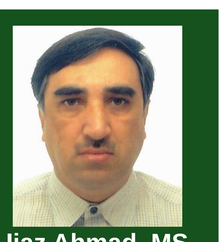
Platelet Involvement in Virus-Induced Inflammatory Demyelinating Disease and Myocarditis



Ijaz Ahmad, MS
ijazahmad383@gmail.com

Ijaz Ahmad[1], Seiichi Omura[1], Fumitaka Sato[1], Koya Mineta[1], Toshiki Izumi[1], Ah-Mee Park[1], Sundar Khadka[1, 2], Cong Thanh Nguyen[1], Reona Shiro[1], Felicity N. E. Gavins[3], Ikuo Tsunoda[1]

[1]Kindai University, Osaka, Japan, [2]Duke University, USA, [3]Brunel University London, UK

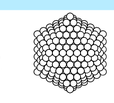
Abstract

Viral infections have been shown to cause damages in specific organs by the host's immune responses to the pathogen (immunopathology). The precise pathomechanism of organ-specific immunopathology in various viral infections remain unknown, although the tissue tropism of viruses is one key factor in these organ-specific immune-mediated disease. Theiler's murine encephalomyelitis virus (TMEV) infection is a unique experimental system to clarify the pathomechanisms of organ-specific viral-induced inflammatory diseases, since TMEV can induce distinct immunopathology in the central nervous system (CNS) and the heart. TMEV-induced demyelinating disease (TMEV-IDD) in the CNS is a chronic inflammatory disease with viral persistence and an animal model of multiple sclerosis (MS) in humans. TMEV infection can also cause myocarditis with immune cell/effector infiltrations in the heart, leading to cardiac fibrosis. Since platelets have been proposed to play immunomodulatory roles in viral infections and immune-mediated diseases, we aimed to determine the role of platelets in TMEV infection. We infected mice with TMEV and harvested sera, platelets, lymphoid organs, the CNS, and the heart. We conducted transcriptome analyses of platelets and found that distinct sets of immune-related genes, including major histocompatibility complex (MHC) class I, were up- or down-regulated in TMEV infection. We depleted platelets from TMEV-infected mice by injecting platelet-specific GPIbα antibodies. Histologically, platelet-depleted mice had significantly fewer viral antigen-positive cells and reduced the severities of TMEV-IDD and myocarditis. Immunologically, platelet-depleted mice had an increase in interferon (IFN)-γ production with a higher anti-TMEV IgG2a/IgG1 ratio. Thus, platelets could play a detrimental role in TMEV infection by modulating IFN-γ and antibody isotype responses; this could interfere with viral clearance, resulting in more severe CNS and cardiac pathologies. Therefore, platelets can be a target in human MS and myocarditis.

Background

Theiler's virus

- Theiler's murine encephalomyelitis virus
- Non-enveloped, single-stranded +RNA virus that belongs to the family *Picornaviridae*
- Isolated in 1934 by Max Theiler
- Two mouse models
  - Central nervous system (CNS) infection causes an inflammatory demyelinating disease, similar to human multiple sclerosis
  - Heart infection causes in myocarditis, similar to human viral myocarditis

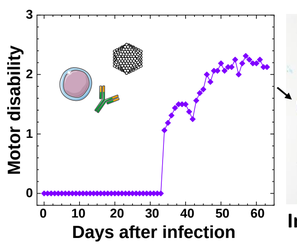


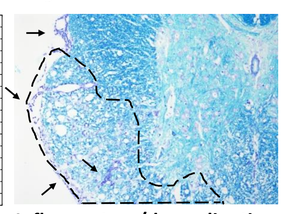
Theiler's virus

Max Theiler
(1899 – 1935)

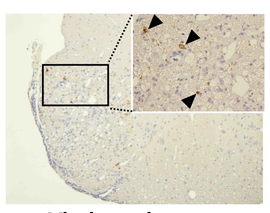
Multiple sclerosis

- Inflammatory demyelinating disease in the CNS
- Two etiologies: 1) autoimmunity and 2) viral infections
- Theiler's virus model of multiple sclerosis
- Inflammatory demyelination, similar to human MS, leading to motor disability in infected mice
  - Both immune-mediated damage and viral persistence contribute to demyelination





