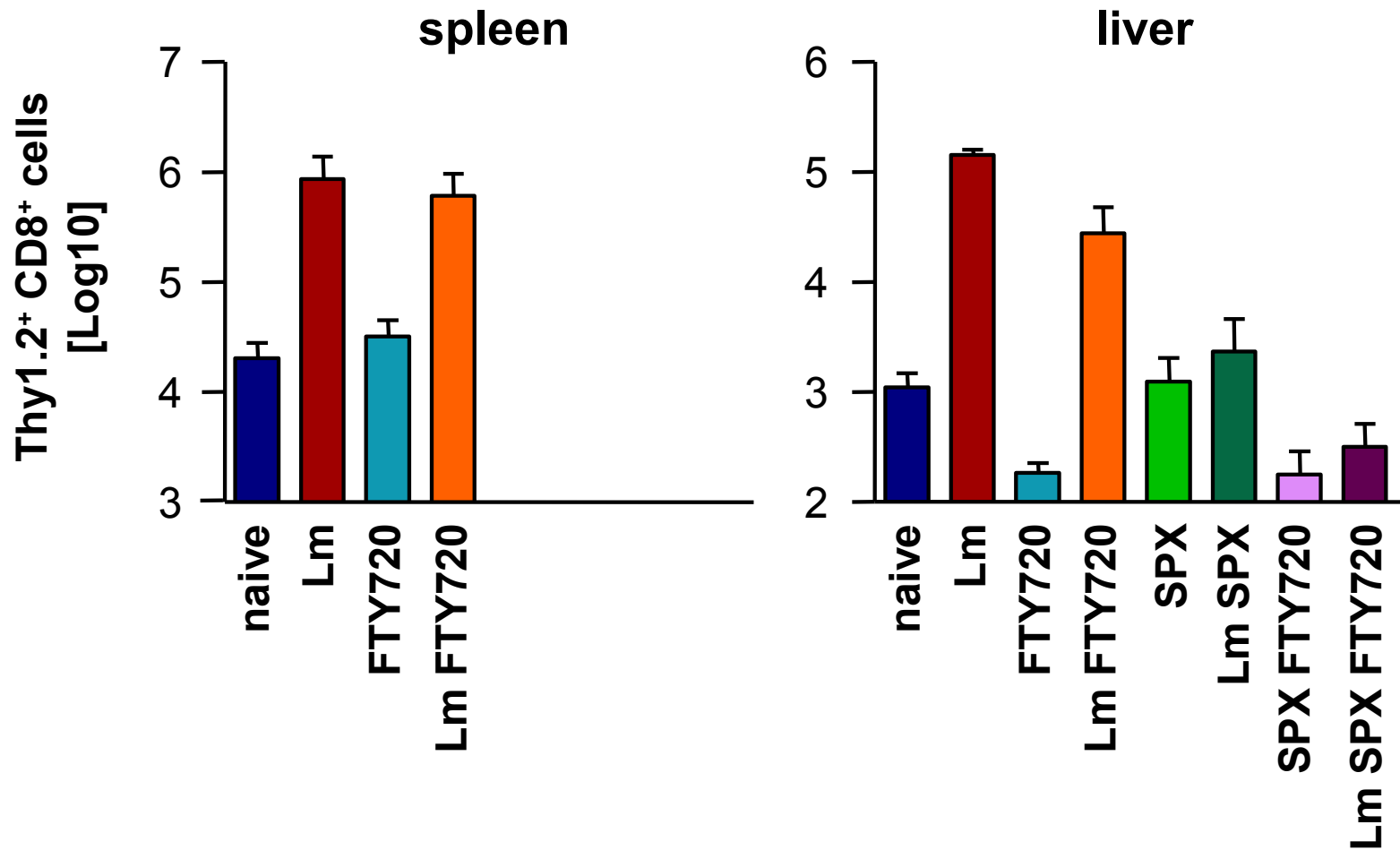
# Listeria-specific T cell responses in FTY720 treated mice



# Listeria-specific T cell responses in FTY720 treated mice



# Listeria-specific T cell responses in FTY720 treated mice



## FTY720: Summary

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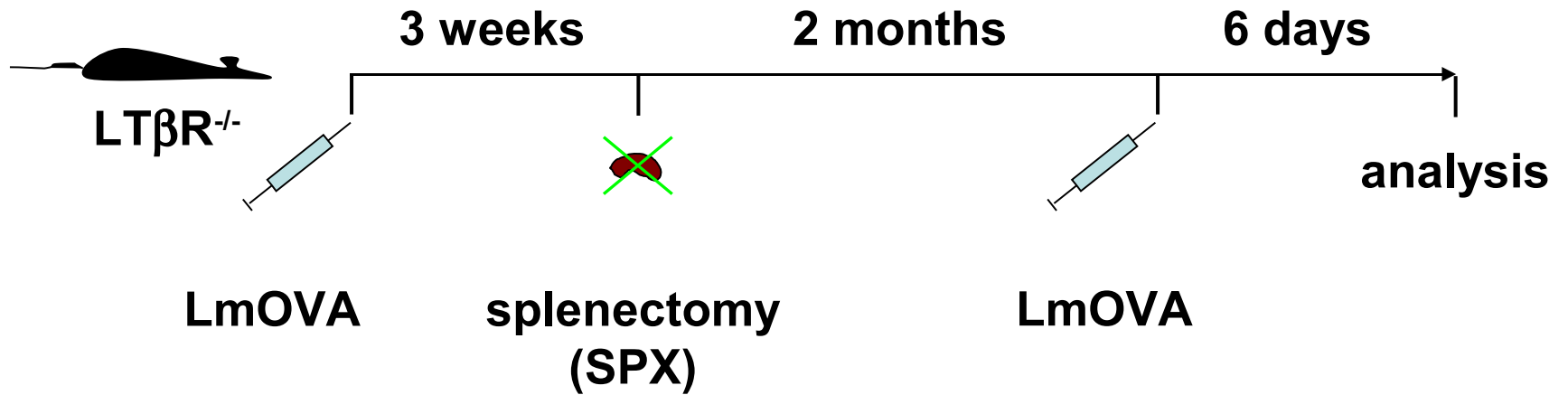
- **The liver is not an inductive site for listeria-specific CD8<sup>+</sup> T cells.**
- **After systemic (i.v.) infection, the spleen is the main inductive site for listeria-specific CD8<sup>+</sup> T cells.**

