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DCIR expression on dendritic cells decelerates early T cell activation in a murine mixed bone marrow chimera model of neurotropic virus infections

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Introduction

The pattern recognition receptor Dendritic cell immunoreceptor (DCIR) is a myeloid C-type lectin receptor that is expressed on antigen-presenting cells, e.g. dendritic cells (DCs), and promotes ambivalent functions depending on the initial trigger[1, 2]. During acute Theiler's murine encephalomyelitis (TME) DCIR expression inhibits antiviral immunity contributing to virus- and immunity-related detrimental neuropathological changes[3].

Here, mixed bone marrow chimeras were utilised to gain insight into DC-specific DCIR expression related effects on antiviral immune responses and neuropathological processes upon TMEV infection in vivo.

Materials and methods

- Generation of mixed bone marrow chimera and intracerebral infection with Theiler's murine encephalomyelitis virus Daniels strain (**Fig. 1**)
- Flow cytometry of blood and spleen
  - detection of green fluorescent protein expressing (GFP^+) cells (**Fig. 2**)
  - splenic peripheral T lymphocyte activation (**Fig. 3**)
- Immunohistochemistry (IHC) of hippocampus (**Fig. 3**)
  - neuronal degeneration: neuronal nuclei (NeuN)-specific IHC
  - viral load: TMEV-specific IHC

