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H. 知的財産権の出願・登録状況
(予定を含む。)

1. 特許取得
なし

2. 実用新案登録
なし

3. その他
なし

厚生労働科学研究委託費
(難治性疾患等克服研究事業(難治性疾患等実用化研究事業(難治性疾患実用化研究事業)))
委託業務成果報告（業務項目）

ネットワーク解析による新規治療標的の探索研究

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研究要旨　多発性硬化症(multiple sclerosis; MS)は中枢神経系白質に炎症性脱髄巣が多発し、様々な神経症状が再発を繰り返して進行する難病である。MS では髄鞘自己抗原反応性ヘルパーT 細胞(Th1, Th17)が血液脳関門を通過して脳・脊髄に浸潤し、マクロファージやミクログリアを活性化し、エフェクター細胞が産生する炎症性サイトカインが脱髄と軸索傷害を惹起する。現在、急性増悪期にはステロイドパルス療法(IVMP), 寛解期にはインターフェロンベータ(IFNβ)の長期投与が標準治療となっている。しかしIFNβノンレスポンダーも多く、第二選択としてフィンゴリモド(Fingolimod)など種々の免疫修飾薬(disease-modifying agent: DMA)が投与されている。MS における治療難航の原因として臨床病理学的多様性(heterogeneity)が挙げられる。個々人の病態に応じた最適な治療法の選択のためには、患者の遺伝的背景や病態の個人差を反映する適切なバイオマーカーを解析する必要がある。本研究では文献学的にMS 個別化治療確立のためのバイオマーカーを調べた。その結果、現時点では個々の患者に最適な治療法をエビデンスに基づき選択するために必要な再現性と信頼性が高いバイオマーカーが十分確立されていないことが判明した。

A. 研究目的

多発性硬化症(multiple sclerosis: MS)は、中枢神経系白質に炎症性脱髄巣が多発して再発を繰り返す難病で、若年成人に好発する。近年日本では患者数は増加傾向にある。MS は遺伝因子･感染因子･環境因子の複雑な相互作用により発症が規定されている。MS では髄鞘自己抗原反応性ヘルパーT 細胞(Th1, Th17)が出現し、血液脳関門を通過して脳･脊髄に浸潤し、マクロファージやミクログリアを活性化し、エフェクター細胞が産生する炎症性サイトカイン(TNF)と活性酸素(reactive oxygen species; ROS)が脱髄(demyelination)と軸索傷害(axonal damage)を早期から惹起する。現在、急性増悪期にはステロイドパルス療法(IVMP), 寛解期にはインターフェロンベータ(IFNβ)の長期投与が標準治療(first line therapy)となっている。しかしIFNβノンレスポンダーも多く、第二選択(second line therapy)としてフィンゴリモド(Fingolimod)

など種々の免疫修飾薬(disease-modifying agent: DMA)が投与されている。MS における治療難航の原因として臨床病理学的多様性(heterogeneity)が挙げられる。

MS は臨床経過から、再発寛解型(relapsing-remitting MS: RRMS), 2 次進行型(secondary-progressive MS: SPMS), 1 次進行型(primary-progressive MS: PPMS)に分類されている。大多数(約 85%)の MS は RRMS で発症し、10-15 年の経過で約半数は再発を繰り返して進行する SPMS に移行する。約 15%では、発症時から再発が不明瞭ながら持続的に進行する PPMS を呈する。RRMS では、炎症性脱髄巣の時間的空間的多発性(dissemination in time and space)が最重要な診断指標となる。初回発作単独のケースは、clinically isolated syndrome(CIS)と呼ばれている。MRI 病巣単独のケースは、radiologically isolated syndrome(RIS)と呼ばれている。MS は病理学的には、T 細胞浸潤、抗体沈着、オリゴデンドロサイトアポトーシスの所見に基づき 4 種類の病型(Lucchinetti の I-IV)に分類されている。病態生理学的には、RRMS は神経炎症(neuroinflammation)が主体で、SPMS, PPMS は神経変性(neurodegeneration)が主体であり、種々の DMA は後者には無効である。現時点では、髄鞘や軸索の再生促進薬はなく、新規標的分子に対する画期的な創薬が待望されている。

ポストゲノム時代を迎え、個別化医療(personalized medicine)の必要性が高まっている。個別化医療は患者の遺伝的背景や病態の個人差を反映するバイオマーカー(biomarker)を解析し、個々の患者に最適な治療法をエビデンスに基づき選択する医療である。患者個人の状態に応じて、副作用を最小限に抑えて最大限の治療効果を発揮する個人にベストな薬物治療の設定が重要な課題である。バイオマーカーとしては、DNA, RNA, タンパク質、代謝物など、オミックス解析技術を駆使して測定可能な全ての生体分子が候補となり、容易に測定可能で再現性が高い指標が求められている(図 1)。本研究では文献学的に MS 個別化治療確立のためのバイオマーカーを調べた。本研究の成果は MS の病態解明に貢献し、厚生労働行政を主導とする患者の QOL 向上につながる。

B. 研究方法

1. MS のバイオマーカー探索

PubMed を駆使して、最近 5 年間に報告された英文論文に記載された MS のバイオマーカーのうち、客観性(症例数・解析手法)の観点から科学的に重要なものを選択した。

(倫理面への配慮)

