性のみ）2例(0.02%)、F)腎障害（形態異常も含む）7855例（92.7%）であった。このうち2つを合併する症例はA+C 1例、A+E 1例、A+F 246例、B+F 7例、C+F 14 例であった。また3つ合併する例がA+B+Fの1例存在し、肝線維化も併せて合併していた。さらに 4 つ合併する例（A+C+D+F）も1例報告された。また常染色体劣性遺伝と思われる症例は2例あり、クラインフェルター症候群（両親いとこ婚）および精神遅滞＋若年性白内障の家族歴があるも精査を拒否した例が報告された。

D．考察

慢性腎臓病の考え方の普及とともに多くの腎臓専門医は成人の腎障害を多く診ているためにバルデー・ビードル症候群に対する認識が強くないと考えられた。

E．結論

腎臓専門医のバルデー・ビードル症候群に対する認識が高くないと推察され、スクリーニング法などを整備して啓蒙する必要性がある。

F．健康危険情報

なし

G．研究発表

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H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
「バルデー・ビードル症候群の実態把握のための奨励研究」班
分担研究報告書

小児神経領域におけるバルデー・ビードル症候群の実態把握

研究分担者　真野利之　大阪府立母子保健総合医療センター小児神経科 副部長

バルデー・ビードル症候群(BBS)は肥満、知能障害、網膜色素変性症、透析に至る慢性腎障害、性腺機能低下症、多指症を特徴とする常染色体劣性疾患である。本邦ではローレンス・ムーン・ビードル症候群と呼ばれることが多いが、それは世界的には肥満のない別の疾患を指すとされる。原因遺伝子はBBS1-BBS14として同定されたが、原因不明例も多い。本疾患は知能・視力障害による介護体制整備や腎・肝障害に対する生命予後改善のために、患者数の把握や疾患の啓蒙は重要だが、小児期発症の疾患にもかかわらず認知度は小児科医、小児神経科医でも低い。本研究では、疾患を全国的に啓発し、正確な診断に基ずく適切な治療・ケアを提供する目的や、基礎研究用のデータ収集のために、全国実態調査を施行したが、我々は小児神経学会（124施設）を中心にデータ収集を行った、

A．研究目的

バルデー・ビードル症候群(BBS)は肥満、知能障害、網膜色素変性症、透析に至る慢性腎障害、性腺機能低下症、多指症を特徴とする常染色体劣性疾患である。本邦ではローレンス・ムーン・ビードル症候群と呼ばれることが多いが、それは世界的には肥満のない別の疾患を指すとされる。すなわち現在本邦では疾患名の混乱により世界水準の情報提供が困難である。原因遺伝子はBBS1-BBS14として同定されたが、原因不明例も多い。小児期発症の疾患にもかかわらず認知度は小児科医、小児神経科医でも非常に低いのが現実である。そのため疾患を全国的に啓発し、正確な診断に基づく適切な治療・ケアを提供する目的や、基礎研究用のデータ収集のための本研究にあたり、小児神経学会の関連施設を中心に全国実態調査を施行した。 また小児神経学会、日本臨床神経生理学会などでビラ等を配布し、疾患の認知に寄与する事を目的とした。

B．研究方法

班員が協議して啓発用文書と調査票を作成し、小児神経学会認定施設（124施設）に啓発用文書と調査票を送付し、過去１年間に診療した肥満、知能障害、網膜色素変性症、腎障害、性腺機能低下、多指症を有する患者数および、それらを合併した症例数を調べた。

C．研究結果

全国47都道府県の合計124施設に送付し、58施設（回収率46.8%）から一次調査票の回答を得た。集計等は主任研究員がまとめて行った。

D．考察

成人を含めた全体の回収率に比して高く、疾患の認知度の低さに関わらず半数

程度の回答は、実際の患者把握とあわせ、小児科領域で一定の啓発になったと考えられる。今後新たな患者の掘り起しに寄与すると考えられる。

他を含めた全国集計では本疾患と考えられる38症例を経験した15施設へ二次調査票を送付した(詳細は総合報告書参照)。

E．結論

バルデー・ビードル症候群(BBS)は、小児科領域でも、認知度が低い疾患で、臨床像を含めアンケートをとるなどの情報を提供する事で、患者の掘り起しにつながり、また疾患自体の研究にもつながっていくと考えられた。

F．健康危険情報

なし

G．研究発表

(共同研究者としての発表のみ)

