多発性硬化症の自己免疫およびウイルスモデルでは抗糖脂質抗体が再発寛解型モデルにのみ関与する
Anti-glycolipid antibodies are associated with clinical courses of four autoimmune and viral models for multiple sclerosis

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Abstract

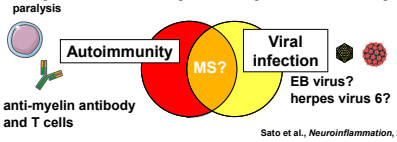
【背景・目的】多発性硬化症 (MS) は、脳・脊髄などの中枢神経系 (CNS) が傷害される病気である。神経線維を覆うミエリン (髄鞘) が炎症によって壊れることで神経の働きに問題が生じ、運動障害、感覚障害などが起こる。疾患の経過 (病型) にはいくつかのパターンがあり、再発と寛解を繰り返す再発寛解型が約 90% を占めるが、持続的に進行する進行型などもある。MS 発症機序は、神経組織に対する自己免疫反応やウイルス感染の関与が想定されている。髄鞘はオリゴデンドロサイトの細胞膜が何層にも重なって形成されている。その細胞膜は他の細胞と異なる多様な糖脂質を表面に有しており、この糖脂質が免疫のターゲットとなっている可能性が考えられる。我々は、MS 病型の違いは発症原因や免疫因子の違いによるのではないか、と仮説した。本研究は、異なる病型をとる 4 つの MS 動物モデルを用いて抗糖脂質抗体の役割を明らかにすることを目的とした。
【方法】4 群の MS モデルのうち 3 群は実験的自己免疫性脳脊髄炎 (EAE) で、髄鞘抗原であるミエリンプロテオリピド蛋白質 ($PLP_{139-151}$)、ミエリンオリゴデンドロサイト糖蛋白質 (MOG_{35-55} もしくは MOG_{92-106}) でマウスを感作し、調製した。1 群はタイラーウイルス (TMEV) を脳内接種することで脳脊髄炎を誘導した。各モデルマウスの血清中抗糖脂質抗体の力価を ELISA 法にて定量した。
【結果・考察】全モデルマウスが運動麻痺を発症し、病理学的には髄鞘の炎症と破壊を認めた。しかし、病型は異なっており、PLP 群は再発寛解型、MOG_{35-55} 群は急性症状のみの単相型、MOG_{92-106} 群と TMEV 群は進行型であった。次に 11 種類の糖脂質 (GM1、GM2、GM3、GM4、GD1a、GD1b、GT1b、GQ1b、GD3、ガラクトセレブロシド、スルファチド) に対する血清抗体価の測定を行った。4 群のモデル中 $PLP_{139-151}$ 群にのみ、GM1、GM3、GM4、スルファチドに対する抗体が有意に高く検出された。PLP 群は、再発寛解型の経過をとるため、この抗糖脂質抗体が髄玉 (寛解) あるいは悪玉 (再発) のいずれかに関与している可能性が考えられた。抗糖脂質抗体はギランバレー症候群 (GBS) の症例の一部では臨床症状に関連していることが証明されている。GBS は新型コロナウイルスでも報告されており、抗糖脂質抗体の測定は MS 患者の臨床型のみならず、新型コロナウイルス関連性の GBS でも病態解明に有用である可能性がある。