本研究は文献学的な考察が主体であり、倫理面への配慮は必要とされない。

C. 研究結果

1. 診断のためのバイオマーカー

現在、MSの診断において、最も鋭敏な病巣検出法はMRIである。また臨床的に重要な所見としては、脳脊髄液(CSF)のオリゴクローナルIgGバンド(OCB)の検出である。CISでは、OCB陽性例はRRMSに進展し易い(Neurology 2008;70:1079-83)。血清抗AQP4抗体の検出は、視神経脊髄炎(NMO)の診断において、特異度100%を呈する重要なバイオマーカーである。最近、MS患者の約半数で病型によらず、血清抗KIR4.1 IgG自己抗体が検出されると報告された(N Engl J Med 2012;367:115-23)。KIR4.1は腎臓尿細管上皮細胞・内耳細胞・アストロサイトに発現しているATP依存性内向き整流性カリウムチャネルである。マウス髄腔内に抗KIR4.1 IgGを投与すると、補体活性化とアストロサイトにおけるKIR4.1の発現低下を惹起する。抗KIR4.1抗体は、MS特異的バイオマーカーとして期待されたが、その後Lennonらのグループにより否定的な報告がなされた。

最近15ヶ国23研究グループが収集した9,772例のMSを含むゲノムワイド関連解析研究(GWAS)の結果が報告され、合計102のnon-MHCリスクSNPが98遺伝子に同定された(Nature 2011;476:214-9)。多くはヘルパーT細胞分化に関与するネットワークを形成していた。しかしGWASで発見されたリスクアレルのオッズ比は全て1.2以下であり、個々のアレルは単独ではMS発症に対する貢献度が低い。現在NGSを用いて全ゲノムや全エクソンを網羅的に解析し、発症リスクの大きなレアバリアントを発見する試みがなされている。NGSとマイクロアレイを併用して、CISやRRMSの血液で低下しているマーカーとして、hsa-miR-20a-5pが同定されたが、再現性は確認されていない(Mult Scler 2014;20:295-303)。

2. 活動性および予後予測のためのバイオマーカー

MS は中枢神経系に限局する炎症性脱髄を特徴とし、血液由来のバイオマーカーは疾患活動性を直接反映しないことが多い。一方、様々なCSF由来のバイオマーカーは、MS活動性の指標となる。CXCL13は受容体CXCR5に結合するケモカインで、B細胞遊走因子として働く。CISでの陽性例はRRMSに進展し易い(Mult Scler 2011;17:335-343)。しかしCXCL13は髄膜炎やNMOでも陽性になることがあり、疾患特異性はない。Chitinase 3-like 1(CHI3L1)とα2-HS-glycoprotein(fetuin-A)は、CSFプロテオーム解析で、MS 疾患活動性を反映して上昇することが見出された糖タンパク質である(Mult Scler 2011;17:335-43)。ニューロフィラメント(低分子量 NFL, 高分子量 NFH)と14-3-3タンパク質は、神経細胞に豊富に存在し、軸索傷害や神経細胞死の際に髄液に放出される。これらが陽性となる症例は、DMAによる治療に抵抗性であり、高度な機能障害を示す傾向がある(Mult Scler 2012;18:552-6)。最近、MS患者血清のNMRによるメタボローム解析と多変量解析(PLS-DA 法)の組み合わせで、RRMS と

SPMS を識別可能なことが報告された(Neurology 2014; 83:1492-9)。MS 進行予測の非侵襲的バイオマーカーとして期待される。

3. 治療効果予測のためのバイオマーカー

MS の IFNβ治療効果予測に関する最も一般的なバイオマーカーは、IFNβ中和抗体である(Mult Scler 2014;20:577-87)。治療開始 1.5 年以内に IFNβ1b 製剤では約 40%, IFNβ1a 製剤では約 20%の症例で、中和抗体が陽性になる。中和抗体が出現すると IFNβの生物学的活性が減弱して再発頻度が増加し、GA や Fingolimod への切り替えが必要となる。IFNβノンレスポンダーは、治療前から I 型 IFN 産生が亢進している遺伝子発現プロフィールを呈している(Brain 2009;132: 3353-65)。細胞増殖に関与する細胞外基質グリピカン 5(GPC5)遺伝子の SNP rs10492503 AA は、IFNβレスポンダーに多い(Mult Scler 2009;15:913-17)。T 細胞活性化に関与し Plexin-B, Tim-2 に結合するセマフォリン Sema4A の血清レベルが上昇している MS では活動性が高く、IFNβノンレスポンダーが多い(J Immunol 2012;188: 4858-65)。しかしながら多くのバイオマーカーは普遍的な再現性が確認されていない。

D. 考察

バイオマーカー(DNA, RNA, タンパク質、代謝物)は、疾患の活動性や進行度の判定、治療効果や予後の予測の客観的指標となり、臨床試験における層別化や新薬開発促進に役立つ重要なメルクマールとなる。MS は臨床病理学的に多様な病態を呈し、個々の患者で治療薬に対する効果や予後には個人差が存在する。従って個別化医療の確立が重要課題となる。しかし残念ながら現時点では、MS 個別化医療に有用なバイオマーカーは確立されていない。特に重要と考えられるバイオマーカーとしては、CIS から臨床的確実 MS(CDMS)への進展を予測するバイオマーカー、RRMS(神経炎症)から SPMS(神経変性)への移行を予測するバイオマーカー、各種治療薬に対するレスポンダー・ノンレスポンダーを識別予測可能なバイオマーカーである。