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H．知的財産権の出願・登録状況

なし

表1　アンケート都道府県別集計

都道府県	送付施設	回答件数	回答率
北海道	8	4	50.0%
青森県	0	0	
秋田県	1	1	100.0%
岩手県	1	0	0.0%
山形県	1	0	0.0%
宮城県	3	2	66.7%
福島県	0	0	
群馬県	1	1	100.0%
栃木県	2	1	50.0%
埼玉県	2	1	50.0%
茨城県	2	0	0.0%
神奈川県	6	2	33.3%
東京都	23	7	30.4%
千葉県	5	5	100.0%
新潟県	3	2	66.7%
富山県	0	0	
石川県	1	1	100.0%
福井県	0	0	
山梨県	3	0	0.0%
長野県	1	1	100.0%
岐阜県	2	2	100.0%
静岡県	5	0	0.0%
愛知県	7	3	42.9%
三重県	0	0	
京都府	4	2	50.0%
滋賀県	2	1	50.0%
兵庫県	3	1	33.3%
大阪府	10	5	50.0%
奈良県	1	1	100.0%
和歌山県	1	1	100.0%
鳥取県	2	2	100.0%
岡山県	3	2	66.7%
島根県	0	0	
広島県	1	0	0.0%
山口県	1	0	0.0%
香川県	2	2	100.0%
徳島県	2	1	50.0%
愛媛県	1	0	0.0%
高知県	0	0	
福岡県	6	3	50.0%
大分県	1	1	100.0%
宮崎県	0	0	
佐賀県	1	1	100.0%
熊本県	1	1	100.0%
鹿児島県	2	1	50.0%
長崎県	1	0	0.0%
沖縄県	2	0	0.0%
計	124	58	46.8%

厚生労働科学研究費補助金（難治性疾患克服研究事業）
「バルデー・ビードル症候群実態把握のための奨励研究」班
分担研究報告書

バルデー・ビードル症候群における分子病態の基礎研究

研究分担者　山下　俊英　大阪大学　大学院医学系研究科　分子神経科学　教授

【研究要旨】

バルデー・ビードル症候群(BBS)は肥満、知能障害、網膜色素変性症、透析に至る慢性腎障害、性腺機能低下症、多指症・合指症を特徴とする常染色体劣性疾患である。肝線維化による肝硬変も合併する。本邦ではローレンス・ムーン症候群、ローレンス・ムーン・ビードル症候群などと呼ばれるが、それらは世界的には肥満のない別の疾患を指す(Obes Rev 2002;3:123)。国際的データベース　PubMedではBBSとされ、原因遺伝子名もBBS1のように表記される。すなわち現在本邦では疾患名の混乱のため、正確かつ世界水準の情報提供が困難な状態にあり疾患名の周知が必要である。原因遺伝子はBBS1-BBS14として同定されているが、未だ原因不明例が多い。本疾患は稀少疾患であるが、現在社会問題となっている小児期の肥満が出現し、また知能障害や失明により社会参加が困難となり、10年以上の長期介護が必要となる。また、腎･肝障害が高度であれば、生命予後も良くない。本疾患の根治療法はなく、この一因に、ヒトの病態を反映するモデルに乏しいこともあげられる。BBSの原因蛋白BBS1-14の少なくともいくつかの蛋白質は複合体を形成し、せん毛機能を維持することが示唆されているが、そのほか、レプチン受容体の細胞内輸送を促進しているとされ、細胞内輸送に重要なsmall GTPaseの活性化を担うGuanine Exchange Factor活性化作用が報告されている。本研究グループは、遺伝子・たんぱく機能解析を分担した。本研究では、iPS細胞を用いて、機能・形態異常について検討し、さらに薬物によるそれらの改善効果を検討することを予定しているが、今年度においては、神経細胞の機能および形態異常の解析法の確立を行うことを目的として、研究を進めた。その結果、２種類の指標を見いだすことができた。一つ目は、Guanine Exchange Factor活性化機能の障害により、神経突起の伸展および軸索先端の成長円錐の形態に変化が惹起されることを明らかにした。さらに軸索内輸送が障害されると、軸索障害後に起こる軸索新生現象が消失することがわかった。これらは、神経細胞におけるGuanine Exchange Factor活性化機能の障害および細胞内輸送の障害に基づく形態的異常であり、このような変化がBBS由来の細胞においても認められる可能性がある。また、その指標を用いて治療薬候補をスクリーニングすることが可能となる。

A．研究目的

バルデー・ビードル症候群(BBS)は肥満、知能障害、網膜色素変性症、透析に至る慢性腎障害、性腺機能低下症、多指症・合指症を特徴とする常染色体劣性疾患である。本研究グループは、遺伝子・たんぱく機能解析を分担した。本研究では、iPSを用いて、機能・形態異常について検討し、さらに薬物によるそれらの改善効果を検討することを予定しているが、今年度においては神経細胞の機能および形態異常の解析法の確立を行うことを目的とした。

B．研究方法

マウス培養大脳皮質神経細胞を用いて、各種ストレスによる神経細胞機能および形態変化の解析を行った。Small GTPaseであるRhoAあるいはRac1がGuanine Exchange Factorによって活性化された時の表現系の解析を行った。さらに神経細胞内で軸索の輸送障害がおこすために、Importinの機能をブロックし、それによりどのような現象が起こるかについて解析した。当該研究によって、次年度以降に行われるiPS細胞から分化させた神経細胞の機

能評価の解析法を確立することを目的とした。

（倫理面への配慮）

組換えDNA実験については、関係法令を遵守し、所属機関の承認を得たうえで行われた。また、動物の取扱いについては文部科学省および所属機関の指針に基づいて、所属機関の承認を得たうえで行われた。

C．研究結果

神経細胞内で Rho の活性を制御する GEF の機能解析を行った。RhoGEF のメンバーである LARG を大脳皮質神経細胞に強制発現させると、RhoA を活性化することで、神経軸索の伸長を阻害し、成長円錐を虚脱させることを見いだした。またこの神経細胞は LARG を内在性に発現しているが、このたんぱく質を siRNA を用いて発現低下させると、脳脊髄内に存在するオリゴデンドロサイトのミエリンに由来する軸索再生阻害因子の効果が失われた。これらの実験結果から、RhoGEF は神経突起の伸展および軸索先端の成長円錐の形態変化に関わることが判明した。