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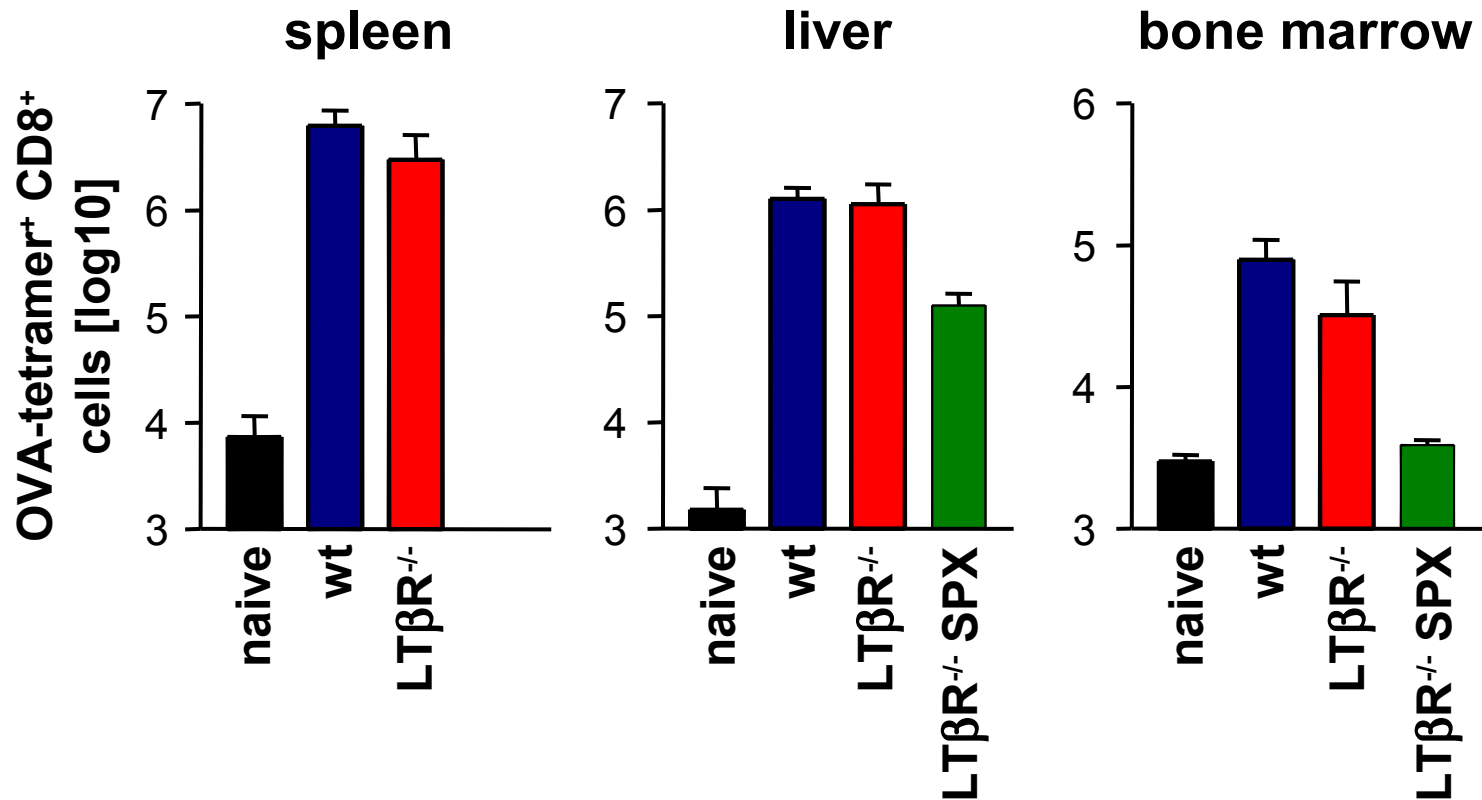
**How important are secondary lymphoid tissues for the induction of memory T cell responses?**

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# How important are secondary lymphoid tissues for the induction of memory T cell responses?



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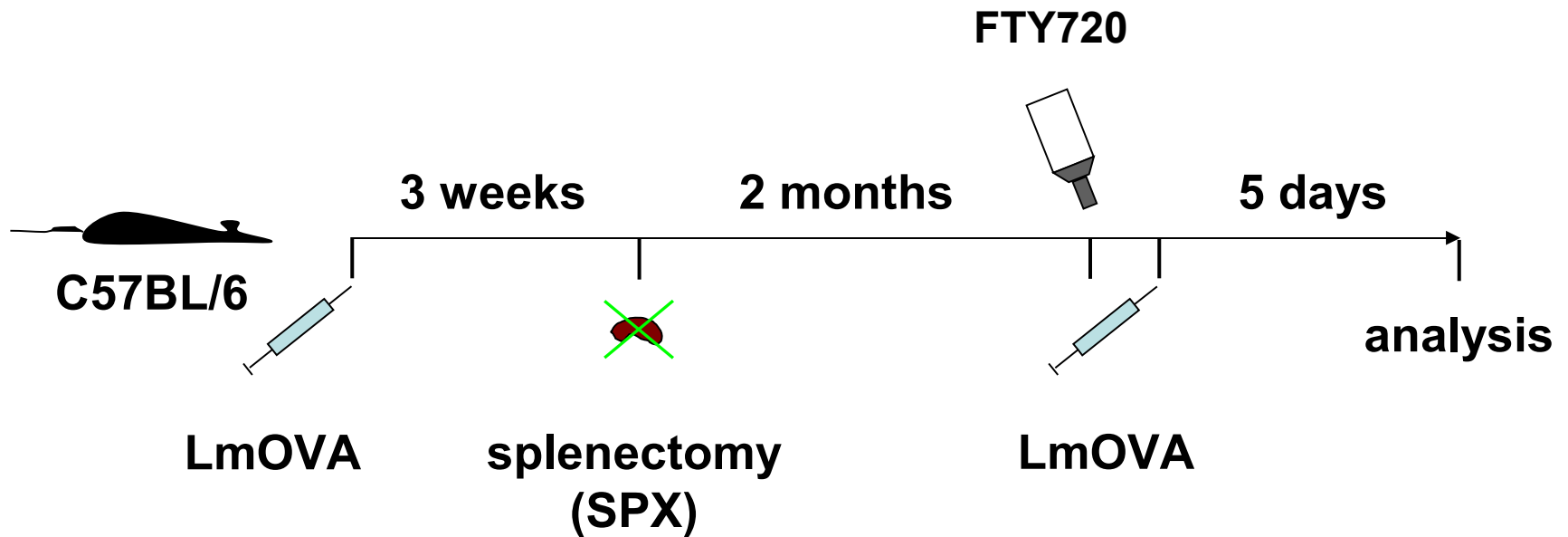