E. 結論

MS は臨床的病理学的多様な病態を呈し、個々の患者で治療効果や予後に関して個人差が存在する。従って MS では個別化医療の確立が最も重要課題となる。しかし残念ながら現時点では、MS 個別化医療に有用なバイオーマーカーは確立されていない。オミックス解析技術を駆使して、再現性や信頼性が高いバイオマーカーを整備する必要がある(図 1)。

F. 健康危険情報

G. 研究発表

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H. 知的所有権の取得状況

1. 特許取得
   該当なし
2. 実用新案登録
   該当なし
3. その他
   なし

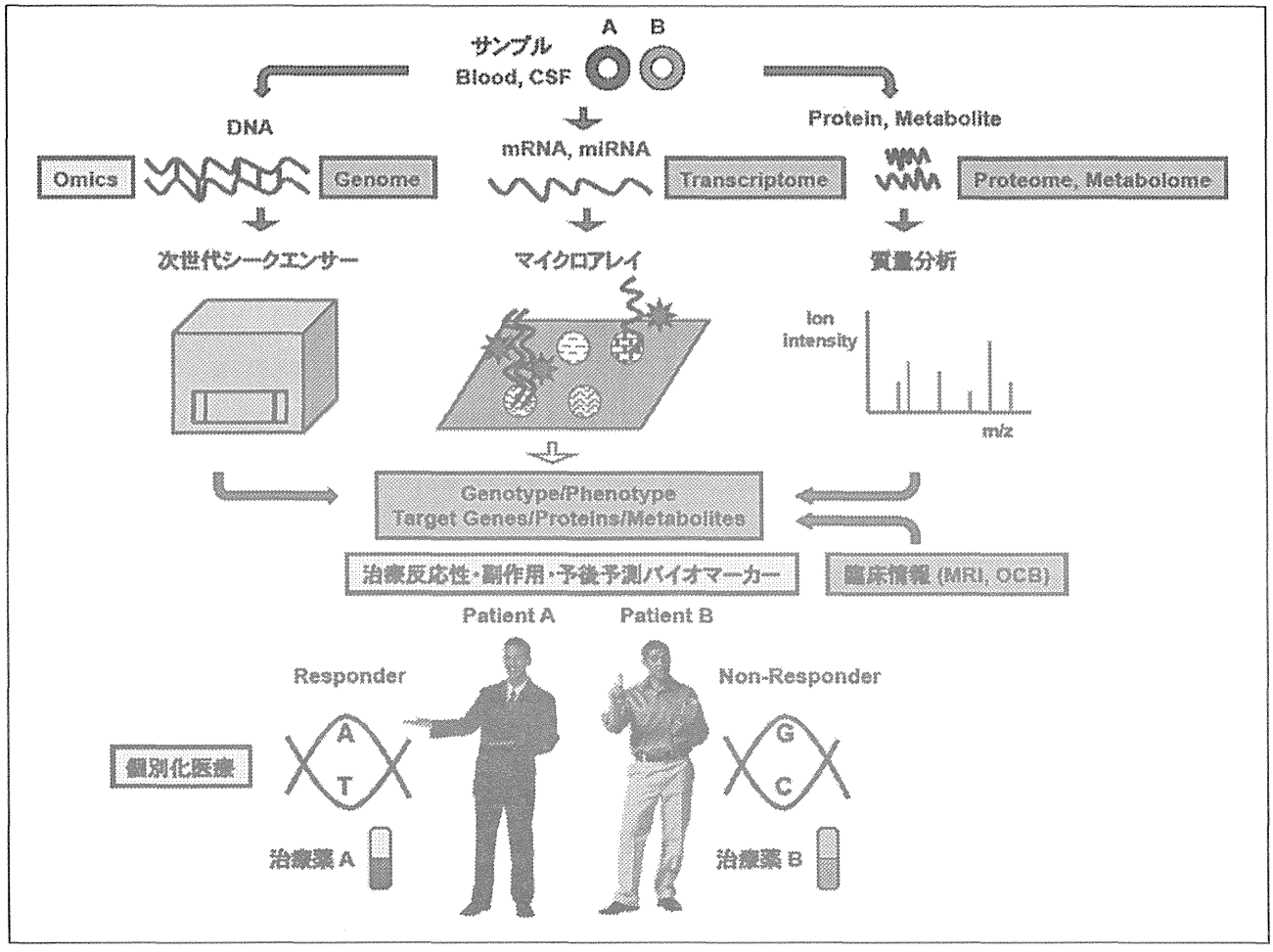

図 1. オミックス解析による個別化医療のためのバイオマーカー探索.