一方で、細胞内輸送の障害によって、神経細胞でどのような変化が生じるかについても解析を行った。軸索内輸送をブロックするために、importin の機能を抑制する peptide inhibitor Bimax および dynein を抑制する p50 を用いた。神経細胞へのストレスとしては、軸索切断という機械的ストレスを用いた。そうすると、通常では、新たな軸索の新生、あるいは樹状突起が軸索に変化するという現象が起こるが、Bimax を投与、あるいは p50 を過剰発現させると、新たな軸索の新生が消失することがわかった（論文８）。この形態変化は、ストレスに対する神経細胞の反応であり、細胞内輸送障害に依存していると示唆される。

D．考察

本研究によって、各種ストレスによる神経細胞機能および形態変化の指標を得ることができた。バルデー・ビードル症候群（BBS）においては、Guanine Exchange Factor 活性化機能の障害、そして細胞内輸送の障害が示されているが、その神経細胞での機能を明らかにし、その障害によってどのような変化が現れるかを見いだすことによって、細胞モデルを用いた治療薬候補の検索が可能になると期待される。本研究では、Guanine Exchange Factor 活性化機能の障害により、神経突起の伸展および軸索先端の成長円錐の形態に変化が惹起されることを明らかにした。さらに軸索内輸送が障害されると、軸索障害後に起こる軸索新生現象が消失することがわかった。これらの知見は基礎研究としても重要であるが、特にこれらの変化を指標として、iPS 細胞から分化させた神経細胞においても同様の変化が認められる可能性がある。またこの反応は、形態変化であり、判定が簡便であるため、その異常をレスキューする治療薬候補をスクリーニングする系として有望である。これらの結果を土台として、次年度以降の iPS 疾患モデルの構築に向けて、研究を進めて行きたい。

E．結論

神経細胞の機能および形態異常の解析法の確立を行うことを目的として、研究を進めた。その結果、２種類の指標を見いだすことができた。これらは、神経細胞における Guanine Exchange Factor 活性化機能の障害および細胞内輸送の障害に基づく形態的異常であり、このような変化がBBS由来の細胞においても認められるか、認められるならばその指標を用いて治療薬候補をスクリーニングすることが可能となる。本研究成果は、病態解明に向けた基礎研究としても重要な知見である。

F．健康危険情報

なし

G．研究発表

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H. 知的財産権の出願・登録状況

なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍：該当なし

雑誌

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Ⅳ. 研究成果の刊行物・別刷

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CASE REPORT

Abnormal Cystatin C Levels in Two Patients with Bardet-Biedl Syndrome

Makito Hirano[1], Mitsuru Ohishi[2], Toshihide Yamashita[3], Yasushi Ikuno[4], Hiromi Iwahashi[5], Toshiyuki Mano[6], Ryu Ishihara[7], Ichiro Tanaka[8], Keiko Yanagihara[6], Chiharu Isono[1], Hikaru Sakamoto[1], Yusaku Nakamura[1] and Susumu Kusunoki[9]

[1]Department of Neurology, Sakai Hospital Kinki University Faculty of Medicine. [2]Department of Geriatric Medicine, Osaka University. [3]Department of Molecular Neuroscience, Osaka University. [4]Department of Ophthalmology, Osaka University. [5]Department of Metabolic Medicine, Osaka University. [6]Division of Pediatric Neurology, Osaka Medical Center and Research Institute for Maternal and Child Health. [7]Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases. [8]Department of Paediatrics, Nara Medical University. [9]Department of Neurology, Kinki University. Corresponding author email: hirano_makito@yahoo.co.jp

Abstract: Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by central obesity, mental impairment, rod-cone dystrophy, polydactyly, hypogonadism in males, and renal abnormalities. The causative genes have been identified as BBS1-14. In the Western countries, the prevalence of this disease ranges from 1/13,500 to 1/160,000, while only a few Japanese patients have been reported in the English-language literature. The incidence of renal dysfunction or anomalies in previous reports varies considerably ranging from ~20% to universal occurrence. We here report that two Japanese patients who had BBS with normal BUN and creatinine levels had elevated levels of cystatin C, a sensitive marker of glomerular filtration rate. A urine albumin level increased only in the elder patient. Thus, cystatin C may be useful for detecting renal abnormalities in patients with an apparent normal renal function. Because this disease is diagnosed by accumulation of symptoms, such a sensitive marker might help early diagnosis of BBS.

Keywords: mental impairment, obesity, cystatin C, renal abnormality, retinitis pigmentosum

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Introduction

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by central obesity, mental impairment, rod-cone dystrophy, polydactyly, hypogonadism in males, and renal abnormalities.[1,2] The causative genes have been identified as BBS1-14 genes that encode proteins possibly linked to cilia function, but more than 20% of patients have no mutations found.[3] The diagnosis is made only by the clinical phenotype with the presence of at least three major symptoms, however, it is often difficult partly because of age-dependent development of some symptoms. In the Western countries, the prevalence of this disease ranges from 1/13,500 to 1/160,000.[3] By contrast, only a few Japanese patients have been reported in the English-language literature.[4–6]

Renal fibrosis is one of the most devastating symptoms, ultimately leading to chronic renal failure requiring hemodialysis.[7] The incidence of renal dysfunction or anomalies in previous reports varies considerably ranging from ~20% to universal occurrence.[2,7] An early detection of such abnormalities may be important for patients and guardians to prepare them. It may also be useful for prompt correct diagnosis of BBS, since the diagnosis of this disease is based on the accumulation of major symptoms as described above. We now report that two Japanese patients with BBS had normal BUN and creatinine level but elevated levels of cystatin C, a sensitive marker of glomerular filtration rate (GFR).