## LT $\beta$ R: Summary

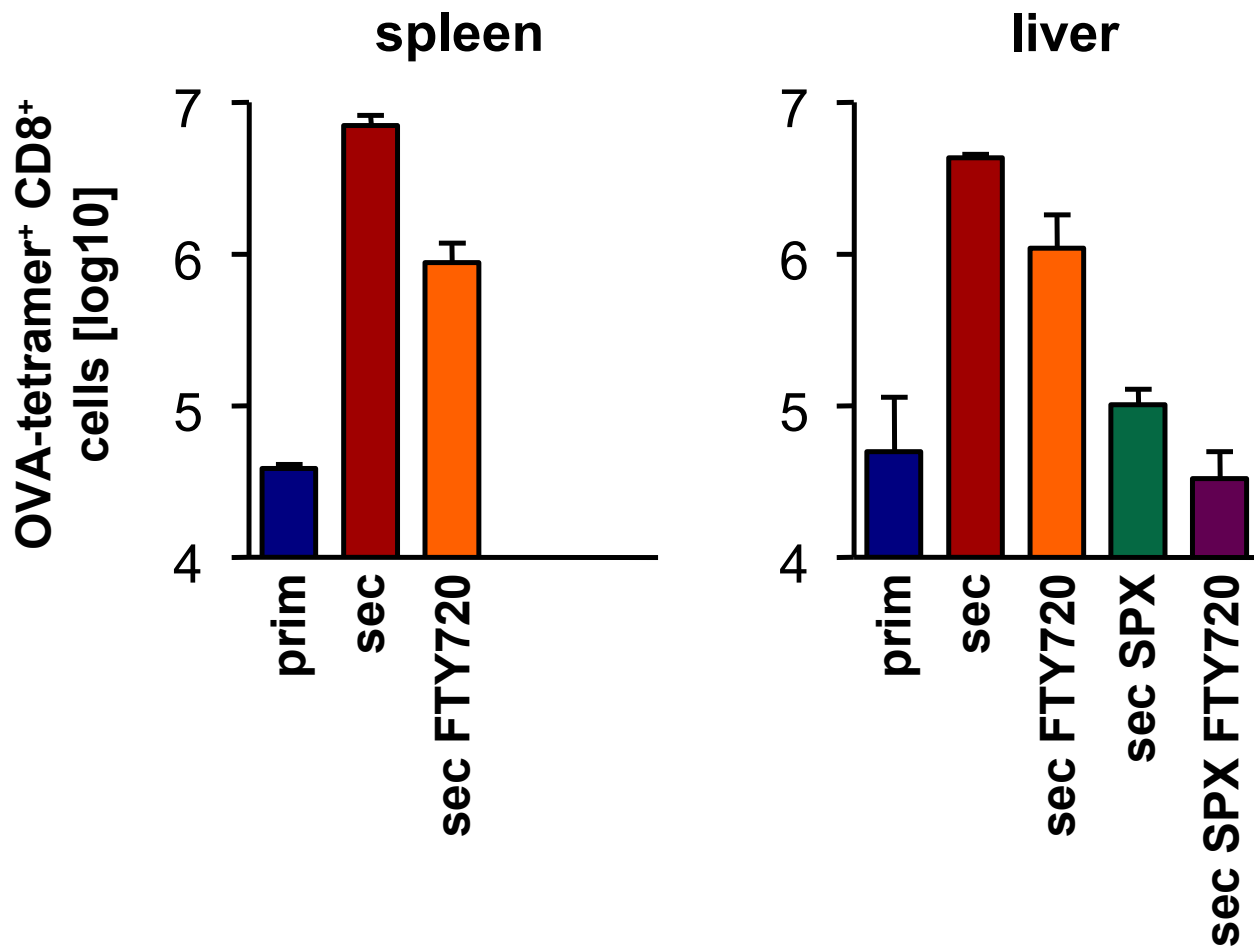
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- **LT $\beta$ R<sup>-/-</sup> mice can generate listeria-specific CD4<sup>+</sup> Th1 and CD8<sup>+</sup> memory T cell responses.**
- **In the absence of spleen, peripheral lymph nodes and Peyer's patches, there is only very inefficient induction of memory T cell responses against *L. monocytogenes*. (in LT $\beta$ R<sup>-/-</sup> mice)**

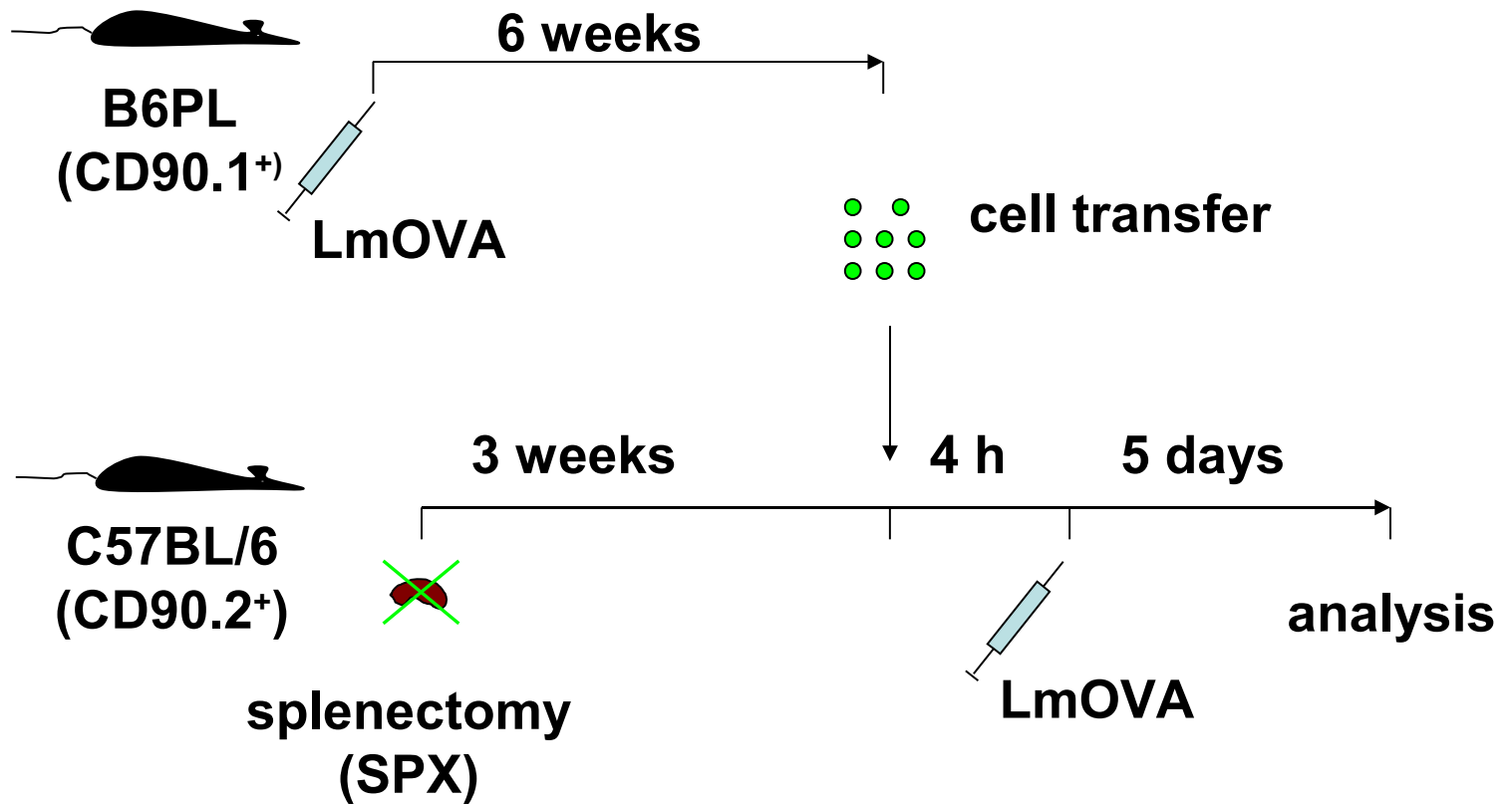
# How important are secondary lymphoid tissues for the induction of memory T cell responses?