Experimental setup

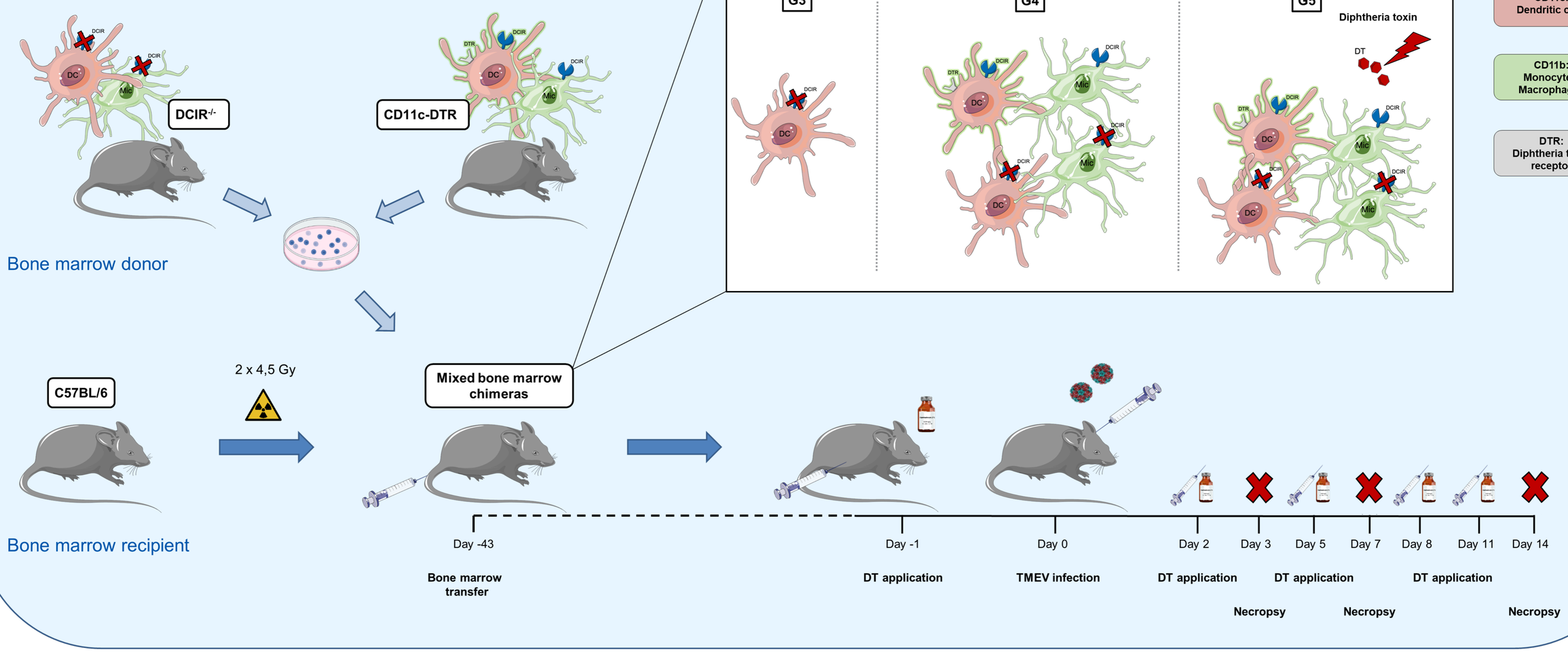

Fig. 1: Experimental setup. Generation of mixed bone marrow chimeras by sublethal irradiation of C57BL/6 mice and transfer of bone marrow cells from $DCIR^{-/-}$- and CD11c-diphtheria toxin receptor (DTR)-transgenic donor mice. Selective depletion of DTR-expressing $DCIR^+CD11c^+$DCs was achieved by repeated diphtheria toxin (DT) applications. 6 weeks post irradiation mice were TMEV-infected intracerebrally and sacrificed 3, 7 and 14 days post infectionem.

Results

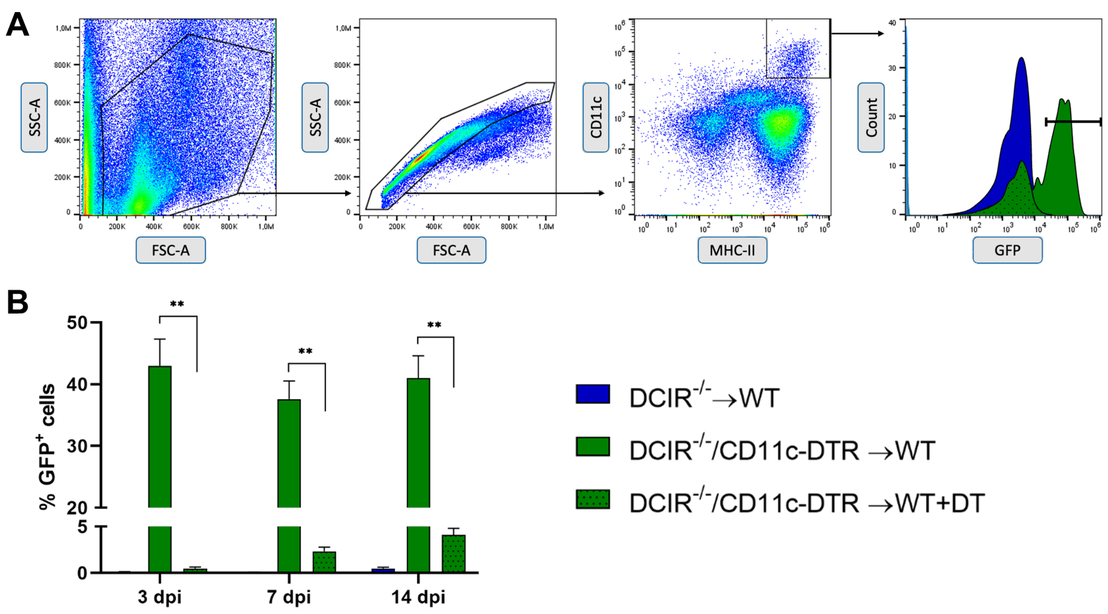

Fig. 2. Flow cytometric analysis of blood samples from TMEV-infected mixed bone marrow chimeras.
Exemplary gating strategy of blood samples to detect green fluorescent expressing (GFP) cells within the $CD11c^+MHC\text{-}II^+$ cell population (**A**) and percentages of GFP^+ cells within the $CD11c^+MHC\text{-}II^+$ cell population of blood samples (**B**).
Statistical analysis: Mann-Whitney U test (* = $p \leq 0.05$, ** = $p \leq 0.01$); data are shown as mean with SEM.
n: 3 dpi = 5 $DCIR^{-/-}$ > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.
n: 7 dpi = 4 $DCIR^{-/-}$ > WT; 5 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.
n: 14 dpi = 6 $DCIR^{-/-}$ > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.