オミックス(Genome, Transcriptome, Proteome, Metabolome)解析により、患者の遺伝的背景や病態の個人差を反映するバイオマーカーを調べ、個々の患者に最適な治療法をエビデンスに基づき選択する。

学会等発表実績

様式第１９

学 会 等 発 表 実 績

委託業務題目「二次進行型多発性硬化症に対する革新的な医薬品の開発を促進させる研究 」

機関名　（独）国立精神・神経医療研究センター

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
多発性硬化症に対する免疫介入試験におけるヒト免疫応答解析	山村　隆	東京（第４２回日本臨床免疫学会総会）	2014.9.25	国内
免疫性神経疾患とPrecision Medicine	山村　隆	東京（第４２回日本臨床免疫学会総会）	2014.9.26	国内
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NR4A2 modulation during autoimmune inflammation of the central nervous system reduces pathogenic T cell responses and ameliorates clinical disease.	Raveney, B., Oki, S., Yamamura, T.	Mainz, Germany (The 12th International Congress of Neuroimmunology)	2014.11.11	国外
NR4A2 controls pathogenic 'switched' Th17 cells in the CNS during autoimmune inflammation.	Raveney, B., Oki, S., Yamamura, T.	京都（第43回 日本免疫学会総会・学術集会）	2014.12.10	国内
Myelin Reactive T cells in the Gut Regulate Experimental Autoimmune Encephalomyelitis (EAE).	Kadowaki A, Miyake S, Chiba A, Yamamura T.	Chicago, USA (FOCIS 2014)	2014.6.26	国外
An Increased Proportion of IL-6-dependent Plasmablasts Characterizes Interferon beta-resistant Patients with Relapsing-remitting Multiple Sclerosis.	Nakamura, T., Matsuoka. T., Araki, M., Sato, W., Lin, Y., Okamoto, T., Murata, M., Miyake, S., Aranami, T., Yamamura, T.	Chicago, USA (FOCIS 2014)	2014.6.26	国外
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視神経脊髄炎に対する抗IL-6受容体抗体トシリズマブ治療の有効性の検討	荒木学、松岡貴子、宮本勝一、楠進、岡本智子、村田美穂、三宅幸子、荒浪利昌、山村隆	福岡（第55回日本神経学会学術大会）	2014.5.22	国内
再発寛解型多発性硬化症病態におけるIL-6依存性プラズマブラストの関与	中村雅一、松岡貴子、荒木学、林幼偉、佐藤和貴郎、岡本智子、村田美穂、下地啓五、佐藤典子、三宅幸子、荒浪利昌、山村隆	福岡（第55回日本神経学会学術大会）	2014.5.24	国内
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MS活動性脳病巣におけるNLRP3 inflammasomeの発現	佐藤準一、川名夏生、山本洋司、朝比奈直弘、北野翔大、紀嘉浩	金沢（第26回日本神経免疫学会学術集会）	2014. 9.5	国内

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Efficacy of the anti-IL-6 receptor antibody tocilizumab in neuromyelitis optica	Araki M, Matsuoka T, Miyamoto K, Kusunoki S, Okamoto T, Murata M, Miyake S, Aranami T, Yamamura T	Neurology	2014	国外
Differential effects of fingolimod on B-cell populations in multiple sclerosis.	Nakamura M, Matsuoka T, Chihara N, Miyake S, Sato W, Araki M, Okamoto, T Lin Y, Ogawa M, Murata M, Aranami T, Yamamura T	Multiple Sclerosis J	2014	国外
How do T cells mediate central nervous system inflammation?	Yamamura T	Clin. Exp. Neuroimmunol.	2014	国外

Towards understanding the role of orphan nuclear receptor NR4A2 in Th17 cell-mediated CNS autoimmunity: An experimental approach using an animal model of multiple sclerosis	Oki S	Clin. Exp. Neuroimmunol.	2014	国外
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ヒト腸内細菌叢のMetagenomics	服部正平	Bio Clinica	2014	国内
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ヒト常在菌叢のメタゲノム解析	服部正平	医学のあゆみ	2014	国内
多発性硬化症の動物モデル - 横断的アプローチによる病態解明と治療標的の探索 -	大木伸司, 山村　隆	日本臨床	2014	国内
免疫動態	佐藤和貴郎, 山村　隆	Clinical Neuroscience	2014	国内

研究成果の刊行物・別刷

Efficacy of the anti–IL-6 receptor antibody tocilizumab in neuromyelitis optica

A pilot study

OPEN

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ABSTRACT

Objective: To evaluate the safety and efficacy of a humanized anti-interleukin-6 receptor antibody, tocilizumab (TCZ), in patients with neuromyelitis optica (NMO).

Methods: Seven patients with anti-aquaporin-4 antibody (AQP4-Ab)-positive NMO or NMO spectrum disorders were recruited on the basis of their limited responsiveness to their current treatment. They were given a monthly injection of TCZ (8 mg/kg) with their current therapy for a year. We evaluated the annualized relapse rate, the Expanded Disability Status Scale score, and numerical rating scales for neurogenic pain and fatigue. Serum levels of anti-AQP4-Ab were measured with AQP4-transfected cells.

Results: Six females and one male with NMO were enrolled. After a year of TCZ treatment, the annualized relapse rate decreased from 2.9 ± 1.1 to 0.4 ± 0.8 ($p < 0.005$). The Expanded Disability Status Scale score, neuropathic pain, and general fatigue also declined significantly. The ameliorating effects on intractable pain exceeded expectations.

Conclusion: Interleukin-6 receptor blockade is a promising therapeutic option for NMO.

Classification of evidence: This study provides Class IV evidence that in patients with NMO, TCZ reduces relapse rate, neuropathic pain, and fatigue. *Neurology*® 2014;82:1302–1306

GLOSSARY

Ab = antibody; **AQP4** = aquaporin-4; **AZA** = azathioprine; **EDSS** = Expanded Disability Status Scale; **IL** = interleukin; **IL-6R** = interleukin-6 receptor; **NMO** = neuromyelitis optica; **PB** = plasmablasts; **PSL** = prednisolone; **TCZ** = tocilizumab.