Patients

A 20-year-old man (patient 1) had mental retardation (minimental state examination 23; normal > 24), rod-cone dystrophy, central obesity (height 158 cm, weight 63 kg, and BMI 25.2) and hypogonadism since the age of 5 years. His waist circumference was 83.5 cm. His blood pressure was 131/85 mmHg, and his heart rate was 61 beats/min. He had normal heart sounds with clear breath sounds. A 16-year-old boy (patient 2), the younger brother of patient 1, had polydactyly in addition to the symptoms described above (height 165 cm, weight 93 kg, and BMI 34.2). His waist circumference was 107 cm. His blood pressure was 128/61 mmHg, and his heart rate was 77 beats/min. He had normal heart sounds with clear breath sounds. Their non-consanguineous parents were apparently healthy. The symptoms of patients and probable autosomal recessive inheritance fulfilled the diagnostic criteria for BBS5. After obtaining informed consent, a DNA chip study was performed at Asper Biotech Ltd. (Tartu, Estonia). The DNA chip (version 5) covered 305 mutations from 14 genes causative for BBS and related diseases (BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7, BBS8, BBS9, BBS10, BBS12, PHF6, ALMS1, and GNAS1), but identified no pathological alterations. Nevertheless, because about one fifth of patients with clinically definite BBS have no identifiable mutations as described above and because the chip covered only mutations previously reported to be pathogenic, these results could not rule out the possibility of a diagnosis of BBS in our family.

Tests for Renal Morphology and Function, and Other Laboratory Tests

To detect morphological renal abnormalities, the patients underwent abdominal CT scans and abdominal sonography, with no apparent anomalies. Blood and urine tests routinely performed in Japan failed to identify any obvious abnormalities (Table 1, upper rows). Other laboratory data of the elder and younger patients included normal blood sugar levels (78 mg/dl and 81 mg/dl, respectively), normal total cholesterol levels (144 mg/dl and 131 mg/dl, normal 120–220 mg/dl), unelevated triglyceride levels (28 mg/dl and 72 mg/dl, normal 30–150 mg/dl), negative serum CRP, and negative urine occult blood or glucose. Creatinine was measured by an enzymatic method. Serum cystatin C

Table 1. Results of sensitive renal function tests.

Patient #	1	2
BUN (mg/dl)	7	9
Cre (mg/dl)	0.6	0.8
Urine protein	– ~ ±	–
Urine albumin (with cre correction normal = <10)	248*	5.2
Cystation C (0.63–0.95 mg/l)	0.96*	0.97*

Note: *Abnormal values.

and urine albumin were then examined. Cystatin C was measured by a colloidal gold agglutination method. The results showed elevated cystatin C concentrations in both patients and microalbuminuria in the elder patient (Table 1, lower rows). Cystatin C levels of the age- and sex-matched controls were also examined, the result of which showed 0.86 mg/L for an elder control and 0.91 mg/L for a younger control.

Discussion

We describe abnormal levels of serum cystatin C in two patients with BBS (Table 1). Cystatin C is a plasma protein with a molecular weight of 13.4 kDa and belongs to the cysteine protease inhibitors.[8] It is constantly synthesized in all types of cells, excreted into plasma, and filtered completely by the glomeruli. Consequently, increasing serum levels of this marker indicate decreasing GFR. Measurement of cystatin C more sensitively detects mild GFR abnormalities than that of creatinine, a more common but less sensitive marker of GFR,[8] probably because the lower molecular weight of creatinine (113 Da) facilitates its easier filtration in the glomeruli. In addition to the sensitivity, cystatin C is a more reliable marker than creatinine for detection of chronic renal disease, since creatinine levels are affected by many extrarenal patient-related factors such as muscle mass and consumption of cooked meat that is a source of creatinine.[8] Our patients had only mild increases in cystatin C. Nevertheless, because cystatin C levels age-dependently increase with decreasing GFR, the values of our young patients seem sufficiently high for their ages.[8]

A urine albumin level increased only in the elder patient. Patients with BBS occasionally manifest proteinuria,[7] suggesting that patients had not only decreased GFR but also increased protein leakage. Urine albumin is used to detect early phases of diabetic or hypertensive nephropathy.[9] Because neither of our patients showed apparent proteinuria, the elder patient may be in an early phase of protein leakage. In diabetes mellitus, timely treatment with an angiotensin-converting enzyme inhibitor, independently of rise in arterial blood pressure, is considered if improvement of glycaemic control and moderate decrease of dietary protein intake for 6–12 months have failed to reduce the albumin excretion rate.[9] Screening programs for microalbuminuria and early intervention can substantially modify the natural history of diabetic renal involvement and disease and possibly reduce the incidence of end-stage renal failure.[9] In BBS, although such intervention has not been tested yet, we may consider similar protective methods for renal dysfunction.