【Background】Multiple sclerosis (MS) is an inflammatory demyelinating disease in the central nervous system (CNS). The clinical courses of MS can differ among patients; most patients initially develop relapsing-remitting (RR) disease, which is often followed by continuous disease progression without remission [secondary progressive (SP) MS]. On the other hand, some MS patients develop disease progression with no remission [primary progressive (PP) MS]. In MS, autoreactive immune effector cells and molecules, including autoantibodies, have been suggested to attack the myelin sheaths and axons, resulting in neurological deficits such as motor paralysis and cognitive disturbance. One of the target molecules of the autoreactive antibodies is glycolipids that are present on nerve fibers. Although anti-glycolipid antibodies have been associated with Guillain-Barré syndrome (GBS), peripheral inflammatory neuropathy, their role in MS is unknown. We hypothesized that induction of anti-glycolipid antibodies could explain the different clinical courses of MS patients.
【Methods】We induced several MS models in mice which have distinct clinical courses, depending on induction methods. Using the sera from MS model mice, we conducted enzyme-linked immunosorbent assays (ELISAs) to quantify antibodies against 11 glycolipids: GM1, GM2, GM3, GM4, GD1a, GD1b, GT1b, GQ1b, GD3, galactocerebroside, and sulfatide.
【Results and Discussions】We induced the autoimmune models of MS, experimental autoimmune encephalomyelitis (EAE), by sensitization with the myelin proteolipid protein ($PLP_{139-151}$ peptide in SJL/J mice, myelin oligodendrocyte protein (MOG_{92-106} peptide in SJL/J mice or A.SW mice, and MOG_{35-55} peptide in C57BL/6 mice. We also induced the viral model of MS by infection of Theiler's murine encephalomyelitis virus (TMEV). Although all MS models developed inflammatory demyelination in the CNS, their clinical courses differed among the models: PLP-induced EAE, RR course; MOG_{92-106}, RR, PP, or SP course; MOG_{35-55}-induced EAE, monophasic course; and TMEV, PP course. Among the MS models, we detected significant increases in four anti-glycolipid antibodies, GM1, GM3, GM4, and sulfatide, only in PLP-induced EAE. These anti-glycolipid antibodies may play either beneficial or detrimental roles associated with remissions or relapses in PLP-EAE, respectively. Therefore, anti-glycolipid antibodies may play a role in the pathophysiology of RR-MS.

Introduction

Multiple sclerosis (MS)

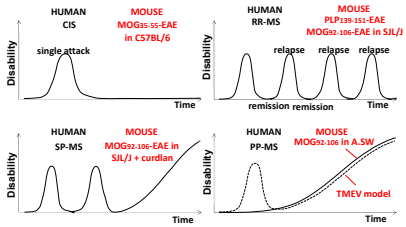
- Inflammatory demyelinating disease in the central nervous system (CNS)
- Anti-CNS autoimmune responses and/or viral infections are proposed to damage nerve fibers, leading to neurological deficits, including motor paralysis



Sato et al., *Neuroinflammation*, 2018

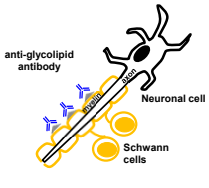
Animal models of MS

- Autoimmune model, experimental autoimmune encephalomyelitis (EAE) by sensitization of the myelin peptides: myelin proteolipid protein ($PLP_{139-151}$, myelin oligodendrocyte glycoprotein (MOG_{35-55}, MOG_{92-106}, which induces myelin-specific immune responses
- Viral model: Theiler's murine encephalomyelitis virus (TMEV) induces inflammation in the CNS
- All animal models develop neurological disability
- Each model has a distinct clinical course, similar to various clinical courses of MS: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and clinically isolated syndrome (CIS, monophasic)



Glycolipid antibodies

- Glycolipids present on nerve fibers, including myelin and axon
- Anti-glycolipid antibodies attack the myelin sheaths and axons, causing nerve damage
- Guillain-Barré syndrome (GBS) is peripheral neuropathy with symmetrical weakness of the limbs, and areflexia
- The induction of anti-glycolipid antibodies results in distinct clinical signs in GBS
- The role of glycolipid antibodies in MS is unknown



Hypothesis

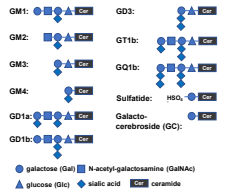
"Anti-glycolipid antibody induction explains the distinct clinical courses of MS"

Materials & Methods

MS models: We induced EAE by sensitization with the myelin proteolipid protein (PLP) $_{139-151}$ peptide in SJL/J mice, myelin oligodendrocyte protein (MOG)$_{92-106}$ peptide in SJL/J mice or A.SW mice, or MOG_{35-55} peptide in C57BL/6 mice, in which myelin peptides were emulsified in complete Freund's adjuvant (CFA). We also induced the viral model of MS by intracerebral injection of TMEV. We harvested sera from the mice.