Neuromyelitis optica (NMO) is a relatively rare autoimmune disease that predominantly affects the spinal cord and optic nerve. Anti–aquaporin-4 antibody (AQP4-Ab), which is a disease marker of NMO, has an important role in causing the destruction of astrocytes that express AQP4.[1] Empirically, the use of disease-modifying drugs for multiple sclerosis, including interferon β, is not recommended for NMO,[2] which is consistent with the distinct pathogenesis of NMO and multiple sclerosis. We have recently described that plasmablasts (PB), which are a subpopulation of B cells, increased in the peripheral blood of patients with NMO and that PB are a major source of anti-AQP4-Ab among peripheral blood B cells.[3] In addition, we observed that exogenous interleukin (IL)-6 promotes the survival of PB and their production of anti-AQP4-Ab in vitro. Given the increased levels of IL-6 in the serum and CSF during relapses of NMO,[1,3] we postulated that blocking IL-6 receptor (IL-6R) pathways might reduce the disease activity of NMO by inactivating the effector functions of PB. A humanized anti–IL-6R monoclonal antibody, tocilizumab (TCZ) (Actemra/RoActemra), has been approved in more than 100 countries for use in the treatment of rheumatoid arthritis.[4] Herein, we describe our clinical study that aimed to explore the efficacy of TCZ in NMO.

Editorial, page 1294

From the Multiple Sclerosis Center (M.A., T.O., S.M., T.A., T.Y.) and Department of Neurology (T.O., M.M.), National Center Hospital, and Department of Immunology, National Institute of Neuroscience (T.M., S.M., T.A., T.Y.), National Center of Neurology and Psychiatry, Tokyo; Department of Neurology (K.M., S.K.), Kinki University School of Medicine, Osaka; and Department of Pediatrics (T.M.), Graduate School of Medicine, University of Tokyo, Japan.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Table Demographics of the patients

	Patient						
	1	2	3	4	5	6	7
Age, y/sex	37/F	38/F	26/F	31/M	55/F	62/F	23/F
Age at onset, y	23	27	21	12	38	60	21
Anti-AQP4-Ab	+	+	+	+	+	+	+
Myelitis	+	+	+	+	+	+	–
Optic neuritis	+	+	+	+	+	+	+
EDSS score	3.5	6.5	3.5	6.0	6.5	6.5	3.0
Total no. of relapses	20	9	6	16	20	3	7
ARR before TCZ	3	2	2	2	3	3	5
Immunotherapies for exacerbations	IVMP, PLEX	IVMP, PLEX	IVMP, PLEX	IVMP, OBP, PLEX	IVMP, PLEX	IVMP, PLEX	IVMP, PLEX
Past immunotherapies	IFNβ, IVIg	IFNβ	—	IFNβ, MITX	IFNβ, AZA	—	AZA
Present immunotherapies	PSL, AZA	AZA	PSL	PSL, AZA	PSL, CyA	PSL, CyA	PSL, tacrolimus
Neuropathic pain (e.g., girdle pain), NRS	4	4	2	4	4	3	0
General fatigue, NRS	5	8	6	7	5	3	9
Pain and antispasticity medication	GBP, CZP, NTP, NSAID	CZP, mexiletine, NTP, tizanidine, NSAID	—	CBZ, baclofen, NSAID	CBZ	PGB	—

Abbreviations: AQP4-Ab = aquaporin-4 antibody; ARR = annualized relapse rate; AZA = azathioprine; CBZ = carbamazepine; CZP = clonazepam; CyA = cyclosporine; EDSS = Expanded Disability Status Scale; GBP = gabapentin; IFNβ = interferon β; IVIg = IV immunoglobulin; IVMP = IV methylprednisolone; MITX = mitoxantrone; NRS = numerical rating scale; NSAID = nonsteroidal anti-inflammatory drug; NTP = Neurotropin (an extract from the inflamed skin of vaccinia virus-inoculated rabbits); OBP = oral betamethasone pulse therapy; PGB = pregabalin; PLEX = plasma exchange; PSL = prednisolone; TCZ = tocilizumab.

METHODS **Level of evidence.** The aim of this Class IV evidence study was to evaluate the effect and safety of a monthly injection of TCZ (8 mg/kg) with their current therapy in patients with NMO. We evaluated the adverse events based on Common Terminology Criteria for Adverse Events, version 4.0.