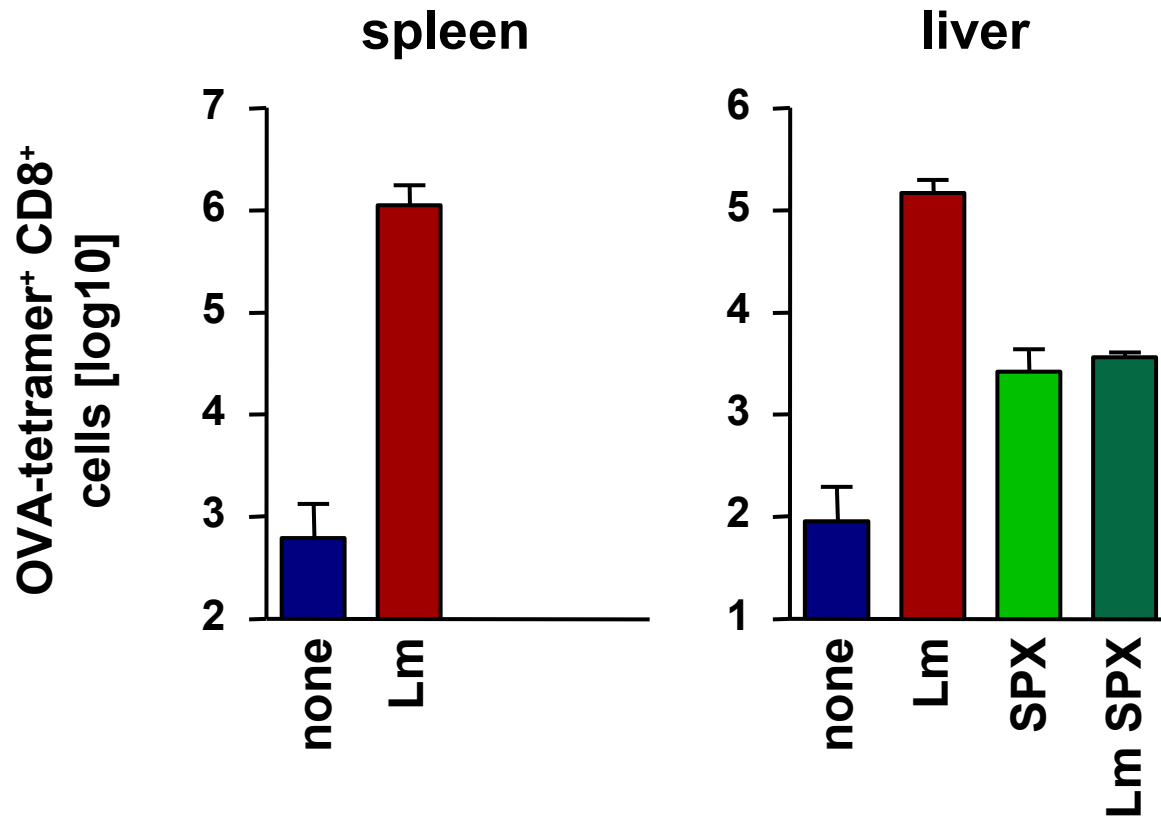
# How important are secondary lymphoid tissues for the induction of memory T cell responses?



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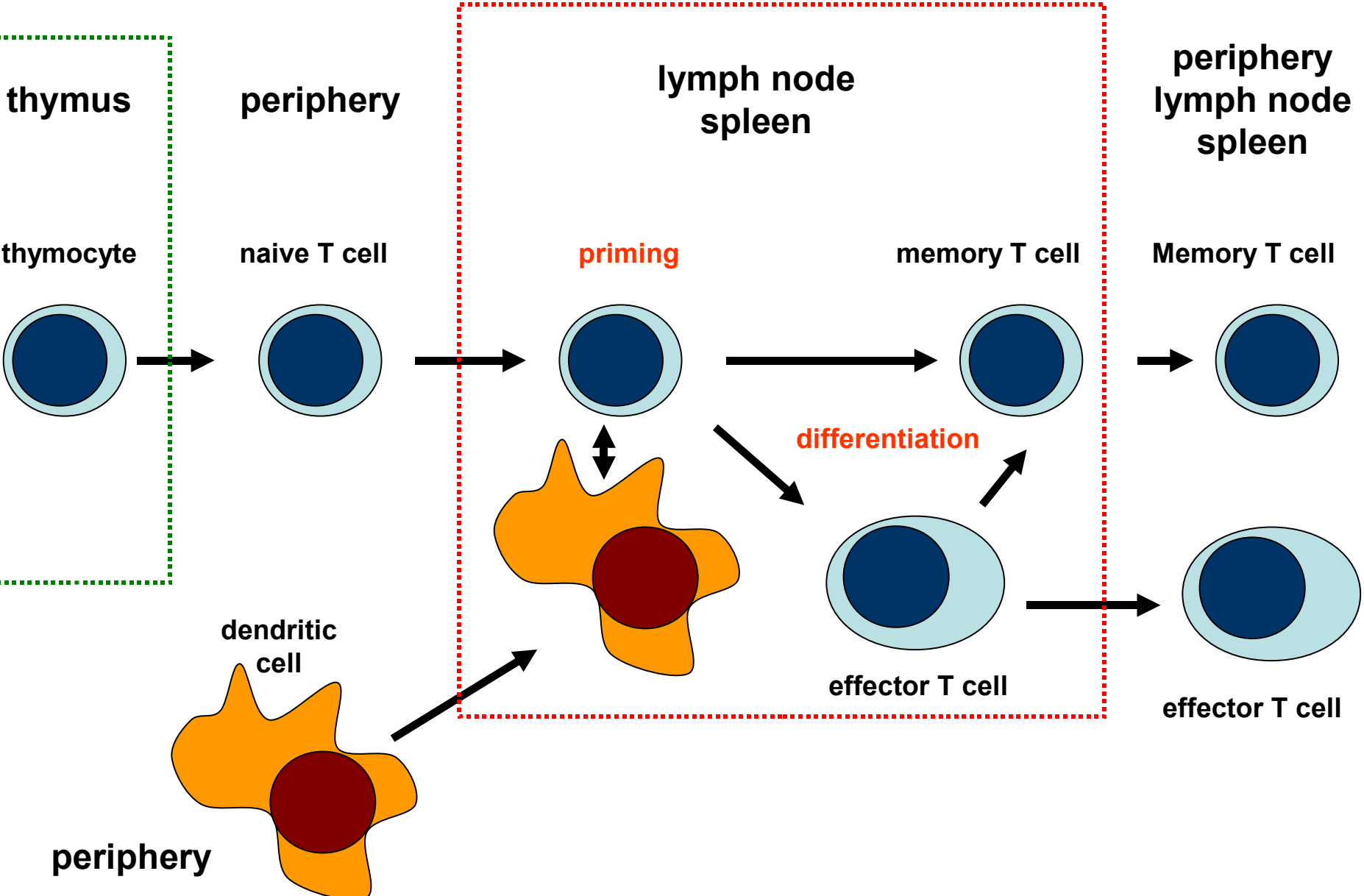