In conclusion, patients who have BBS with apparently normal kidney functions may have abnormal levels of cystatin C, facilitating an early detection of kidney dysfunctions that might be helpful for prompt correct diagnosis of BBS. However, because our study is based on the results of the small number of patients, conclusion must await further studies.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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nature medicine

RGMa modulates T cell responses and is involved in autoimmune encephalomyelitis

Rieko Muramatsu[1,2,8], Takekazu Kubo[3,8], Masahiro Mori[4], Yuka Nakamura[1,2], Yuki Fujita[1,2], Tsugio Akutsu[5], Tatsusada Okuno[6], Junko Taniguchi[4], Atsushi Kumanogoh[6], Mari Yoshida[7], Hideki Mochizuki[2,5], Satoshi Kuwabara[4] & Toshihide Yamashita[1–3]

In multiple sclerosis, activated CD4$^+$ T cells initiate an immune response in the brain and spinal cord, resulting in demyelination, degeneration and progressive paralysis. Repulsive guidance molecule-a (RGMa) is an axon guidance molecule that has a role in the visual system and in neural tube closure. Our study shows that RGMa is expressed in bone marrow–derived dendritic cells (BMDCs) and that CD4$^+$ T cells express neogenin, a receptor for RGMa. Binding of RGMa to CD4$^+$ T cells led to activation of the small GTPase Rap1 and increased adhesion of T cells to intracellular adhesion molecule-1 (ICAM-1). Neutralizing antibodies to RGMa attenuated clinical symptoms of mouse myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) and reduced invasion of inflammatory cells into the CNS. Silencing of RGMa in MOG-pulsed BMDCs reduced their capacity to induce EAE following adoptive transfer to naive C57BL/6 mice. CD4$^+$ T cells isolated from mice treated with an RGMa-specific antibody showed diminished proliferative responses and reduced interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-4 and IL-17 secretion. Incubation of PBMCs from patients with multiple sclerosis with an RGMa-specific antibody reduced proliferative responses and pro-inflammatory cytokine expression. These results demonstrate that an RGMa-specific antibody suppresses T cell responses, and suggest that RGMa could be a promising molecular target for the treatment of multiple sclerosis.

In multiple sclerosis, activated CD4$^+$ T cells specific for components of the myelin sheath initiate an immune response in the white matter of the brain and spinal cord[1], resulting in demyelination, degeneration and progressive paralysis. Dendritic cells in the peripheral tissues and the central nervous system (CNS) are responsible for T cell activation and helper cell differentiation. T cell activation depends on the interaction of T cell receptors (TCRs) with their cognate antigen peptide presented on the surface of antigen-presenting cells (APCs), including dendritic cells and macrophages. Regulated adhesion of T cells to APCs through leukocyte function-associated antigen-1 (LFA-1) is a crucial step in the generation of a sustained TCR-mediated signal. The binding of integrins, including LFA-1, is also important for T cell trafficking into the brain[2]. The small GTPase Rap1, which is activated by antigens and chemokines, is a potent stimulator of integrins, including LFA-1 (refs. 3,4), and promotes immunological synapse formation and leukocyte migration[5].

RGMa is a membrane-bound protein that was originally identified as an axon guidance molecule in the visual system[6]. RGMa also has a role in laminar patterning in *Xenopus laevis* and chick embryos and in cephalic neural tube closure in mouse embryos[7]. Although RGMa is recognized as having a crucial role in the nervous system, we found that RGMa was expressed in dendritic cells by expression analysis in mice. This finding prompted us to investigate the role of RGMa in the immune system. This study describes a previously unknown role of RGMa in modulating T cell–mediated immune responses.

RESULTS

RGMa regulates CD4$^+$ T cell adhesion

We first examined whether RGMa is expressed in dendritic cells. Following stimulation with lipopolysaccharide (LPS), the level of mRNA encoding RGMa was increased in the BMDCs (**Fig. 1a**). Western blot analysis confirmed that full-length RGMa (50-kDa bands) and the proteolytically cleaved mature form of RGMa (35-kDa bands) were upregulated in LPS-stimulated BDMCs (**Fig. 1b**). We then assessed whether CD4$^+$ T cells express receptors for RGMa. Human RGMa-Fc bound to splenic CD4$^+$ T cells in a concentration-dependent manner (**Fig. 1c**). Therefore, RGMa is induced in activated BMDCs and CD4$^+$ T cells express receptors for RGMa.

Next, we explored the effects of RGMa on CD4$^+$ T cell adhesion. In T lymphocytes, TCR ligation results in the transient activation of Rap1 and an increase in the GTP-bound form of Rap1 at the interface of T cells and APCs, which potentiates subsequent T cell activation[3,5]. Rap1 was activated 5 min after stimulation of splenocytes and CD4$^+$ T cells with RGMa (**Fig. 1d**). As TCR-induced adhesion requires Rap1 activation[3,4,8], we determined whether the RGMa-induced

[1]Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, Osaka, Japan. [2]Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Tokyo, Japan. [3]Department of Neurobiology, Graduate School of Medicine, Chiba University, Chiba, Japan. [4]Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan. [5]Department of Neurology, Kitasato University School of Medicine, Kanagawa, Japan. [6]Department of Immunopathology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. [7]Institute for Medical Science of Aging, Aichi Medical University, Aichi, Japan. [8]These authors contributed equally to this work. Correspondence should be addressed to T.Y. (yamashita@molneu.med.osaka-u.ac.jp).

