IgA deficiency exacerbates inflammatory demyelination in a viral model of multiple sclerosis

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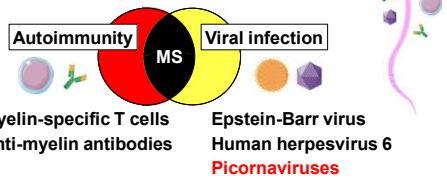
Abstract

[Background] Multiple sclerosis (MS) is an immune-mediated disease characterized by inflammatory demyelination in the central nervous system (CNS). Although the precise pathomechanism of MS is unclear, immunoglobulin (Ig) A has been proposed to play a role in CNS lesions. In human MS and its autoimmune model, experimental autoimmune encephalomyelitis (EAE), IgA-producing B cells have been detected in demyelinating lesions. Since Theiler's murine encephalomyelitis virus (TMEV) infection in mice can induce MS-like demyelination by viral persistence and immunopathology, TMEV infection has been widely used as a viral model of MS. Using the TMEV model, we previously demonstrated the IgA mRNA upregulation and IgA-producing B-cell infiltration in demyelinating lesions. Although the physiological role of IgA in mucosal immunity including regulation of the gut microbiota is well-known, the role of IgA in MS and CNS viral infections remains unknown. Thus, we aimed to determine the role of IgA in the TMEV model. [Methods] We generated IgA-deficient (IgA-/-) mice on the background of SJL/J mice. We infected SJL/J wild-type (WT) and IgA-/- mice intracerebrally with TMEV and monitored their clinical signs by evaluating impaired righting reflex scores for 2 months. We compared neuropathology and antiviral cellular and humoral responses between WT and IgA-/- mice. [Results] During the observation period, we found that the clinical scores were higher in IgA-/- mice than in WT mice. Histologically, IgA-/- mice developed more severe inflammatory demyelination with a larger number of viral antigen-positive cells in the CNS, compared with WT mice. Immunologically, IgA-/- mice had greater amounts of interleukin (IL)-17 and IL-10 than WT mice. On the other hand, there were no differences in interferon (IFN)-γ and IL-4 levels, lymphoproliferative responses to TMEV, or anti-viral IgG1 and IgG2c antibody titers. [Conclusions] Since viral persistence plays a pathogenic role in the TMEV model, the higher levels of viral antigen-positive cells in the CNS would cause more severe inflammatory demyelination in IgA-/- mice than in WT mice. This suggests that IgA-producing B cells could play a protective role in the CNS, where anti-viral IgA could neutralize TMEV. Alternatively, since the gut microbiota compositions have been known to be regulated by IgA, dysbiosis of the gut microbiota in IgA-/- mice may contribute to the exacerbation of inflammatory demyelination in the TMEV model.

Background

Multiple sclerosis (MS)

- Inflammatory demyelinating disease in the central nervous system (CNS)
- Approximately 20,000 patients in Japan with the ratio of women to men of 3 : 1
- Etiology: autoimmunity and viral infection

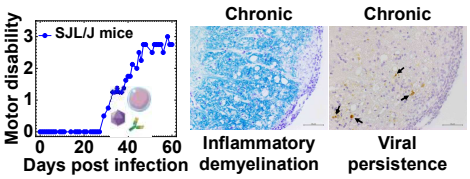


Myelin-specific T cells
Anti-myelin antibodies

Epstein-Barr virus
Human herpesvirus 6
Picornaviruses

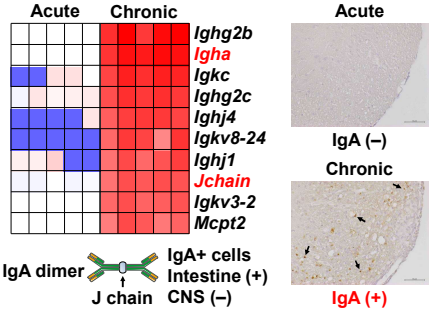
Viral model of MS: Theiler's virus

- Non-enveloped, positive-sense, single-stranded RNA virus belonging to the picornavirus family
- Inflammatory demyelination with viral persistence in the CNS of SJL/J mice during the chronic phase
- Immune effectors: T cells and IgG



IgA/IgA+ cells in Theiler's virus infection

- IgA-related gene upregulation, IgA depositions, and IgA+ cells in demyelinating lesions

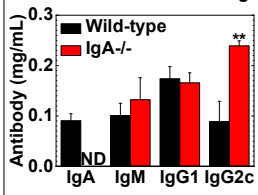


IgA/IgA+ cells in MS and experimental autoimmune encephalomyelitis (EAE)

- Higher IgA levels in cerebrospinal fluid of active MS patients compared with inactive MS patients
- IgA+ cells in active demyelinating lesions of MS
- Amelioration of EAE via interleukin (IL)-10 production from IgA+ cells
- Precise roles of IgA in MS?

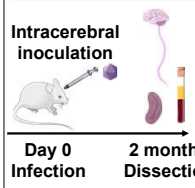
Aim & IgA deficient (IgA-/-) mice

To determine the roles of IgA in the Theiler's virus model of MS using SJL/J IgA-/- mice



We generated IgA-/- mice on the SJL/J mouse background and confirmed lack of IgA in serum by enzyme-linked immunosorbent assays (ELISAs).