## **How important are secondary lymphoid tissues for the induction of memory T cell responses?**

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- **In the absence of functional secondary lymphoid tissues memory CD8<sup>+</sup> T cell responses are significantly impaired (but not completely absent).**
- **At least a subpopulation of CD8<sup>+</sup> memory T cells requires a lymphoid environment for efficient activation.**

# Maturation and activation of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes



# The chemokine receptor CCR7

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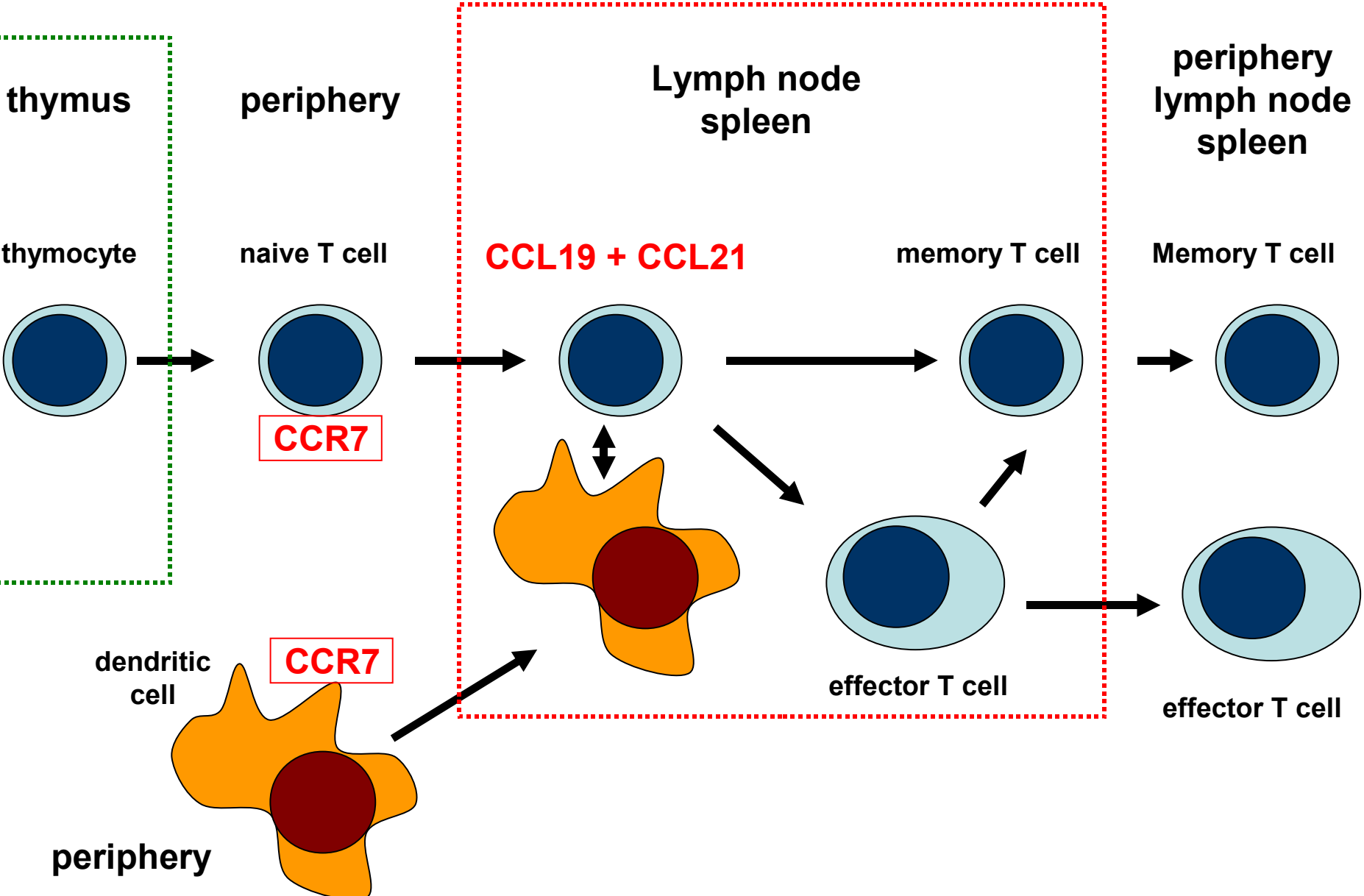
## Expression:

- naive T cells and subpopulations of memory T cells
- activated dendritic cells

## Ligands:

- CCL19  
expressed on high endothelial venules and stromal cells in the T cell zones of lymph nodes and spleen
- CCL21  
expressed in stromal cells and in the T cell zones of lymph nodes and spleen