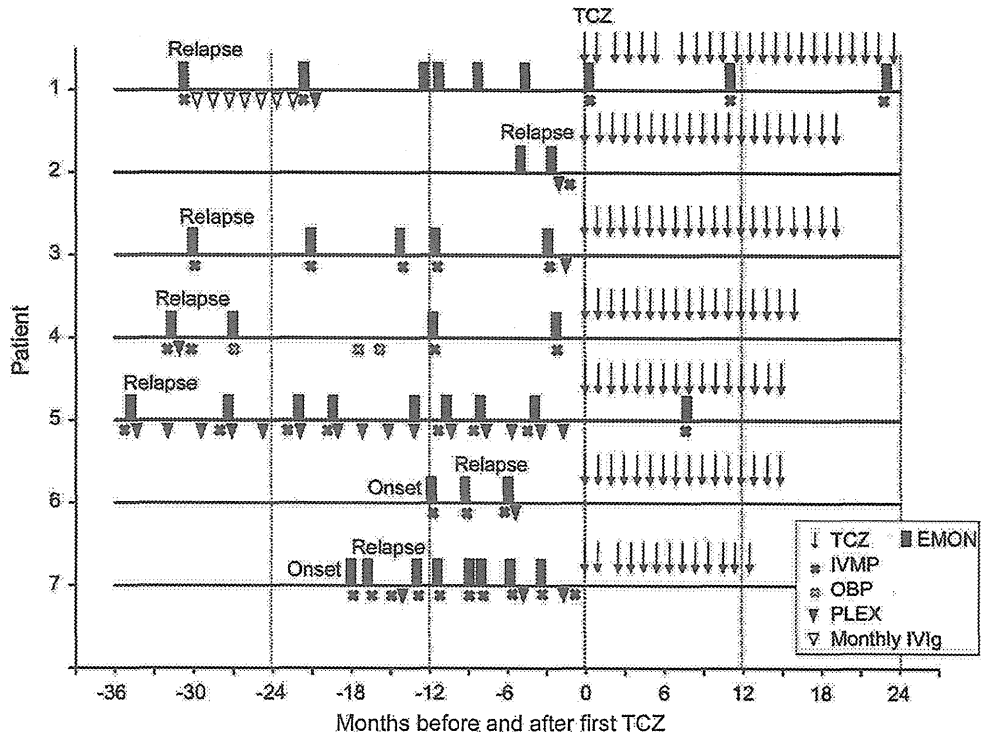
Standard protocol approvals, registrations, and patient consents. All patients gave written informed consent before the first treatment with TCZ. The institutional ethical standards committee on human experimentation approved this clinical study. The study is registered with University Hospital Medical Information Network Clinical Trials Registry, numbers UMIN000005889 and UMIN000007866.

Patients and treatment. Seven patients who met the diagnostic criteria of NMO in 2006 were enrolled after providing informed consent (table). Results of chest x-rays, interferon γ release assays, and plasma 1,3-β-D-glucan measurement excluded latent tuberculosis and fungal infection. All of the patients had been treated with combinations of oral prednisolone (PSL) and immunosuppressants, including azathioprine (AZA). Nevertheless, they had at least 2 relapses during the year before enrollment (figure 1). Among their past immunomodulatory medications, interferon β had been prescribed in 4 patients before the anti-AQP4-Ab assay became available. Although symptomatic treatments had been provided, the patients experienced general fatigue and intractable pain in their trunk and limbs. There were no abnormalities in their routine laboratory blood tests. Neither pleocytosis nor increased levels of IL-6 were observed in the CSF. MRI revealed high-intensity signals in the optic nerves and longitudinally extensive lesions in the spinal cord. All patients except one had scattered brain lesions. A monthly dose (8 mg/kg) of TCZ was added to the patients' oral corticosteroid and immunosuppressive drug regimen.

Clinical and laboratory assessment. As clinical outcome measures, we evaluated alterations in the number of relapses, Expanded Disability Status Scale (EDSS) scores, and pain and fatigue severity scores (numerical rating scales). A relapse was defined as an objective exacerbation in neurologic findings that lasted for longer than 24 hours with an increase in the EDSS score of more than 0.5. Brain and spinal cord MRI scans were examined every 4 or 6 months. CSF examinations, sensory-evoked potentials, and visual-evoked potentials were also evaluated at the time of entry into the study and 12 months later. We measured serum anti-AQP4-Ab levels by evaluating the binding of serum immunoglobulin G to AQP4 transfectants, as previously described.[5] All outcome measures were analyzed with nonparametric Wilcoxon rank-sum tests, with the use of 2-tailed statistical tests at a significance level of 0.05.

RESULTS After starting TCZ treatment, the total number of annual relapses in the patients significantly reduced (figures 1 and 2). Notably, 5 of the 7 patients were relapse-free after starting TCZ. The relapses observed in patients 1 and 5 were mild and their symptoms recovered after IV methylprednisolone. On average, the annualized relapse rate reduced from 2.9 ± 1.1 (range, 2–5) during the year before study to 0.4 ± 0.8 (range, 0–2) during the year after

Figure 1 Clinical course of the patients before and after tocilizumab treatment



The zero on the x-axis represents the first administration of tocilizumab (TCZ). Dark gray bars: exacerbations of myelitis or optic neuritis (EMON); downward arrow: TCZ treatment; black X: IV methylprednisolone (IVMP); white X: oral betamethasone pulse (OBP) therapy; black triangle: plasma exchange (PLEX); white triangle: IV immunoglobulin (IVIg). After receiving 12 injections, all patients continued treatment with TCZ by entering an extension study that evaluates the long-term safety and efficacy of TCZ. We showed the clinical status after completion of the 1-year study to indicate the continuation of remission.

