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Signalling of the C-type lectin receptor CLEC12A restrains protective immunity during acute Theiler's murine encephalomyelitis virus infection

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Introduction

Theiler's murine encephalomyelitis virus (TMEV) is an enteric pathogen of rodents belonging to the family *Picornaviridae*. Intracerebral TMEV infection represents a reliable model to study virus-induced hippocampal damage and seizure development[1]. C-type lectin domain family 12 member A (CLEC12A) is an inhibitory C-type lectin receptor which negatively regulates the functions of innate immune cells[2,3] but its role in neurotropic viral infection has not yet been determined. The current study aims at characterizing the effect of CLEC12A upon antiviral immunity and virus-mediated neuropathology in a knockout mouse model.

Materials and Methods

Animal experiment

- Five week old wild type (WT) C57BL/6 and CLEC12A$^{-/-}$ mice
- Intracerebral infection with 1×10^5 PFU DA strain of TMEV
- Necropsy at 3, 7 and 14 days post infection

Techniques

- Histology – Serial sections of formalin-fixed paraffin-embedded (FFPE) cerebral tissues were used for hematoxylin and eosin (HE) staining. Hippocampal evaluation was done by densitometry using the QuPath software (version 0.3.2).
- Immunohistochemistry – FFPE cerebral tissues were used for NeuN- (neurons), TMEV- (virus), CD45R- (B cells), CD3- (T cells), CD107b- (microglia/macrophages), and GFAP-specific (astrocytes) immunohistochemistry. Densitometric analyses was performed by using the QuPath software (version 0.3.2).
- Flow cytometry – Phenotypical changes of splenic lymphocytes were determined by flow cytometry using CD4-, CD44-, CD62L-, CD69- and CD19-specific markers.
- Statistical Analysis – Mann-Whitney *U*-test was used for statistical analysis (SPSS Statistics 27)

Results

Histological and immunohistochemical analyses revealed increased inflammatory responses and enhanced CD3$^+$ T cell infiltration and increase of GFAP in the hippocampus of infected CLEC12A$^{-/-}$ mice at 3 dpi (Fig.1). Significantly reduced numbers of TMEV-infected cells were observed in the hippocampus of CLEC12A$^{-/-}$ mice at 7 dpi (Fig.1). At 7 and 14 dpi increased numbers of GFAF$^+$ astrocytes were found in brains of WT mice indicative of astrocytosis. No differences were found between CLEC12A$^{-/-}$ mice and WT mice regarding the number of NeuN$^+$ hippocampal neurons as well as of brain-infiltrating CD45R$^+$ B cells and CD107b$^+$ macrophages/microglia. Flow cytometric analyses of spleens showed increased frequencies of CD4$^+$CD69$^+$ T cells in CLEC12A$^{-/-}$ mice at 3 dpi (Fig.2)