# Maturation and activation of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes



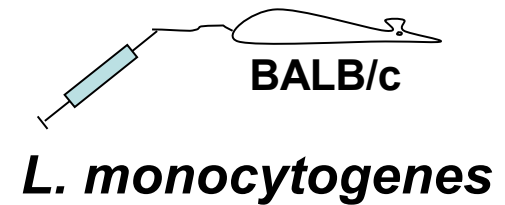
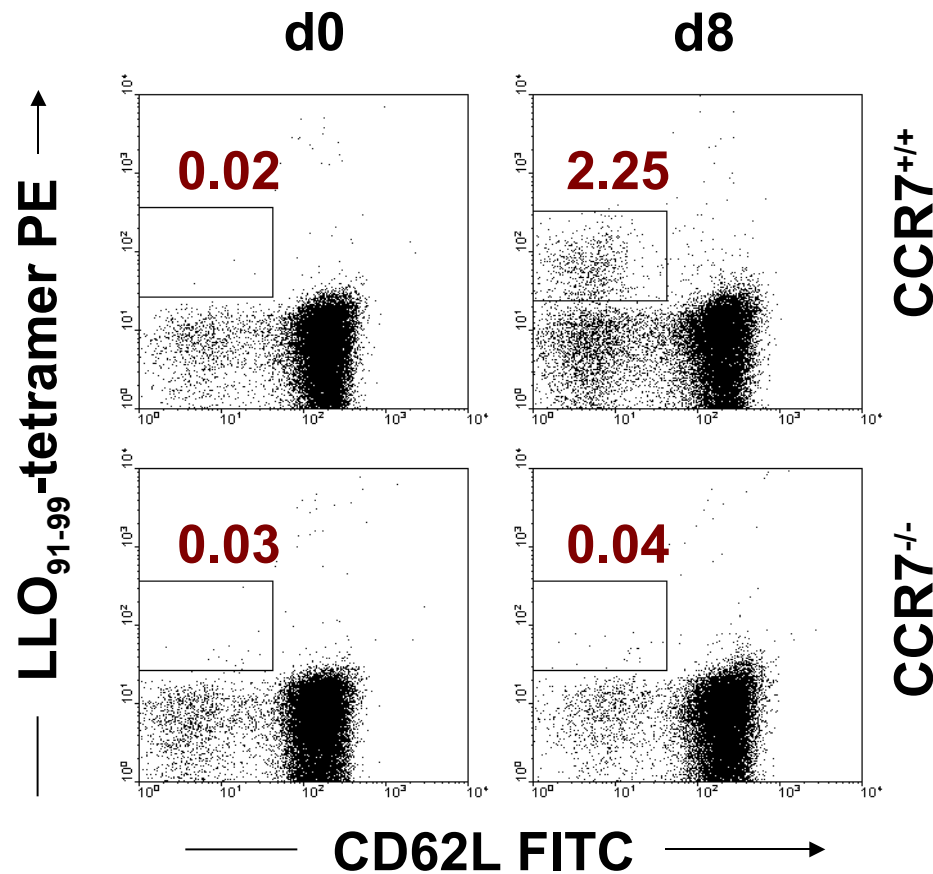
# Function of the CCR7/CCR7-ligand system

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## Hypothesis:

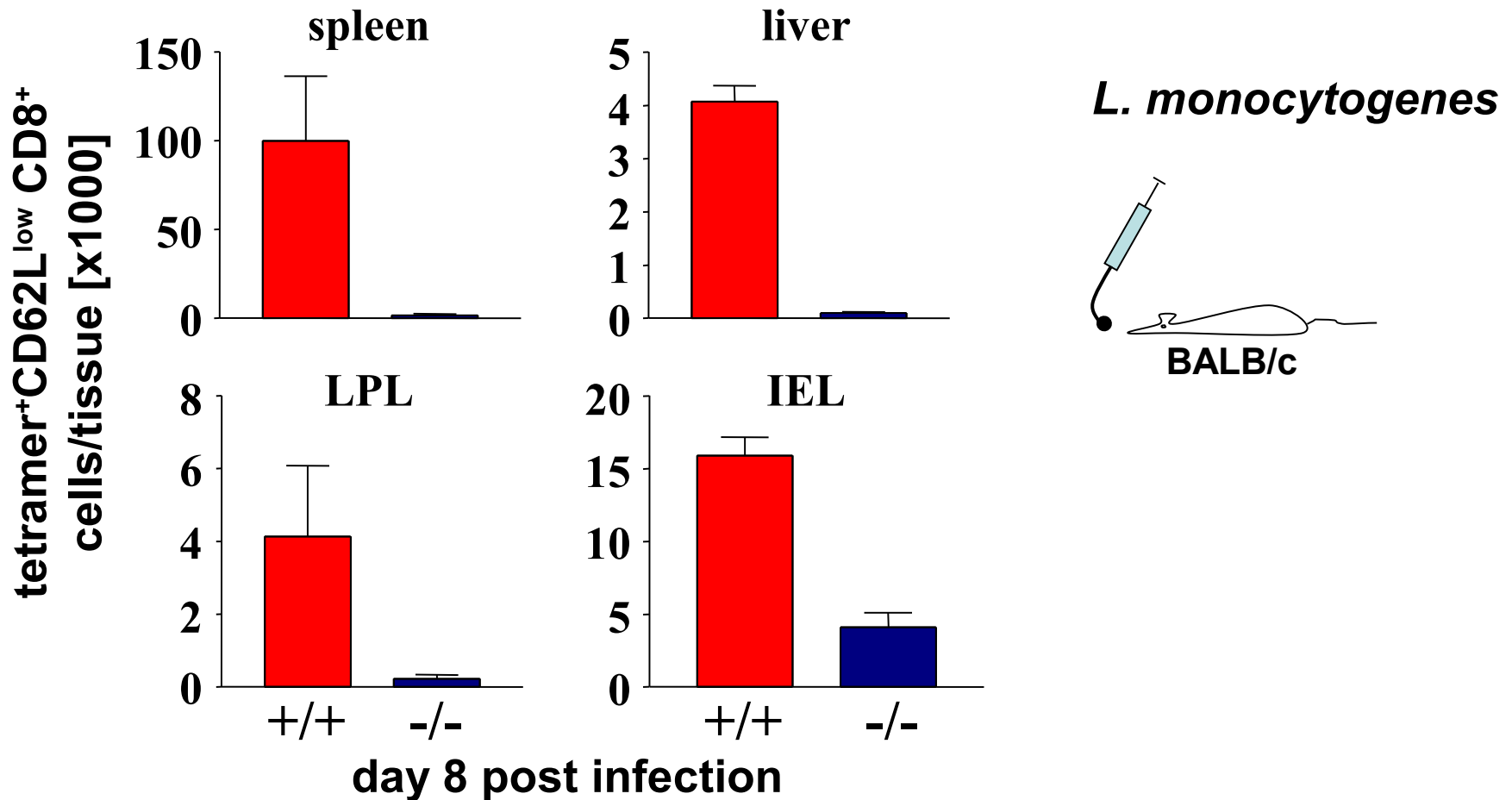
- **Naive T cells and activated dendritic cells migrate along the CCL19/CCL21 gradient to defined areas in secondary lymphoid tissues.**
- **This migration is essential for efficient priming of naive T cells.**

# LLO<sub>91-99</sub>-specific CD8<sup>+</sup> T cell responses during primary *L. monocytogenes*-infection of CCR7<sup>-/-</sup> mice