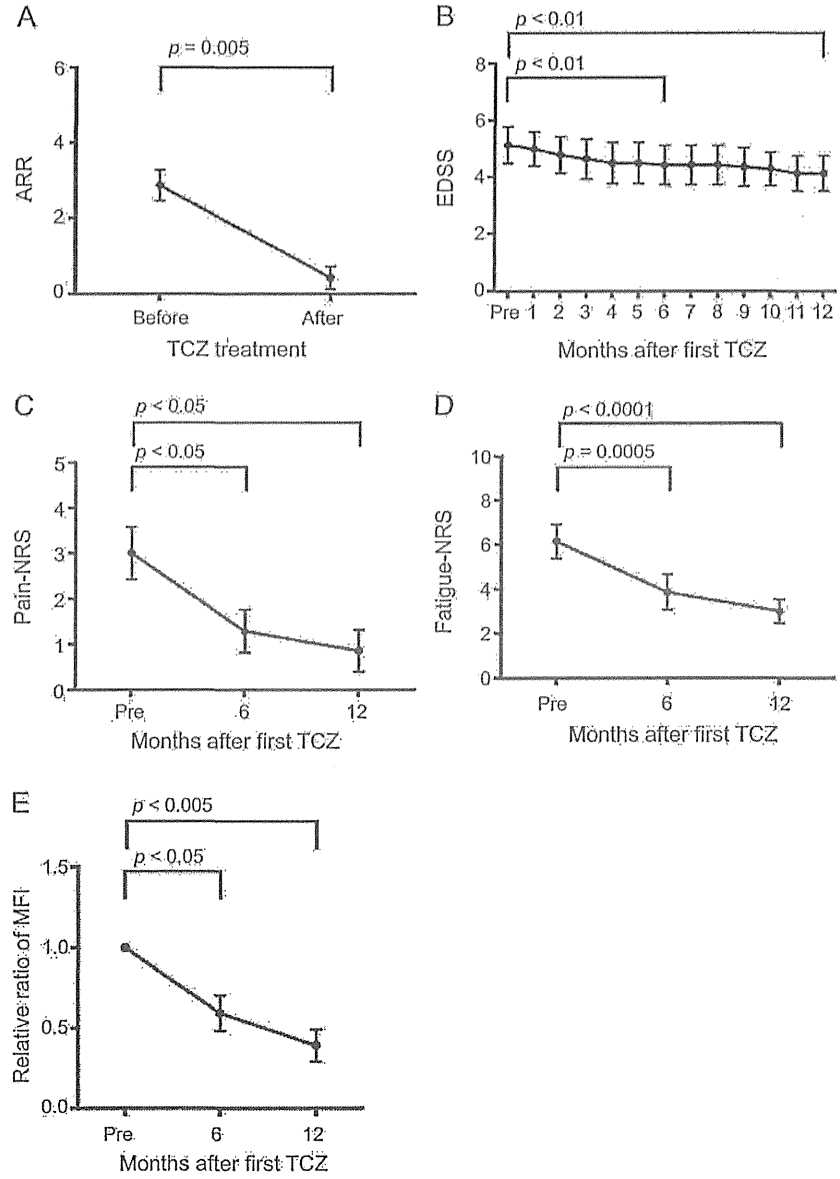
starting TCZ (figure 2). The EDSS score decreased modestly but significantly from 5.1 ± 1.7 (range, 3.0–6.5) to 4.1 ± 1.6 (range, 2.0–6.0) at 12 months. The chronic neurogenic pain in their trunk and extremities, which is characteristic of NMO[6,7] (table), gradually lessened after the patients started TCZ. Consequently, the numerical rating scale for pain reduced from 3.0 ± 1.5 upon study entry to 1.3 ± 1.3 after 6 months and 0.9 ± 1.2 after 12 months. General fatigue also improved from 6.1 ± 2.0 to 3.9 ± 2.1 at 6 months and 3.0 ± 1.4 at 12 months. The MRI scans, sensory- and visual-evoked potentials, and CSF observations did not show any interval changes. Serum anti-AQP4-Ab levels represented by the relative mean fluorescence intensity were significantly reduced (figure 2E).

Adverse events included upper respiratory infections (patients 1 and 7), acute enterocolitis (patients 1 and 4), acute pyelonephritis (patient 1), leukocytopenia and/or lymphocytopenia (patients 1, 4, and 7), anemia (patients 3 and 7), and a slight decline in systolic blood pressure (patient 1). However, none of the events was severe. Oral PSL and AZA were tapered in patients 1, 3, 4, and 7, resulting in a reduction of the mean doses (PSL from 19.5 ± 7.6 to 8.8 ± 5.6 mg/d [average of patients 1, 3, 4, and 7], AZA from 37.5 to 5.4 mg/d [average of patients 1 and 4]).

DISCUSSION Pain management is a difficult problem in patients with NMO. In fact, a retrospective study of 29 patients with NMO who experienced pain has documented that 22 of the 29 patients were taking pain medications, but none of them rated their current pain as 0 out of 10 on a 10-point scale.[6] In the present study, the intractable pain reduced gradually after the patients started TCZ treatment. After 6 or 12 months of therapy, 3 of the 6 patients with pain were completely free of pain. These results suggested a role of IL-6 in NMO pain and the possible merits of the use of TCZ in clinical practice as a pain reliever.