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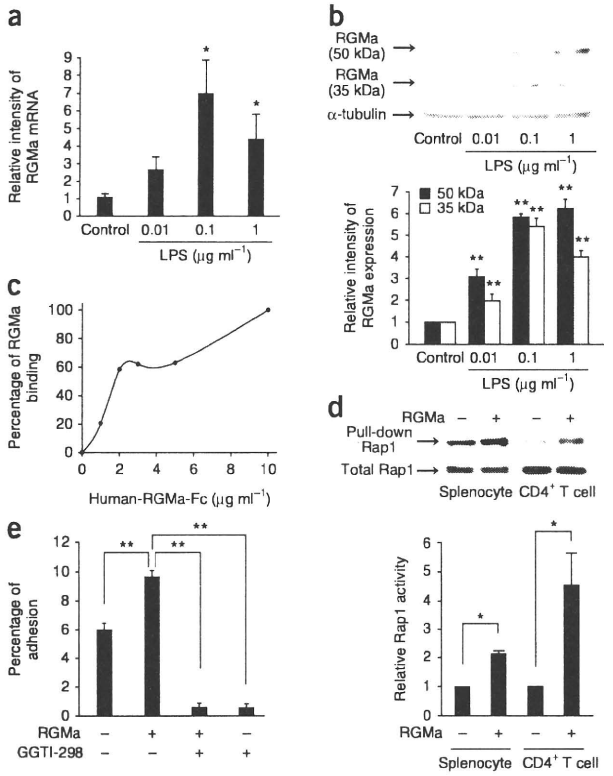

Figure 1 RGMa activates Rap1 and regulates CD4+ T cell adhesion. (**a**) Quantitative RT-PCR showing relative expression level of mRNA encoding RGMa in LPS-stimulated BMDCs at the indicated concentrations for 24 h. (**b**) Western blot analysis of RGMa (50-kDa and 35-kDa bands; top rows) and α-tubulin (bottom row). Relative expression of RGMa in the BMDCs. (**c**) Binding of human RGMa-Fc to splenic CD4+ T cells. (**d**) Top, representative western blot images obtained with a Rap1 pull-down assay. The bottom graph shows the relative Rap1 activity, as determined by the band intensity of RalGDS-bound Rap1 normalized to that of total Rap1 in the lysates. (**e**) CD4+ T cell adhesion to ICAM-1 in the presence and absence of GGTI-298, a selective Rap1 inhibitor. Error bars are the mean ± s.e.m. of three or four independent experiments. $*P < 0.05$ and $**P < 0.01$ by one-way analysis of variance followed by Tukey's test for **a**,**b** and **e** and by Student's *t* test for **d**.

Rap1 activation alters T cell adhesion. RGMa-stimulated CD4+ T cells showed stronger adhesion to ICAM-1 when compared with the control CD4+ T cells (**Fig. 1e**). Moreover, a selective inhibitor of Rap1, GGTI-298, abolished RGMa-induced adhesive activity in CD4+ T cells. These results suggest that RGMa enhances the adhesive activity of CD4+ T cells through Rap1 activation.

Expression of RGMa and neogenin in EAE and multiple sclerosis

To assess the role of RGMa *in vivo*, we examined the expression of RGMa and neogenin, a receptor for RGMa, in the spleens, lymph nodes and spinal cord sections of C57BL/6 mice with EAE induced by MOG. Immunohistochemical analyses reveal that the majority of the CD11c+ cells in these tissues expressed RGMa weakly (**Fig. 2a** and **Supplementary Fig. 1a**). RGMa expression increased in CD11c+ cells after the induction of EAE (**Fig. 2a** and **Supplementary Fig. 1a**). Although RGMa was also expressed in mouse plasmacytoid dendritic cell antigen-1 (mPDCA-1)-positive cells in these tissues, its expression was unchanged after the induction of EAE (**Fig. 2b** and **Supplementary Fig. 1b**). Furthermore, CD4+ T cells in these tissues expressed neogenin. Expression of neogenin did not change during the observation period after the induction of EAE (**Fig. 2c** and **Supplementary Fig. 1c**). Next, we assessed Rap1 activity *in situ* to determine whether Rap1 is activated in CD4+ T cells in the CNS after induction of EAE. Activated Rap1 was present in CD4+ T cells in the cervical spinal cords of EAE mice but not in control mice (**Fig. 2d**).

We performed immunohistochemical analyses on autopsied samples of brain and spinal cord obtained from eight individuals with multiple sclerosis. We evaluated the presence of mature and immature dendritic cells in these tissues by using antibodies to CD83 and CD209 (also known as DC-SIGN), respectively[9]. CD83+ and CD209+ dendritic cells were immunoreactive for RGMa in the brain and spinal cord sections of individuals with multiple sclerosis (**Fig. 2e**), but not in sections from control brains (data not shown). To examine the expression of neogenin in human cells, we purified peripheral blood mononuclear cells (PBMCs) from individuals with relapsing-remitting multiple sclerosis. CD3+ T cells from these samples expressed neogenin at the time of relapse and during the remission phase (**Fig. 2f**). Neogenin expression did not differ in PBMCs from individuals with multiple sclerosis or healthy controls (**Fig. 2g**). Furthermore, neogenin was expressed in CD3+ T cells in brain and spinal cord sections from individuals with multiple sclerosis (**Supplementary Fig. 1d**).