Enzyme-linked immunosorbent assay (ELISA): We coated 11 glycolipids associated with GBS on 96-well plates, and detected anti-glycolipid antibodies by anti-mouse $F(ab')_2$ antibody.

Lymphoproliferative response: We harvested mononuclear cells (MNCs) from the spleen, cultured and stimulated MNCs with glycolipid antigens at 5 μg/ml for 5 days. We quantified glycolipid-specific proliferative responses by absorption (optical density, O.D.) measured using the CCK-8 reagents at 450 nm.

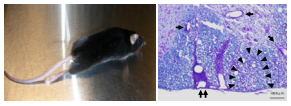


Minac K and Bognar S. (2010) , 特表 (2013) .

Results

Clinical courses of MS models

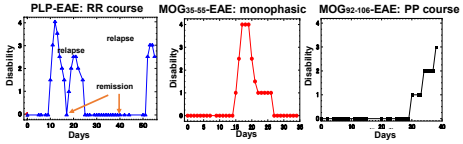
- All MS models developed neurological signs with inflammatory demyelination
- Each MS model had a distinct clinical course



Hind-limb paralysis

Inflammatory demyelination

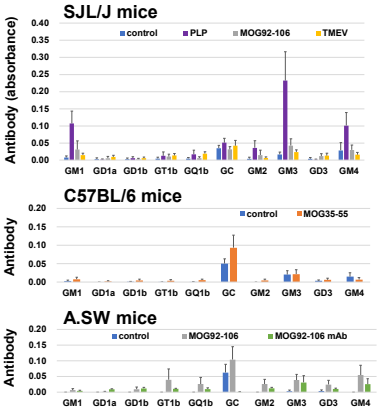
Meningitis (↑)
Perivascular cuffing (↑)
Demyelination (▲)



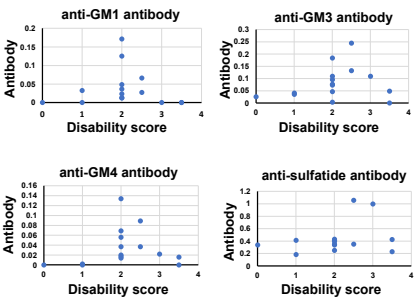
Inoculum	Mouse strain	Model	Clinical course
$PLP_{139-151}$	SJL/J	EAE	RR
MOG_{35-55}	C57BL/6	EAE	monophasic
MOG_{92-106}	SJL/J	EAE	PP, SP, or RR
MOG_{92-106}	A.SW	EAE	PP
TMEV	SJL/J	virus	PP

RR, relapsing-remitting; PP, primary progressive; and SP, secondary progressive

SJL/J mice with PLP-EAE have anti-glycolipid antibodies

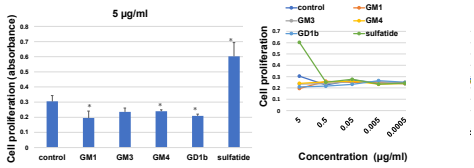


Glycolipid antibodies and disability



- Anti-GM1, GM3, and GM4 antibody titers were lower in EAE mice with high disease severity
- Anti-sulfatide antibody titers were higher than any other anti-glycolipid antibodies regardless of disease severity

Glycolipid-specific cell proliferation



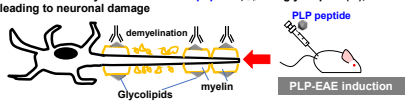
- Glycolipid-specific lymphoproliferation (cell proliferation) was observed only by sulfatide stimulation at 5 μg/ml.
- Control cultures were stimulated with vehicle.