The pathophysiology of neurogenic pain is now understood in the context of interactions between the immune and nervous systems,[8] which involve proinflammatory cytokines such as IL-6 as well as immune cells, activated glia cells, and neurons. Supportive for the role of IL-6 in pain, recent work in

Figure 2 Effects of tocilizumab on clinical and immunologic parameters



(A) Annualized relapse rate (ARR) before and after tocilizumab (TCZ) treatment. (B) Expanded Disability Status Scale (EDSS) score during the 1-year study period. Pain severity (numerical rating scale [NRS]) (C) and fatigue severity (D) scores before, 6 months after, and 12 months after the start of TCZ treatment. The dots and I bars indicate means ± SEM. We analyzed only data obtained during the first year of TCZ treatment. (E) The alterations in the serum anti-aquaporin-4 antibody (AQP4-Ab) were evaluated by the relative ratio of the mean fluorescence intensity (MFI), which was based on the MFI before TCZ treatment. Serum anti-AQP4-Ab detection assay was performed as described previously[3,5] with minor modifications. In brief, optimally diluted serum was added to human AQP4-expressing Chinese hamster ovary (CHO) cells. CHO cell-bound anti-AQP4-Ab was detected using fluorescein isothiocyanate-anti-human immunoglobulin G antibody by flow cytometry. For comparison, the MFI of each sample was divided by the MFI of the sample before the start of TCZ treatment.

rodents showed that gp130 expressed by nociceptive neurons might have a key role in pathologic pain.[9] Although expression of membrane-bound IL-6R is restricted to hepatocytes, neutrophils, and subsets of T cells, the gp130, ubiquitously expressed in cellular membranes, can transduce IL-6R signaling via binding to the IL-6/soluble IL-6R complex.[4] This indicates that IL-6 trans-signaling via the soluble IL-6R could be pivotal in causing pain in NMO, although alternative possibilities cannot be excluded.

TCZ treatment recently showed efficacy for patients with aggressive NMO who were refractory to the anti-CD20 antibody rituximab.[10] The efficacy of TCZ could result from its effect on IL-6–dependent inflammatory

processes, involving CD20-negative PB, pathogenic T cells, and regulatory T cells. This work, however, does not restrict the use of TCZ in serious NMO. Although the need for monitoring latent infection and adverse events is obvious, we propose that the use of TCZ may be considered at an early stage of NMO before disability or a lower quality of life becomes evident.

AUTHOR CONTRIBUTIONS

T.Y., S.M., S.K., M.M., and M.A.: design and conceptualization of the study. M.A., K.M., T.O., and T.Y.: analysis and internalization of the data. T.M. and T.A.: flow cytometry analysis and anti-AQP4-Ab assay. M.A. and T.Y.: drafting and revising of the manuscript. T.Y.: supervising the entire project.

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Efficacy of the anti–IL-6 receptor antibody tocilizumab in neuromyelitis optica: A pilot study

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Research Paper

MULTIPLE SCLEROSIS JOURNAL | MSJ

Differential effects of fingolimod on B-cell populations in multiple sclerosis

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Abstract

Background: Fingolimod is an oral drug approved for multiple sclerosis (MS) with an ability to trap central memory T cells in secondary lymphoid tissues; however, its variable effectiveness in individual patients indicates the need to evaluate its effects on other lymphoid cells.

Objective: To clarify the effects of fingolimod on B-cell populations in patients with MS.

Methods: We analysed blood samples from 9 fingolimod-treated and 19 control patients with MS by flow cytometry, to determine the frequencies and activation states of naive B cells, memory B cells, and plasmablasts.

Results: The frequencies of each B-cell population in peripheral blood mononuclear cells (PBMC) were greatly reduced 2 weeks after starting fingolimod treatment. Detailed analysis revealed a significant reduction in activated memory B cells ($CD38^{int\text{-}high}$), particularly those expressing Ki-67, a marker of cell proliferation. Also, we noted an increased proportion of activated plasmablasts ($CD138^{+}$) among whole plasmablasts, in the patients treated with fingolimod.

Conclusions: The marked reduction of $Ki\text{-}67^{+}$ memory B cells may be directly linked with the effectiveness of fingolimod in treating MS. In contrast, the relative resistance of $CD138^{+}$ plasmablasts to fingolimod may be of relevance for understanding the differential effectiveness of fingolimod in individual patients.

Keywords

B cells, CD38, CD138, fingolimod, memory B cell, multiple sclerosis, plasmablast, proliferation, resistance, sphingosine 1-phosphate receptor 1

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Introduction

It is currently assumed that a large proportion of autoreactive T cells in multiple sclerosis (MS) is derived from a pool of $CCR7^{+}$ central memory T cells that are passing through the secondary lymphoid tissues (SLT).[1] Accordingly, egress of the T cells from the SLT represents a key process in MS pathogenesis. This process follows a rule of chemotaxis, in which the sphingosine 1-phosphate (S1P) receptor 1 (S1P1) expressed by lymphocytes is critically involved.[2] Fingolimod, an oral drug for treating relapsing–remitting MS (RRMS), serves as a functional antagonist for S1P1: Fingolimod induces internalisation and degradation of S1P1 in lymphocytes, causing the lymphocytes to lose the ability to respond to S1P and consequently, to become trapped in the SLT.[3] Analysis of large cohorts of patients with RRMS demonstrate the overall effectiveness of fingolimod in reducing the annualised relapse rate (ARR), as well as the appearance of new brain lesions in the patients' magnetic resonance imaging (MRI) scans.[4,5]

The number of central memory interleukin 17-producing $CD4^{+}$ T cells (Th17 cells) is reduced in the peripheral blood of fingolimod-treated patients. This is now being interpreted as a major mechanism of drug action;[6] however, fingolimod is not able to prevent relapses nor exhibit

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