RGMa-specific antibodies attenuate clinical signs in EAE

To determine whether RGMa inhibition alters the clinical severity of EAE, we intraperitoneally administered RGMa-specific antibodies[10] or control rabbit IgGs to mice on days 7 and 10 after immunization with MOG. The antibody was detectable in the spleen and lymph nodes at day 7 after administration (**Supplementary Fig. 2a**). RGMa-specific antibody treatment reduced the clinical severity of the disease (**Fig. 3a**) and the percentage of mice that presented with clinical signs of EAE (**Fig. 3b**), but did not delay the day of onset of EAE clinical symptoms (**Fig. 3c**). However, the mean maximum EAE score (**Fig. 3d**) and the cumulative EAE scores (**Fig. 3e**) were lower in RGMa-specific antibody–treated mice as compared with control IgG–treated mice. These data show that RGMa-specific antibody treatment attenuates the severity of EAE.

RGMa-specific antibody treatment reduced the infiltration of cells in the spinal cord at day 21 after the induction of EAE in mice (**Fig. 3f,g**). The accumulation of CD4+, CD11b+, F4/80+, B220+, CD11c+ and mPDCA-1+ cells in the spinal cord (**Fig. 3h**) of EAE mice decreased as a result of treatment with the RGMa-specific antibody. Myelin loss and axonal damage in EAE mice was also reduced following treatment with the RGMa-specific antibody (**Fig. 3i,j**) Thus, RGMa-specific antibody treatment reduces inflammatory cell accumulation and histological damage following the induction of EAE.

A role for RGMa in T cell activation in EAE

To confirm whether RGMa expressed on dendritic cells modulates EAE, we carried out adoptive transfer experiments with MOG-pulsed BMDCs following RGMa knockdown. Transfection of BMDCs with RGMa siRNA downregulated RGMa expression (**Supplementary Fig. 2b**). Recipient C57BL/6 mice injected intravenously with RGMa siRNA-transfected, MOG-pulsed BMDCs had moderately reduced clinical disease scores as compared with mice injected with control siRNA–transfected, MOG-pulsed BMDCs