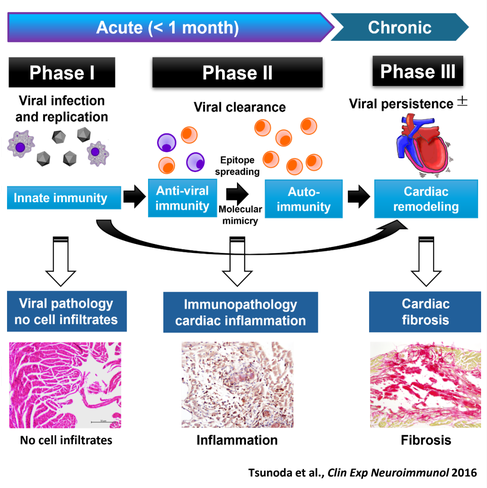
Inflammatory/demyelination



Viral persistence

Viral myocarditis

- Inflammatory disease in the heart with three phases
- Human myocarditis caused by several viral infections: adenovirus, SARS-CoV-2, and Coxsackie B virus
- Animal models: Coxsackie B virus and Theiler's virus
- Theiler's virus-induced myocarditis, similar to human myocarditis

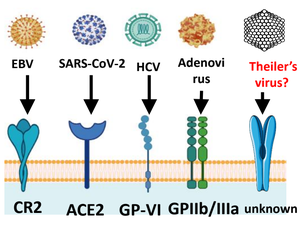


Tsunoda et al., *Clin Exp Neuroimmunol* 2016

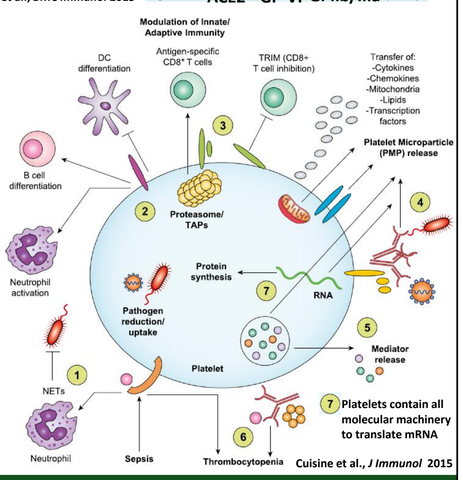
New roles of platelets in viral infections

Platelets

- express viral receptors
  - ➡ Viral clearance or delivery/replication in other organs
- contain immune molecules/mRNAs
  - ➡ Expression/delivery of immune molecules in other organs
- interreact with various immune cells
  - ➡ Modulation of anti-viral immunities, e.g., MHC class I expression and IgG isotype switch



Chabert et al., *BMC Immunol* 2015



Cuisine et al., *J Immunol* 2015

Aim and Methods

Aim: Determine the role of platelets in viral models of multiple sclerosis and myocarditis

- Viral quantification and transcriptome in platelets by RNA sequencing on days 4, 7, and 35 after virus infection
- Platelet-depletion by anti-GPIbα antibodies at early or late time points
- CNS and cardiac histology, anti-viral IgG isotypes, and cytokines
- Adverse effects: anemia and fecal occult hemorrhage

Day 0: Theiler's virus infection

Day 35

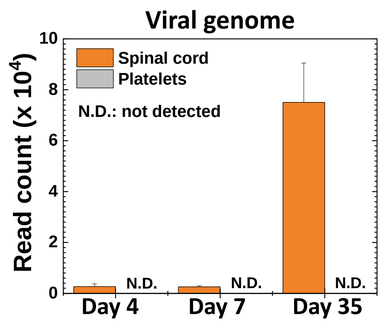
Early: days 0 and 5

Late: days 18 and 22

- Viral quantification
- Platelet transcriptome
- CNS/heart histology
- IgG and cytokines