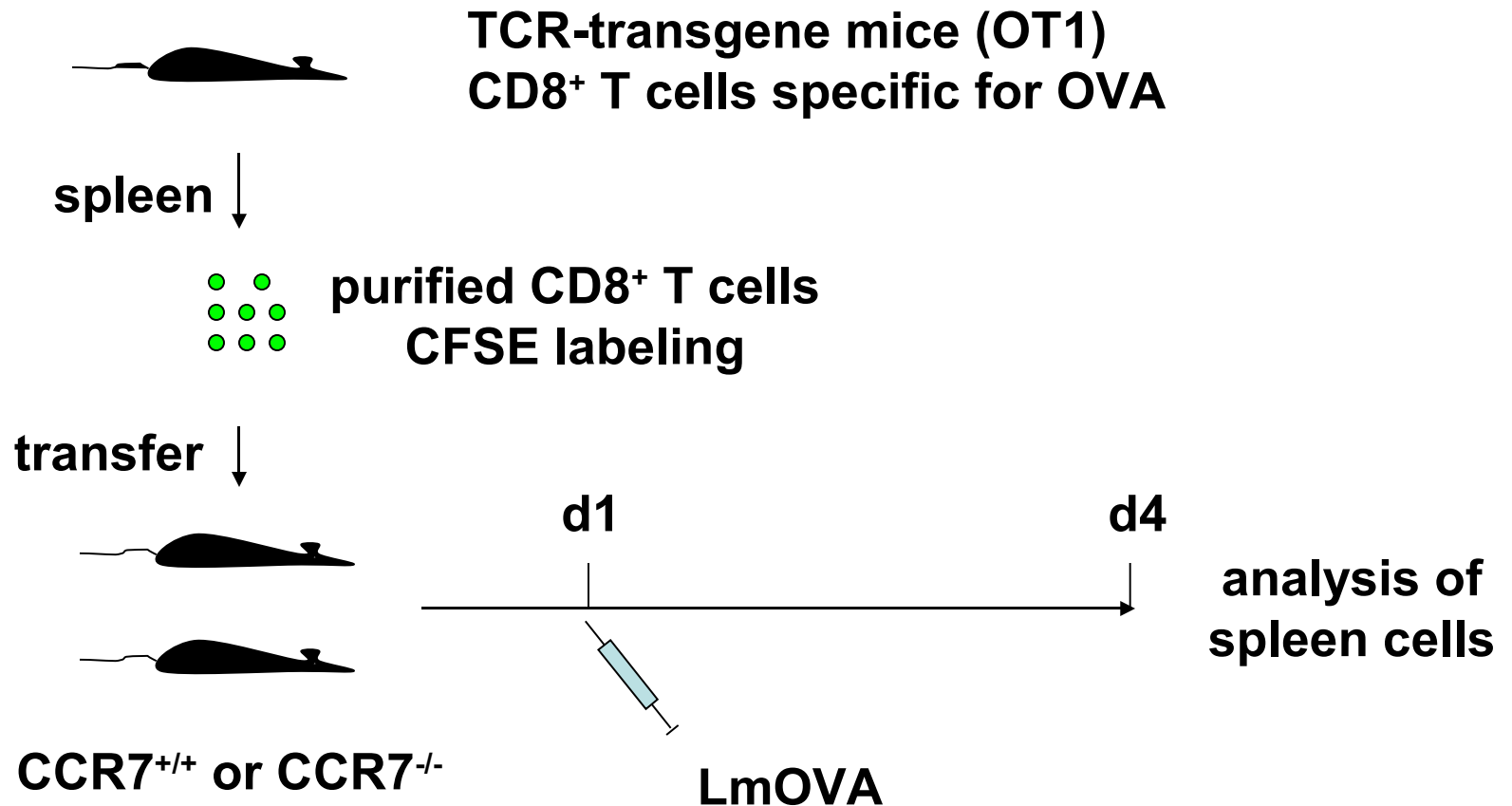


CD8 gated cells

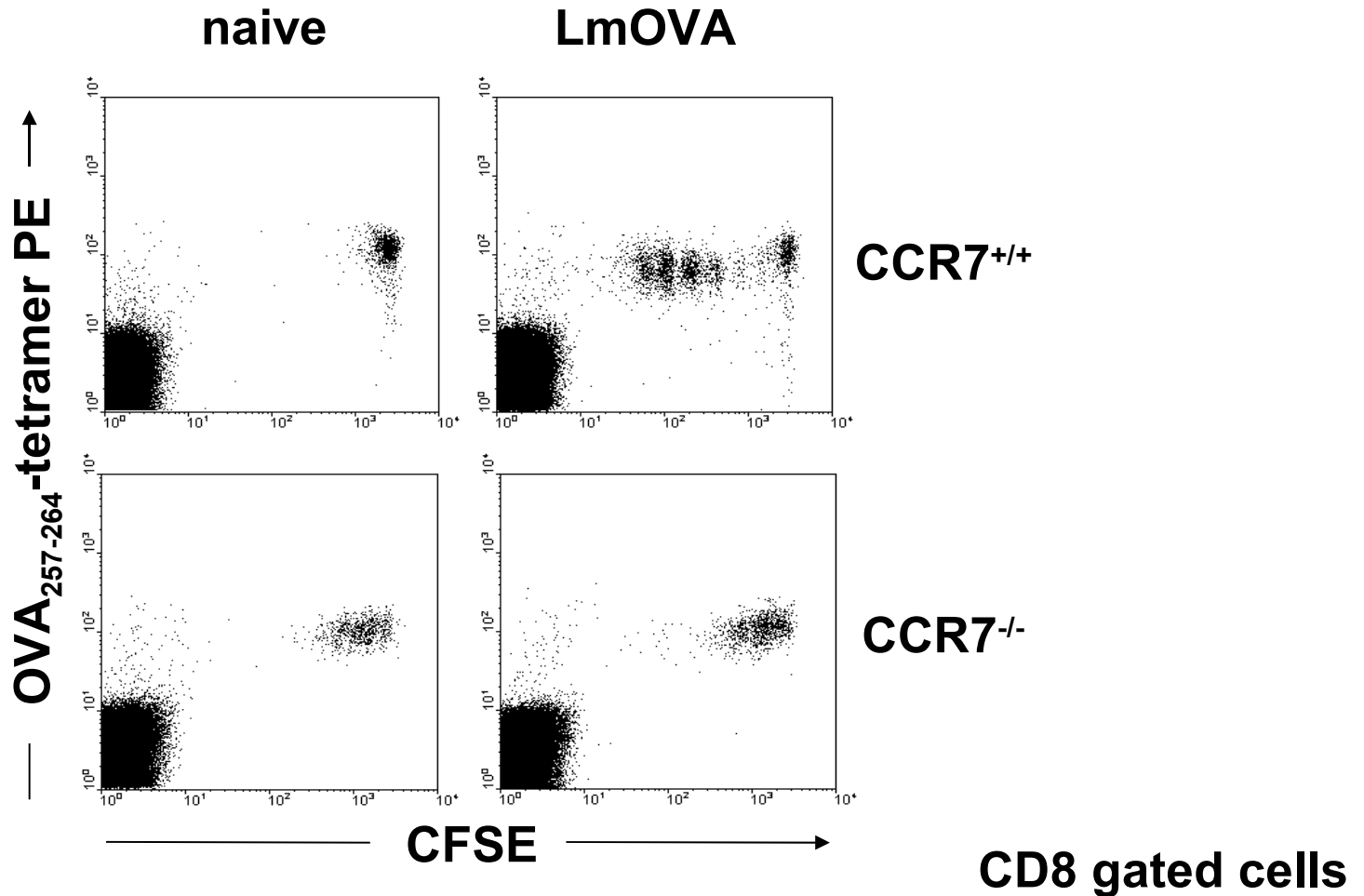
# LLO<sub>91-99</sub>-specific CD8<sup>+</sup> T cell responses during primary *L. monocytogenes*-infection of CCR7<sup>-/-</sup> mice