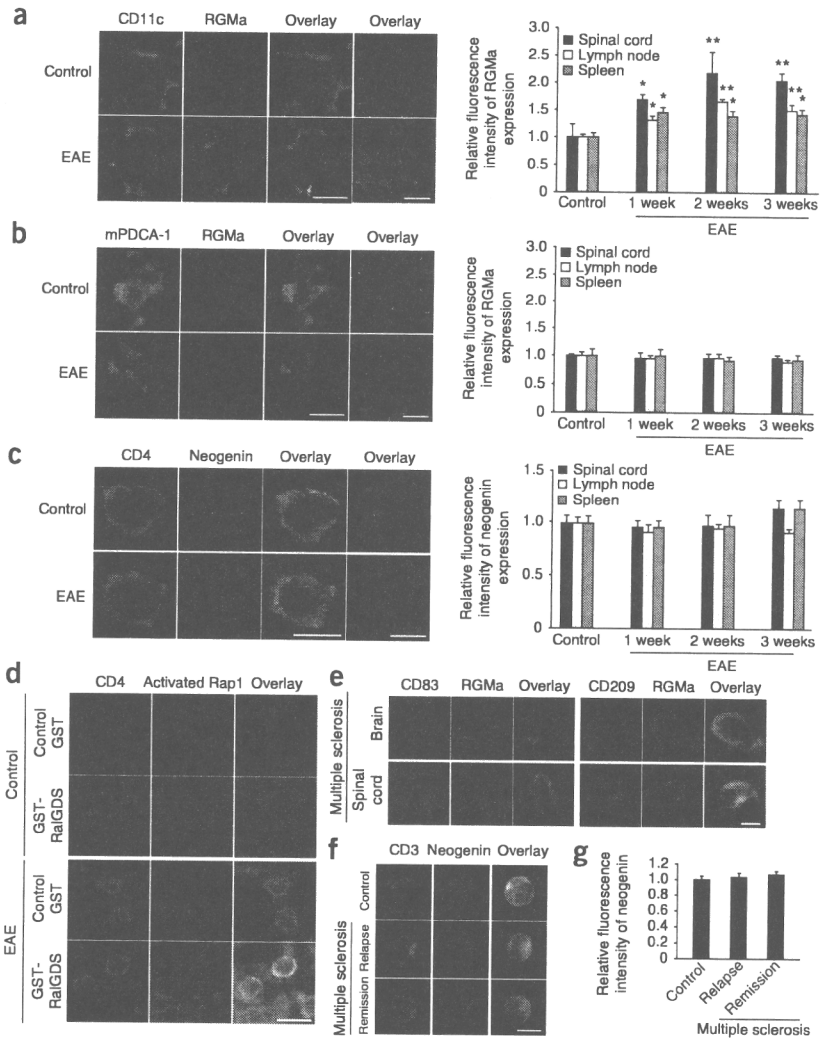

Figure 2 Expression of RGMa and neogenin in MOG-induced EAE and multiple sclerosis tissue. (**a**) Frozen sections of the spleen immunostained for RGMa (labeled with Alexa Fluor 568) and CD11c (labeled with Alexa Fluor 488) in EAE and control mice. The graph shows the relative expression of RGMa in CD11c$^+$ cells in the lymph node, spleen and spinal cord before (control) and 1, 2 and 3 weeks after immunization with MOG. $n = 37$–51 cells for each mouse. $^*P < 0.05$ and $^{**}P < 0.01$ by one-way analysis of variance followed by Tukey's test. (**b**) The sections (same sections as shown in **a**) of the spleen immunostained for RGMa (labeled with Alexa Fluor 568) and plasmacytoid dendritic cells (mPDCA-1) (labeled with Alexa Fluor 488). The graph shows the relative expression of RGMa in mPDCA-1$^+$ cells. $n = 40$–48 cells for each mouse. (**c**) Expression of neogenin (labeled with Alexa Fluor 568) in CD4$^+$ T cells (labeled with Alexa Fluor 488) in the spleen. The graph shows the relative expression of neogenin in CD4$^+$ T cells. (**d**) *In situ* Rap1 pull-down assay (labeled with Alexa Fluor 568) in CD4$^+$ T cells (labeled with Alexa Fluor 488) in cervical spinal cord tissue sections of EAE and control mice. (**e**) Multiple sclerosis brain and spinal cord tissues sections double-labeled for RGMa (with Alexa Fluor 488) in combination with CD83 or CD209 (DC-SIGN) (labeled with Alexa Fluor 568) $n = 8$. (**f**) Expression of neogenin (labeled with Alexa Fluor 488) in human CD3$^+$ cells (labeled with Alexa Fluor 568) in relapsing-remitting multiple sclerosis and healthy control PBMCs. (**g**) Relative fluorescence intensity of neogenin in the immunohistochemical analysis. Error bars represent the mean ± s.e.m. of 3 or 4 independent experiments. Scale bars in **a**–**e**, 50 μm for low (overlay images in **a**, **b**, and **c**) and 10 μm for high (all other images) magnification images; scale bar in **f**, 5 μm.

(**Fig. 4a**). The adoptive transfer of RGMa siRNA–transfected BMDCs resulted in reduced F4/80$^+$ cell infiltration into the spinal cord at day 21 after EAE induction (**Supplementary Fig. 2c**).

To further address whether dendritic cell–derived RGMa has a role in T cell activation, we immunized C57BL/6 mice with MOG, followed by treatment with RGMa-specific antibodies or control antibodies at days −2, 0 and 5 after immunization. On day 10 after immunization, we collected cells from the spleen and draining lymph nodes of the treated mice, re-stimulated these cells with MOG peptide, purified CD4$^+$ T cells from these cells and then adoptively transferred them into naïve recipient mice. The EAE clinical scores (see **Supplementary Methods**) were moderately reduced in C57BL/6 mice that were injected with CD4$^+$ T cells from RGMa-specific antibody–treated EAE mice as compared with mice injected with CD4$^+$ T cells from the IgG control antibody–treated mice (**Fig. 4b**).

Next, we assessed whether the RGMa-specific antibody directly inhibits T cell trafficking to the CNS. We immunized transgenic mice that ubiquitously express EGFP (CAG-EGFP mice) with MOG, isolated splenocytes from these mice 7 d after immunization and re-stimulated splenocytes with MOG for 3 d. We treated naïve recipient C57BL/6 mice with control IgG or RGMa-specific antibody 3 d before and at the time of transfer of the re-stimulated CD4$^+$ T cells. At day 10 after adoptive transfer, there was no significant difference in the infiltration of EGFP-labeled T cells into the CNS of control or RGMa-specific antibody–treated mice (**Fig. 4c**). Consistent with these *in vivo* observations, the RGMa-specific antibody did not inhibit adhesion of splenic CD4$^+$ T cells from EAE mice to ICAM-1 *in vitro* (**Fig. 4d**). This result excludes the possibility that the antibody directly interfered with the adhesion of CD4$^+$ T cells to ICAM-1. Furthermore, using an *in vitro* model of the blood-brain barrier consisting of brain-derived capillary endothelial b-End3 cells, splenic CD4$^+$ T cells from MOG-EAE mice transmigrated more readily across the b-End3 cells than did CD4$^+$ T cells isolated from EAE mice treated (*in vivo*) with the RGMa-specific antibody (**Fig. 4e**). However, transmigration of T cells was not altered following direct addition of RGMa-specific antibody *in vitro* to splenic CD4$^+$ T cells (**Fig. 4e**). Because Rap1 activity is associated with increased adhesion of T cells, we measured Rap1 activity by pull-down assay and found that Rap1 activity was reduced in CD4$^+$ T cells isolated from EAE-mice treated with the RGMa-specific antibody (**Fig. 4f**). However, we did not observe marked suppression of Rap1 activity following *in vitro* treatment of CD4$^+$ T cells with the RGMa-specific antibody (**Fig. 4f**). Thus, we obtained no evidence suggesting that the RGMa-specific antibody directly modulates the trafficking of T cells to the CNS.