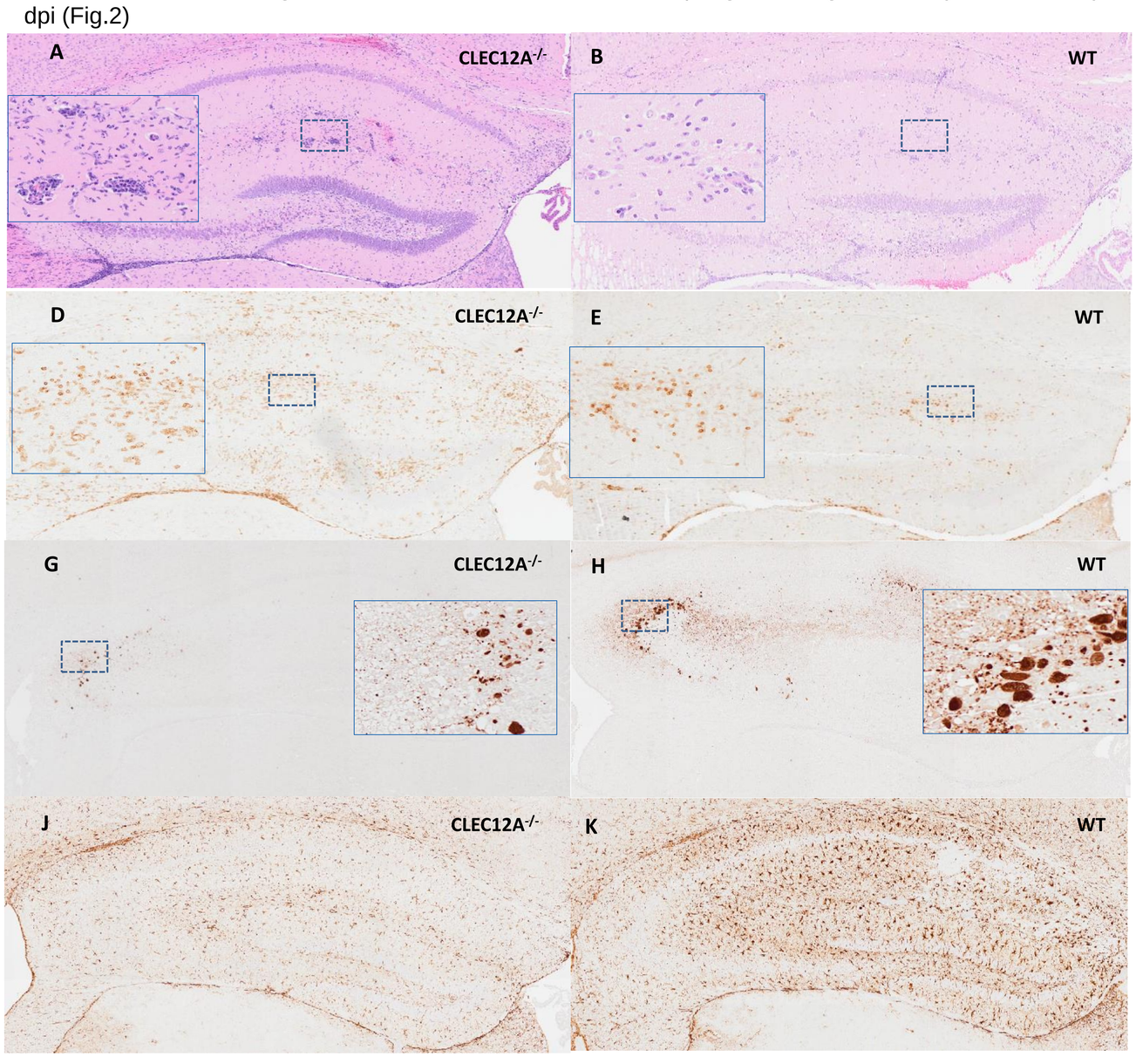

Fig.1 Evaluation of the hippocampus

A-C: Significant increase of inflammatory responses in the hippocampus of infected CLEC12A$^{-/-}$ mice (C12A) compared to wild type mice (WT) at 3 dpi (HE staining). Perivascular inflammatory infiltrates (•). (*$p \leq 0.05$; Mann-Whitney *U*-test).

D-F: CLEC12A$^{-/-}$ mice (C12A) show a significant increase of CD3$^+$ cells in the hippocampus as compared to wild type mice (WT) at 3 dpi. (*$p \leq 0.05$; Mann-Whitney *U*-test).

G-I: CLEC12A$^{-/-}$ mice (C12A) show significantly less TMEV$^+$ cells in the hippocampus as compared to wild type mice (WT) at 7 dpi. (*$p \leq 0.05$; Mann-Whitney *U*-test).

J-L: CLEC12A$^{-/-}$ mice (C12A) show significantly more GFAP$^+$ cells in the hippocampus as compared to wild type mice (WT) at 3 dpi and increased numbers of GFAP$^+$ cells in WT mice at 7 and 14 dpi. (*$p \leq 0.05$; Mann-Whitney *U*-test).

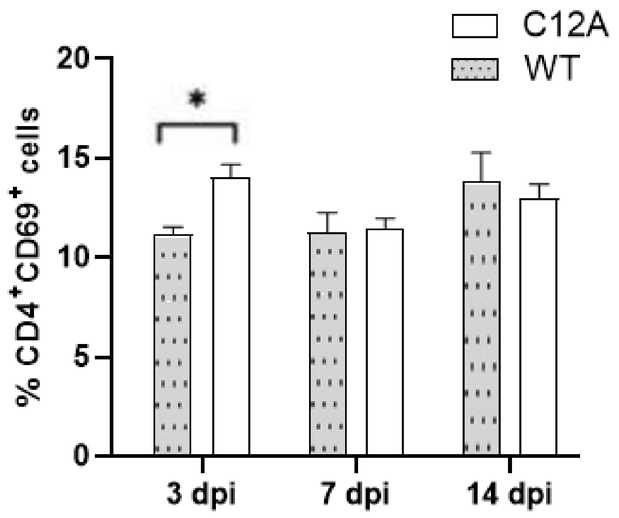

Fig.2: Flow cytometric analysis of spleen tissue
Significant increase of CD4$^+$CD69$^+$ T cells in CLEC12A$^{-/-}$ mice (C12A) at 3 dpi as compared to wild type mice (WT) following TMEV infection (*$p \leq 0.05$; Mann-Whitney *U*-test).

- CLEC12A deficiency leads to transiently enhanced neuroinflammation and T cell recruitment to the brain.
- CLEC12A deficiency supports viral elimination in C57BL/6 mice following TMEV infection without increasing virus-induced immunopathology
- CLEC12A inhibits peripheral T cell activation and antiviral immunity in acute neurotropic virus infection.
- C-type lectin receptors represents potential targets for intervention strategies to selectively enhance protective immunity in neurotropic virus infection.

References

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