Viral infection activates myelin-specific T cells, triggering MS-like CNS inflammatory demyelination

Fumitaka Sato, Ph.D.
fumi_taka_sato@yahoo.com

Fumitaka Sato[1,2], Seiichi Omura[1,2], Ah-Mee Park[1], Mitsugu Fujita[1], Karen Y. Stokes[2], Ikuo Tsunoda[1,2]

[1]Kindai University Faculty of Medicine, Osaka, Japan

[2]Louisiana State University Health Shreveport, LA, USA

Abstract

Background: Multiple sclerosis (MS) has been suggested to be triggered by microbial infections in genetically susceptible hosts harboring anti-myelin autoimmune T cells. Myelin oligodendrocyte glycoprotein (MOG)-specific T cell receptor (TCR) transgenic (tg) 2D2 mice develop experimental autoimmune encephalomyelitis (EAE) following MOG sensitization. In contrast, only 4% of 2D2-tg mice develop spontaneous EAE with mild inflammation in the central nervous system (CNS) after 3 months of age.

Objective: To determine whether microbial infection can activate MOG-specific T cells as an adjuvant and confer susceptibility to EAE in 2D2-tg mice.

Materials and Methods: We injected 6-week-old 2D2-tg or wild-type C57BL/6 mice intraperitoneally with the following microbes and microbe mimics: Theiler's murine encephalomyelitis virus (TMEV, RNA virus), poly(I:C) (RNA virus mimic), murine cytomegalovirus (MCMV, DNA virus), and curdlan (bacteria/fungus component, Th17 inducer).

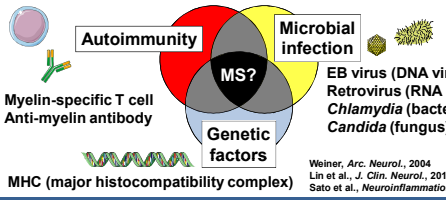
Results: During the 2-months observation period, intraperitoneal TMEV injection induced hind-limb paralysis in 43% of 2D2-tg mice (mean onset 36.7 ± 9.6 days) with severe inflammatory demyelination and axonal degeneration in the CNS. Among other groups, only a few 2D2-tg mice injected with MCMV or curdlan had mild CNS involvement. Intracerebral injection of TMEV was more effective for inducing CNS disease; 83% of 2D2-tg mice developed severe inflammatory demyelination with earlier onset time (13.1 ± 1.7 days). No wild-type mice developed CNS demyelination following any treatment (Note: C57BL/6 mice are TMEV-resistant, while susceptible SJL/J mice develop demyelination 1 month after intracerebral, but not intraperitoneal, TMEV infection).

Conclusions: Virus infection may activate anti-myelin T cells as an adjuvant, triggering CNS inflammatory demyelination.

Background

Multiple sclerosis (MS)

- Inflammatory demyelinating disease in the central nervous system (CNS)
- Certain microbial infections in genetically susceptible hosts harboring autoimmune responses to myelin

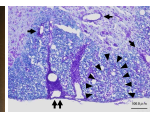


Weiner, *Arc. Neurol.*, 2004
Lin et al., *J. Clin. Neurol.*, 2015
Sato et al., *Neuroinflammation*, in press

Experimental autoimmune encephalomyelitis (EAE)

- Animal model of MS
- Similar to MS clinically and histologically
- Sensitizing mice with myelin antigen with complete Freund's adjuvant and pertussis toxin injection for inducing myelin-specific immune responses
- Complicated data interpretation due to the artificial sensitization protocol


Hind-limb paralysis

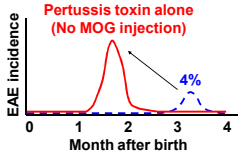

Inflammatory demyelination

Meningitis (↔)
Perivascular cuffing (↑)
Demyelination (▲)

Ransohoff, *J. Clin. Invest.*, 2006
Tsunoda et al., *J. Neuropathol. Exp. Neurol.*, 1996

Spontaneous EAE: 2D2-transgenic (tg) mice

- Have myelin oligodendrocyte glycoprotein (MOG)-specific T cell receptors (TCR) in the majority of $CD4^+$ T cells
- No disease in the first 3 months after birth
- Accelerate EAE with a higher incidence by injecting pertussis toxin alone
- Useful for investigating interactions between autoimmune responses and microbial infections

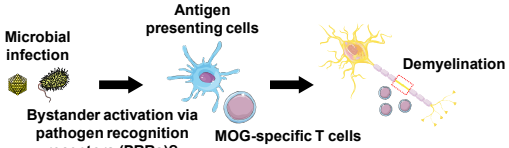


Bettelli et al., *J. Exp. Med.*, 2003

Gaps in knowledge and *"hypothesis"*

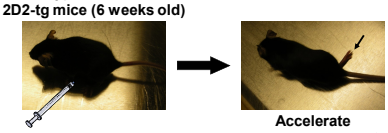
How certain microbial infections trigger MS?

"Microbes or microbial components could activate MOG-specific T cells as an adjuvant, causing EAE?"



Materials & Methods

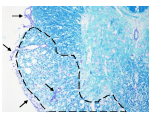
Experimental design

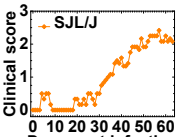


Wild-type C57BL/6 and 2D2-tg mice were intraperitoneally injected with the following microbes or microbe mimics; Theiler's virus, poly(I:C), murine cytomegalovirus, or curdlan. EAE signs and CNS pathology were evaluated.