Results

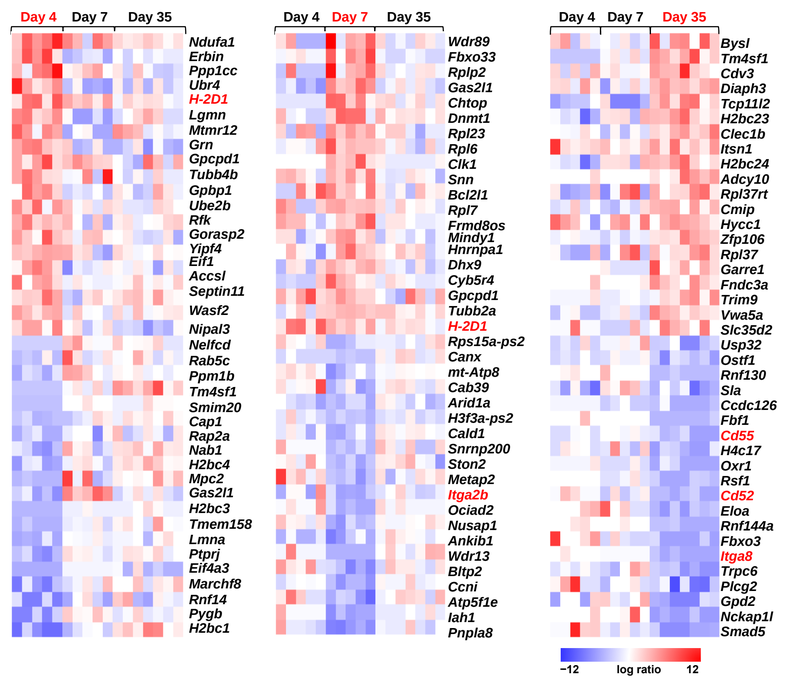
No Theiler's virus genome in the platelets



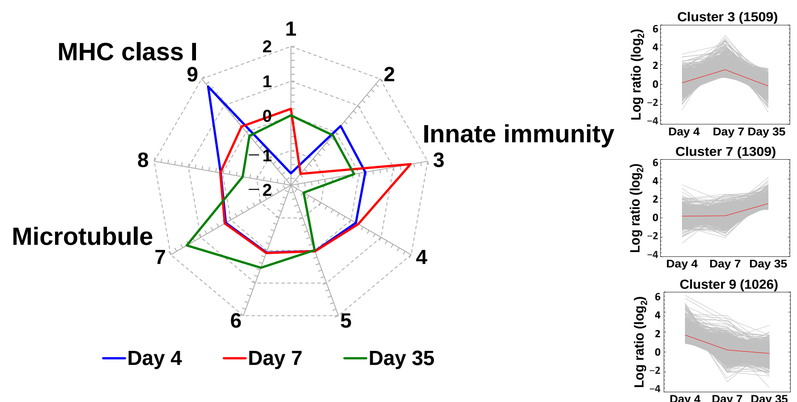
- In the spinal cord, we detected a small number of viral genomes on days 4 and 7 and a large number of viral genomes on day 35.
- In platelets, viral genome was not detectable at any time points.

Platelet transcriptome changes in virus infection

Top 20 up- or down-regulated genes in platelets of Theiler's virus-infected mice

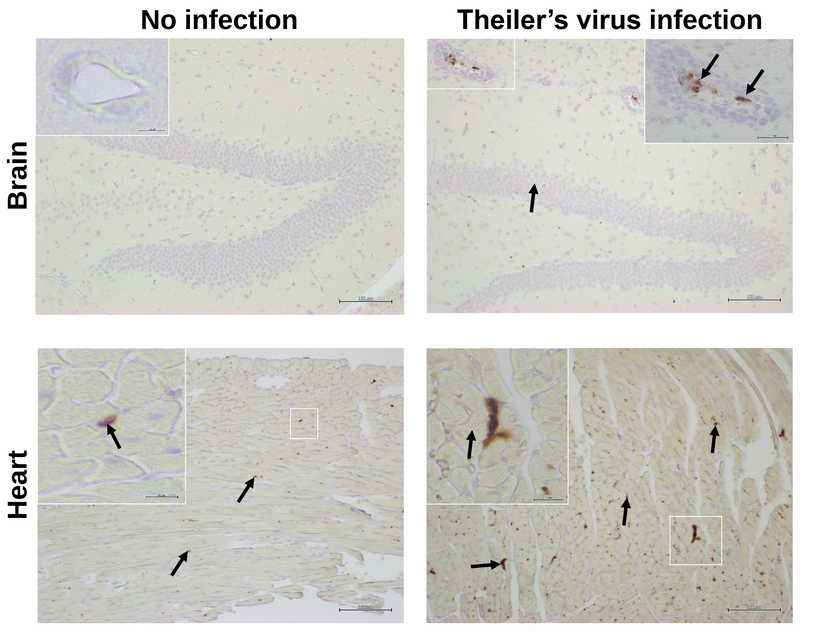


K-means clustering of gene expression patterns divided the genes into nine clusters