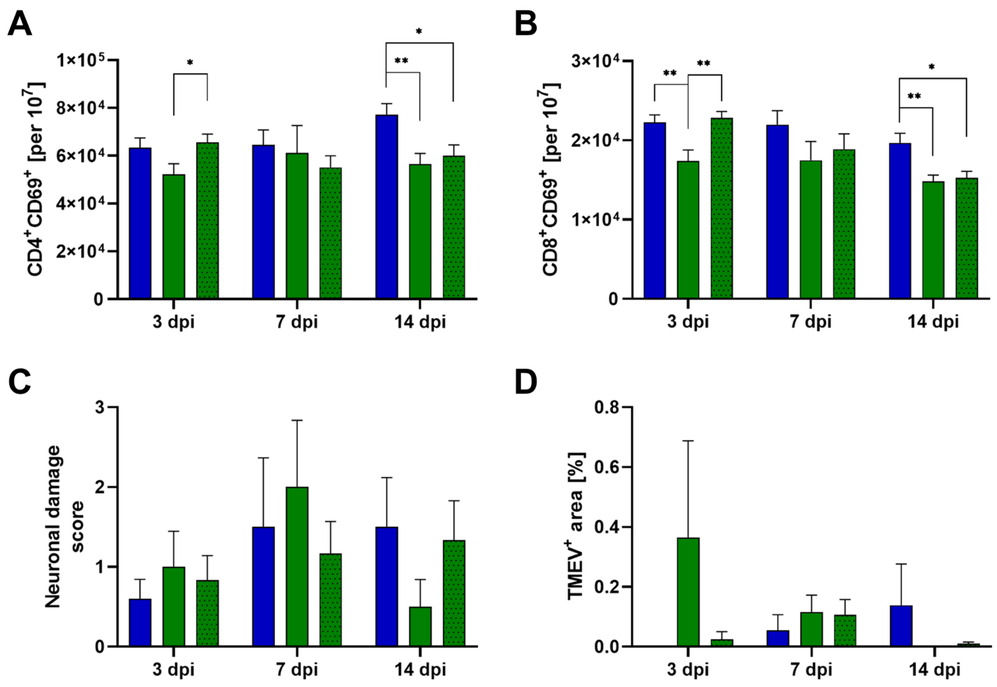

Fig. 3. Flow cytometric analysis of spleens (**A, B**) and immunohistochemical assessment of hippocampal neuronal damage (**C**) and viral load (**D**) from TMEV-infected mixed bone marrow chimeras.
The color coding legend of the groups is shown in Figure 2.
Statistical analysis: Mann-Whitney U test (* = $p \leq 0.05$, ** = $p \leq 0.01$); data are shown as mean with SEM.
n: 3 dpi = 5 $DCIR^{-/-}$ > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.
n: 7 dpi = 4 $DCIR^{-/-}$ > WT; 5 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.
n: 14 dpi = 6 $DCIR^{-/-}$ > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT; 6 $DCIR^{-/-}$/CD11c-DTR > WT+DT.

Conclusion & Outlook

- Depletion of diphteria toxin (DT) sensitive dendritic cells with DT application every 3 days is successful and verified by flow cytometric detection of green fluorescent protein (GFP) within the $CD11^+MHC\text{-}II^+$ cell population.
- DC-specific expression of DCIR promotes the inhibitory impact on antiviral immunity upon TMEV infection affecting peripheral T cell activation and cerebral integrity.
- Group sizes will be increased, additional control groups of irradiated C57BL/6 with homologous bone marrow transfer will be considered, and further cytokine expression and viral RNA quantity will be determined.

References

[1] Mayer, Raulf and Lepenies, Histochemistry and Cellbiology, 2016. [2] Maglinao et al, Journal of Immunology, 191, 2013.
[3] Stoff et al., Scientific Reports, 11, 2021

Figure 1 contains modified images from Servier Medical Art (https://smart.servier.com) licensed by CC BY 3.0 and modified icons created with BioRender.com.

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