Methods

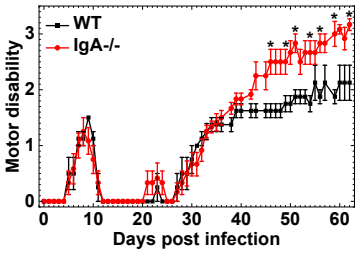


- CNS
  - Luxol fast blue stain
  - Immunohistochemistry
- Spleen/serum
  - Cytokine ELISA
  - Lymphoproliferative assay
  - Antibody ELISA

We infected SJL/J wild-type (WT) and IgA-/- mice with Theiler's virus and monitored the clinical signs by evaluating impaired righting reflexes for 2 months. The CNS tissue and spleen/serum were harvested for neuropathological and immunological analyses.

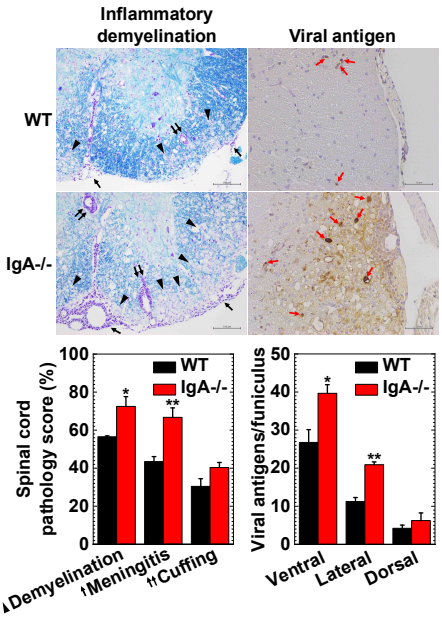
Results

IgA-/- mice exhibit more severe motor disability in the Theiler's virus model of MS



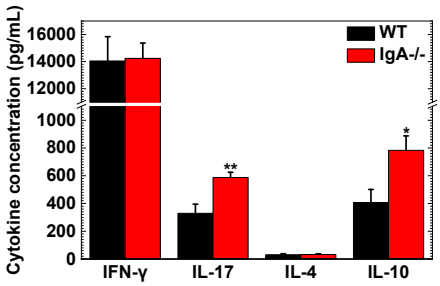
After Theiler's virus infection, motor disability was evaluated by impaired righting reflexes for 2 months.
* $P < 0.05$, Mann-Whitney U test.

IgA-/- mice have more severe inflammatory demyelination with a larger number of viral antigen-positive cells in the CNS



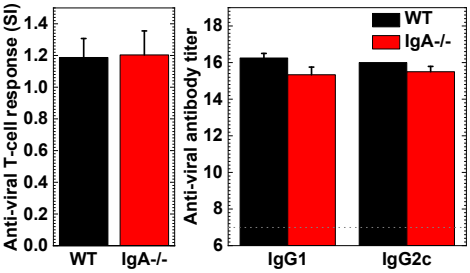
The spinal cord sections were stained with Luxol fast blue for myelin visualization. Viral antigens were detected by immunohistochemistry with an antibody against Theiler's virus. * $P < 0.05$ and ** $P < 0.01$, Student's t-test.

Interleukin (IL)-17 and IL-10 concentrations are higher in IgA-/- mice



Splenic mononuclear cells (MNCs) were stimulated with a mitogen, concanavalin A. The concentrations of IFN-γ, IL-17, IL-4, and IL-10 in the supernatants were quantified by ELISAs. * $P < 0.05$ and ** $P < 0.01$, Student's t-test.

Anti-viral T-cell and antibody responses are similar between WT and IgA-/- mice

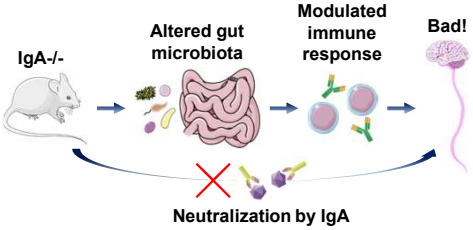


Anti-viral T-cell responses were determined using the Cell Counting Kit-8; anti-viral antibody titers were quantified by ELISAs. The dotted line indicates the detection limit.

Conclusions

- IgA deficiency worsened Theiler's virus-induced demyelinating disease.
- IgA deficiency exacerbated both inflammatory demyelination and viral persistence in the CNS.
- IgA deficiency enhanced both proinflammatory IL-17 and anti-inflammatory IL-10 productions.
- IgA deficiency did not alter anti-viral immunity.

Working hypothesis



- Since IgA is known to regulate the gut microbiota, IgA-/- mice would have the altered gut microbiota compositions, which modulate immune responses.
- IgA deficiency may reduce viral clearance in the CNS.
- Future experiments
  - 16S rRNA sequencing
  - Double staining of IgA and viral antigens

References, Grant, & COI

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- COI: The authors have no financial relationships to disclose