Discussion

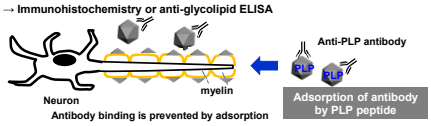
Mechanisms of anti-glycolipid antibody induction

1. Molecular mimicry between PLP and glycolipids

Anti-PLP antibody recognizes glycolipid antigens on nerve fibers by molecular mimicry between the PLP peptide (●) and glycolipids (▬), leading to neuronal damage

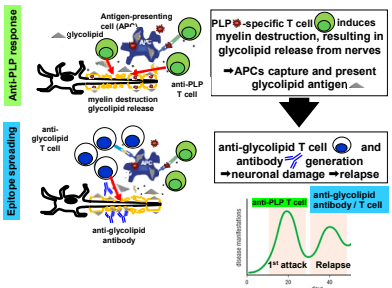


Future experiment: Adsorption of serum by the PLP peptide → Immunohistochemistry or anti-glycolipid ELISA



Antibody binding is prevented by adsorption

2. Epitope spreading from PLP to glycolipid antigen



Future experiment: Kinetic study to associate between clinical signs and anti-glycolipid antibody titers

Limitation of the study

- Isotype of anti-glycolipid antibodies; several anti-ganglioside antibodies have been reported to be IgM, not IgG, in GBS
- Serum adsorption by each glycolipid ➡ glycolipid ELISA; the epitope can be common between glycolipid antibodies
- Generation of hybridoma producing the glycolipid antibody ➡ adoptive transfer of the antibody to naïve or EAE mice to determine whether the glycolipid play a protective or detrimental role

Conclusions

- PLP-induced EAE mice had a relapsing-remitting (RR) disease; the other MS models had a monophasic or progressive disease course
- Among MS models, only PLP-induced EAE mice with RR disease course mounted antibodies against four glycolipids: GM1, GM3, GM4, and sulfatide.
- Anti-glycolipid antibodies may play either beneficial or detrimental roles, which are associated with remissions or relapses in PLP-EAE, respectively
- Anti-glycolipid antibodies may play a role in the pathophysiology of RR-MS
- Molecular mimicry and/or epitope spreading will be explored as possible mechanisms by which anti-glycolipid antibody is produced in RR-EAE

References

- 中村優美和, 朴雅美, 角田郁生. (2020). 多発性硬化症ウイルスモデルにおける唾液 IgA 検出システムの確立：新型コロナウイルス・ワクチン開発への応用. 別冊 BIO Clinica 25: 98-102.
- Morikawa M, Kuwahara M, Ueno R, Samukawa M, Hamada Y and Kusunoki S. (2016) . Serological study using glycoarray for detecting antibodies to glycolipids and glycolipid complexes in immune-mediated neuropathies. *J Neuroimmunol.* 301: 35-40.
- Sato F, Omura S, Martinez NE and Tsunoda I. (2018). Animal models for multiple sclerosis. In: *Neuroinflammation* (2nd edition). Minagar A (Ed), Elsevier, Burlington, MA. pp. 37-72.
- Minac K and Bognar S. (2010). Role of gangliosides in brain aging and neurodegeneration. *Translational Neuroscience.* 1: 300-307.
- 角田郁生. (2021). ウイルス感染・ワクチン接種による免疫性神経疾患：神経免疫学・ウイルス学の立場から HPV ワクチンの接種. 思春期学 39: 20-27, 2021
- 楠進. (2013). Guillan-Barré 症候群 (GBS) と Fisher 症候群. 楠進編集「免疫性神経疾患ハンドブック」南江堂 東京 日本 pp128-145.
- 角田郁生. (2018). MSの動物モデルについて教えてください. 吉良潤一編集 <シリーズ> 神経内科 Clinical Questions & Pearls「中枢脱髄性疾患」中外医学社 東京 日本 pp115-120.

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