Theiler's murine encephalomyelitis virus (Theiler's virus)

- Non-enveloped, positive-sense, single-stranded RNA virus that belongs to the family *Picornaviridae*
- Recognized by toll-like receptor (TLR) 3 and TLR7
- Induces immune-mediated demyelination 1 month after infection by only intracerebral injection
- Causes demyelination depending on mouse strains, MHC: susceptible SJL/J mice versus resistant C57BL/6 mice


Inflammatory demyelination



No disease
- Before 1 month
- By peripheral injection
- In C57BL/6 mice

Sato et al. *Sci. Rep.*, in press
Martinez et al., *Brain Behav. Immun.*, 2015
Sato et al., *Am. J. Pathol.*, 2013

Poly(I:C)

- Synthetic analog of double-stranded RNA
- Mimics RNA virus infections
- Activates the anti-viral pattern recognition receptor, TLR3

Murine cytomegalovirus (MCMV)

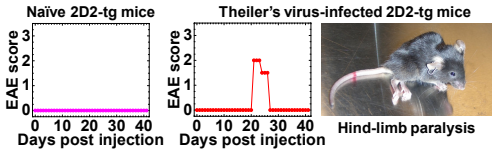
- Enveloped, double-stranded DNA virus that belongs to the family *Herpesviridae*
- Recognized by TLR9 and cyclic GMP-AMP synthase (cGAS)

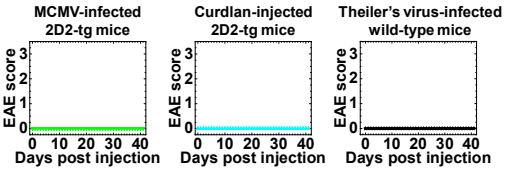
Curdlan

- Linear β-1,3-glucan, a component of bacteria and fungi
- Activates Dectin-1, a C-type lectin, on dendritic cells, enhancing Th17 cell differentiation

Results

Intraperitoneal injection of Theiler's virus accelerates spontaneous EAE in 2D2-tg mice

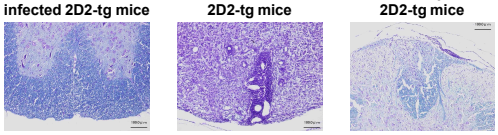




EAE sign	Naïve	Theiler's virus	MCMV	Curdlan
2D2-tg	–	+	–	–
Wild-type	–	–	–	–

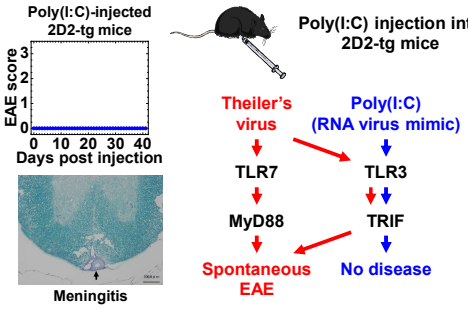
During the 6 week observation period, naïve 2D2-tg mice did not exhibit EAE signs. In contrast, Theiler's virus infected-2D2-tg mice had EAE signs, including hind-limb paralysis. On the other hand, we did not see EAE signs in 2D2-tg mice treated with MCMV or curdlan and wild-type mice with or without microbe injection.

Intraperitoneal injection of Theiler's virus causes severe demyelination in 2D2-tg mice



Luxol fast blue staining showed no demyelination in naïve or MCMV-infected 2D2-tg mice (left). In contrast, Theiler's virus-infected 2D2-tg mice with EAE developed severe inflammatory demyelination (middle). Although curdlan-treated 2D2-tg mice did not exhibit EAE signs, a few mice from the group had mild inflammation in the CNS (right).

Poly(I:C), an RNA virus mimic, does not induce EAE in 2D2-tg mice



A few poly(I:C)-injected 2D2-tg mice had mild inflammation in the CNS, but not EAE signs. Theiler's virus is recognized by both TLR7/MyD88 and TLR3/TRIF pathways. In contrast, poly(I:C) activates the TLR3/TRIF pathway. This may explain why Theiler's virus infection is more effective for inducing EAE in 2D2-tg mice.

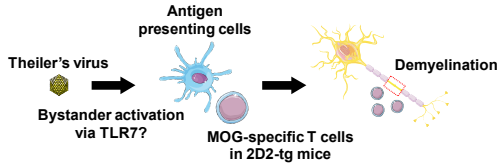
Intracerebral injection of Theiler's virus is more effective for inducing EAE

Injection route	EAE incidence	Onset days
Intracerebral	83%	13.1 ± 1.7
Intraperitoneal	43%	36.1 ± 9.6

2D2-tg mice were infected with Theiler's virus either intracerebrally or intraperitoneally. While the incidence of EAE was higher in the intracerebral injection than in the intraperitoneal injection, the mean onset days of EAE was earlier in intracerebral injection compared with intraperitoneal injection.

Conclusions

- Theiler's virus infection accelerates spontaneous EAE
- Other microbes and microbe mimics also act as adjuvants, inducing subclinical EAE
- Theiler's virus may act as an effective adjuvant, triggering demyelination by bystander activation
- Which PRRs and what factors are critical for inducing bystander activation?



Acknowledgements

Grants
- National Institute of General Medical Sciences COBRE Grant
- National Institute of Neurological Disorders and Stroke of the NIH
- National Center for Research Resources of the NIH
- KAKENHI from the Japan Society for the Promotion of Science
- Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan

Excellent technical assistance
- Namie Sakiyama (Kindai University Faculty of Medicine)
- Kimberly N. Legaux, M.S. (Louisiana State Health Shreveport)
- Tawsha G. Creason (Louisiana State Health Shreveport)