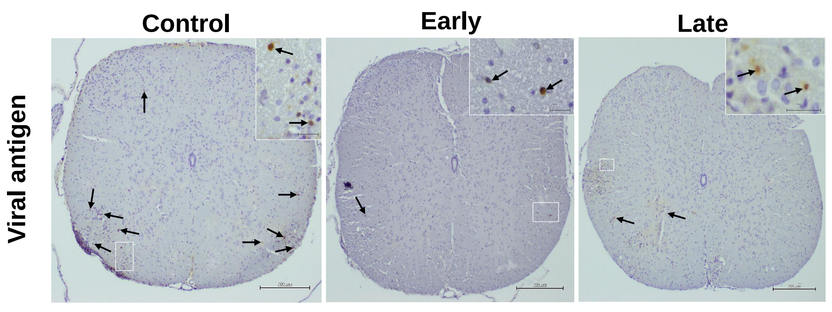
- Cluster 3: innate immune genes (*Oas3*, *Tlr12*, and *Tlr13*) upregulated on day 7
- Cluster 7: microtubule-related genes (tubulin, dynein, and kinesin) upregulated on day 35
- Cluster 9: MHC class I-related genes (*H-2D1*, *H-2K1*, and *β2M*) upregulated on day 4

Platelets are present in the brain and heart of Theiler's virus-infected mice



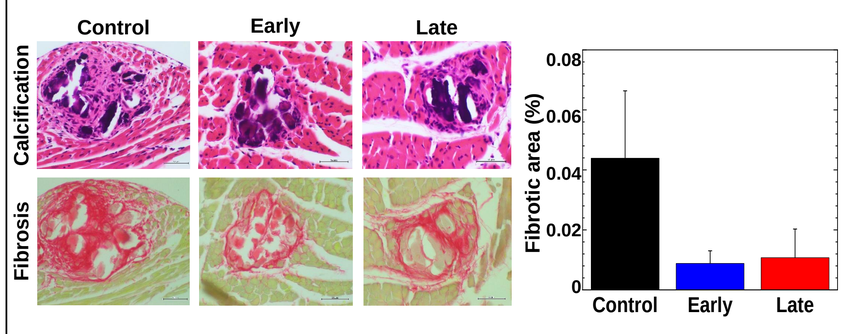
Immunohistochemistry using anti-platelet antibodies in Theiler's virus infection. In the brain, platelets attached to the luminal side of vascular endothelia. In the heart, platelet infiltrated diffusely.

Platelet depletion suppresses viral persistence and pathology in the spinal cord



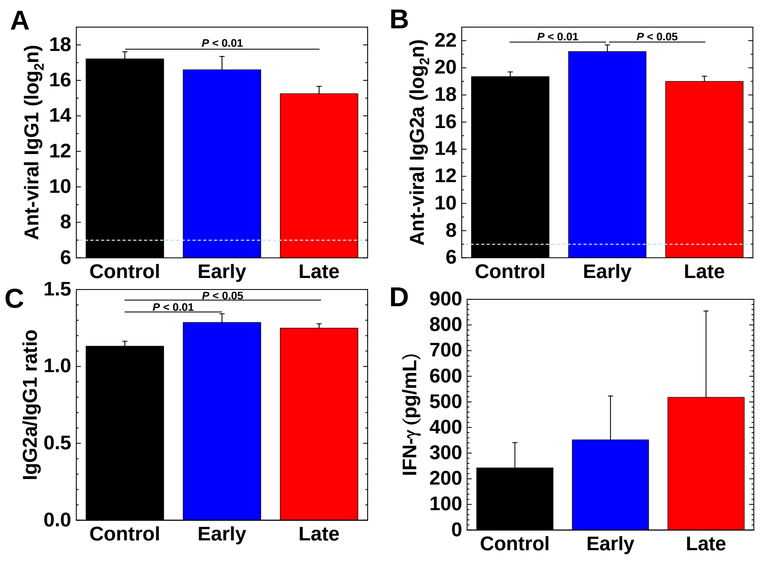
In the spinal cord, platelet-depleted groups (Early and Late) had fewer numbers of viral antigen+ cells. Platelet depletion also suppressed spinal cord pathology: meningitis, demyelination, and overall pathology.

Platelet depletion suppresses myocarditis



In the heart, platelet-depleted groups (Early and Late) had less severe calcification (dark purple, H&E stain) and fibrosis (picrosirius red stain) than the control group. Fibrotic areas were quantified by ImageJ.