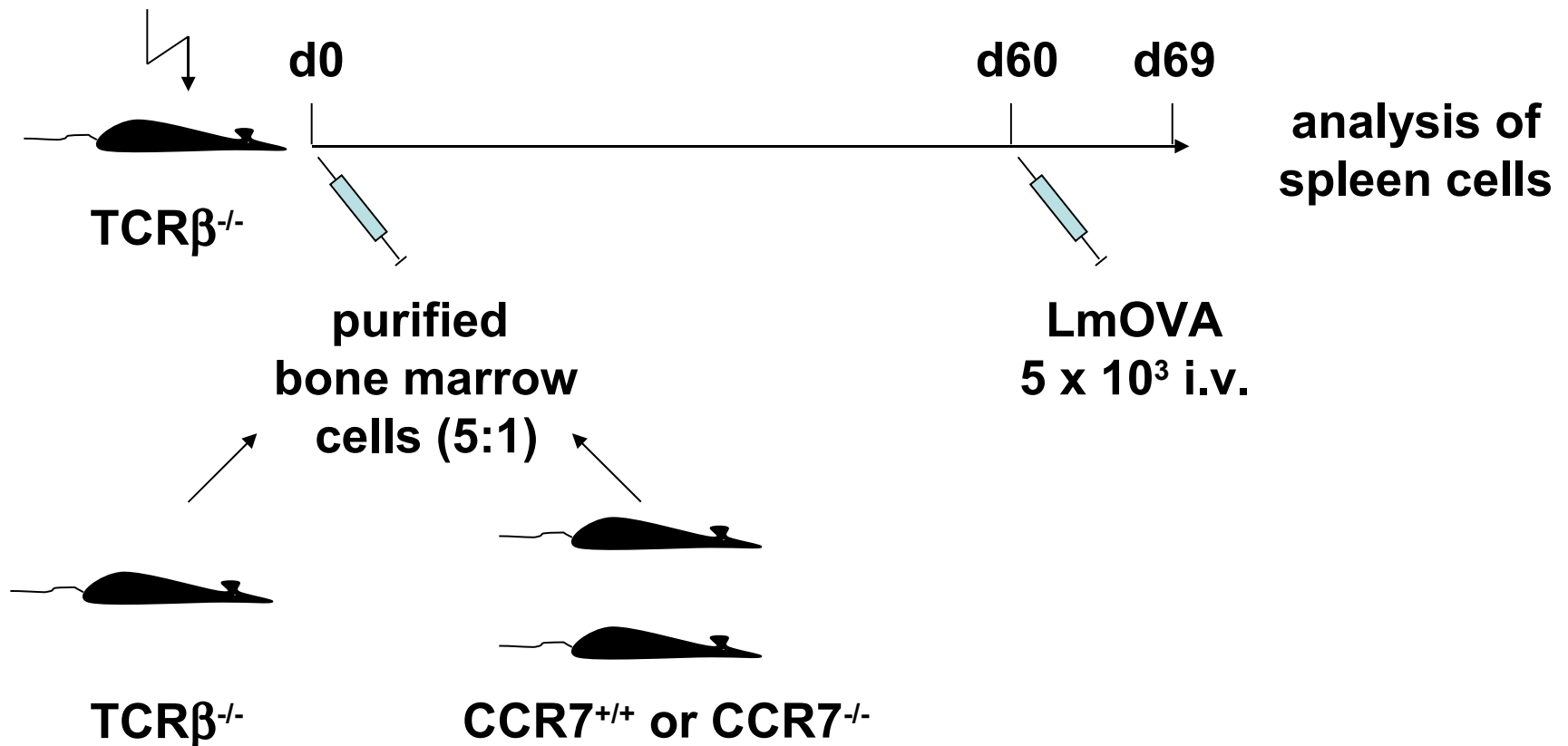
# Is expression of CCR7 on CD8<sup>+</sup> T cells sufficient for efficient T cell priming?



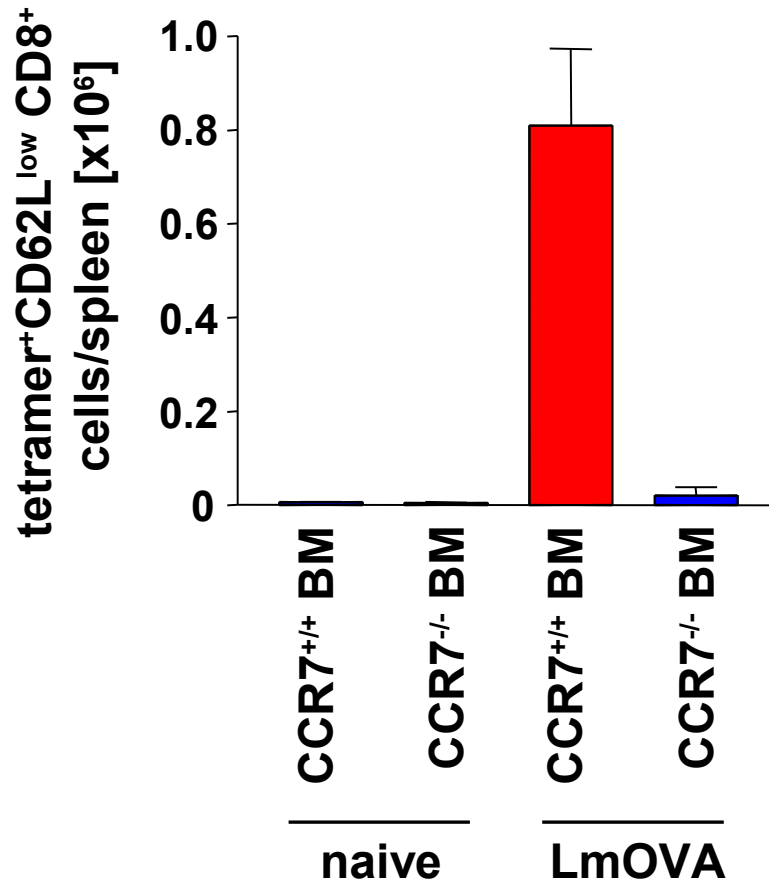
# Expression of CCR7 on CD8<sup>+</sup> T cells only is not sufficient for efficient T cell priming



# Is expression of CCR7 on antigen-presenting cells sufficient for priming of CD8<sup>+</sup> T cells?



# Expression of CCR7 on antigen-presenting cells is not sufficient for priming of CD8<sup>+</sup> T cells



## CCR7: Summary

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- **CCR7 is necessary for the priming of MHC class Ia restricted CD8<sup>+</sup> T cells.**
- **CCR7 is not required for the priming MHC class II restricted CD4<sup>+</sup> T cells.**
- **Memory T cell responses against *L. monocytogenes* are independent of CCR7.**
- **The expression of CCR7 on CD8<sup>+</sup> T cells or on professional antigen-presenting cells alone is not sufficient for priming.**

# Maturation and activation of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes