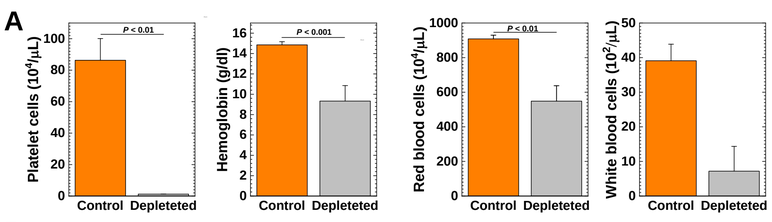
Platelet depletion alters IgG isotypes and interferon-γ production

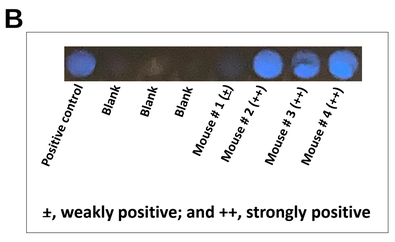


(A) Anti-viral IgG1 titers were lower in the Late group.
(B) Anti-viral IgG2a titers were higher in the Early group.
(C) IgG2a vs IgG1 ratios (Th1/Th2 balance) were higher in platelet-depleted groups (Early and Late).
(D) Platelet-depleted groups (Early and Late) had higher interferon (IFN)-γ production. No differences in productions of other cytokines (IL-4, IL-10, or IL-17) among the groups.

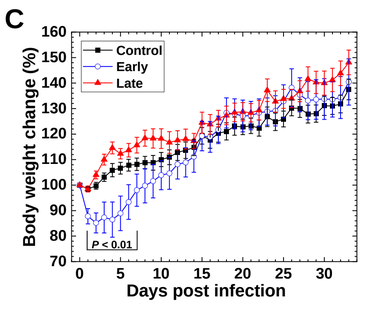
Discussion

Shortcomings of platelet depletion





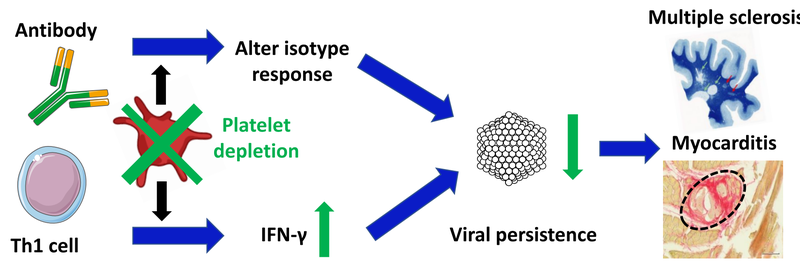
Fecal occult blood



(A) Platelet-depleted mice had severe anemia with low hemoglobin concentrations and reduced RBC counts; WBC counts were also decreased.
(B) Fecal occult blood in platelet-depleted mice was detected by the luminol reaction (Park and Tsunoda, *Biotechniques* 2018).
(C) Intracerebral viral inoculation with platelet-depletion resulted in body weight loss in the Early group, in which 45% (5/9) of mice died due to brain hemorrhage.

- Antibody injections more than two per mouse became less efficient platelet-depletion, likely due to the generation of neutralizing anti-rat IgG.
- Possible troubleshooting: functional blocking of platelets once the platelet target molecule is discovered.

Conclusions



- No virus was detected in platelets
- Platelet transcriptome was changed during the time course of Theiler's virus infection
- Platelet depletion suppressed spinal cord and cardiac pathologies
- Platelet depletion altered anti-viral IgG isotypes and IFN-γ production
- Platelets may play a detrimental role in multiple sclerosis and viral myocarditis

Reference

- Ahmad I, Omura S, Sato F, Park A-M, Khadka S, Gavins FNE, Tsunoda I. (2024). Exploring the role of platelets in virus-induced inflammatory demyelinating disease and myocarditis. *Int Mol Sci*, 25(6), 3460.
- Omura S, Kawai E, Sato F, Martinez NE, Tsunoda I. (2018). Theiler's virus-mediated immunopathology in the CNS and heart: Roles of organ-specific cytokine and lymphatic responses. *Front Immunol*, 9: 2870.

Acknowledgements

- The authors have no financial relationships to disclose
- Monbukagakusho (MEXT 文部科学省) Scholarship, Japan
- Ijaz Ahmad is a PhD MEXT (2020) student from Pakistan