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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

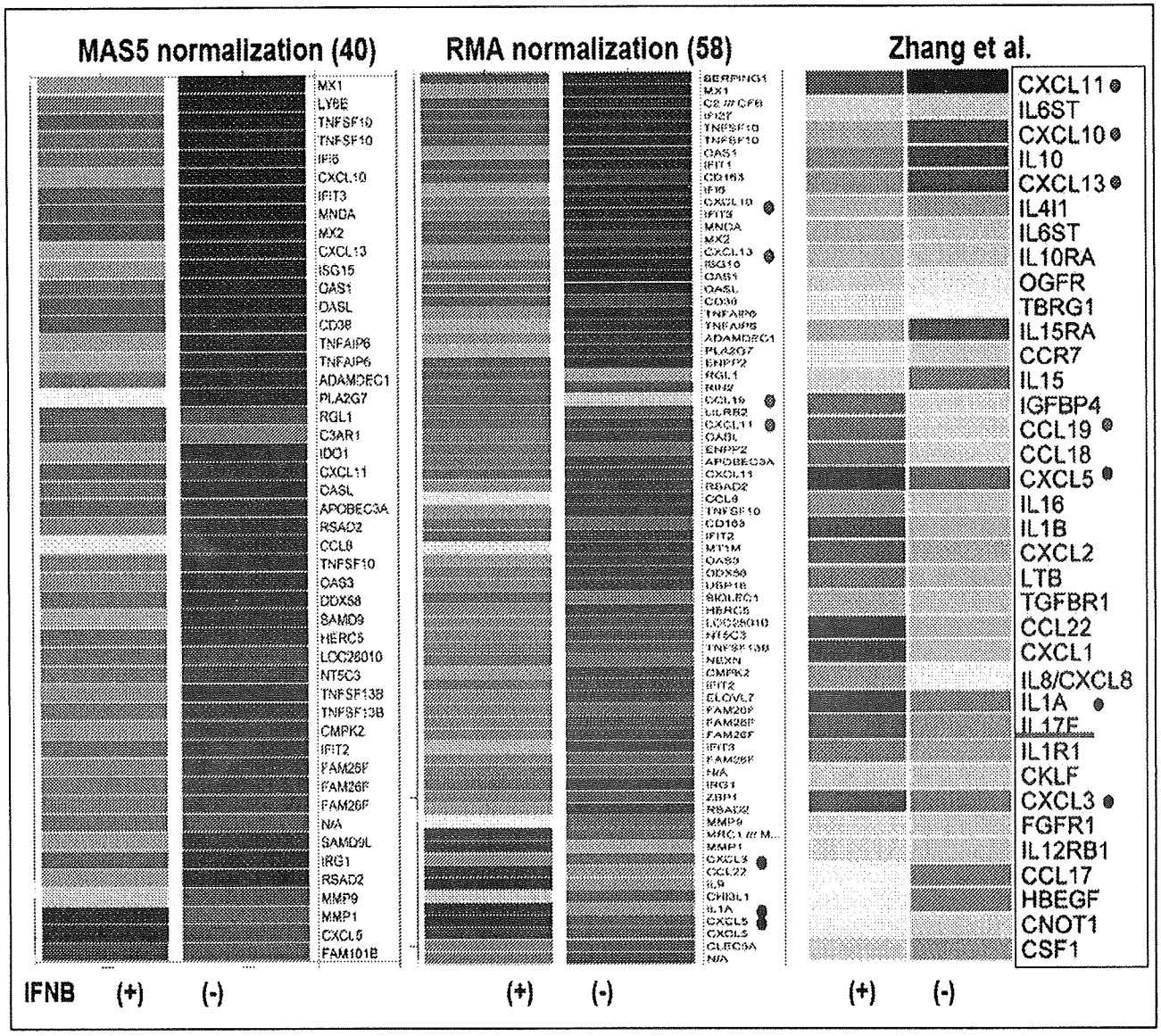

Fig. 1. Clustering analysis of differentially expressed genes in activated T-cells incubated in the presence or absence of IFNB. The microarray dataset of GSE14386 is composed of gene expression profiles by Affymeirix HG-133 Plus 2.0 array of cultured PBMC isolated from 15 CIS patients activated by anti-CD3 and CD-28 anibodies in the presence or absence of IFNB. The CEL data was analyzed by GeneSpring GX10.0.2 following normalization by robust multiarray average (RMA) or MicroArray Suite 5 (MAS5). The statistical evaluation was performed by paired T-test and multiple testing correction with Benjamini Hochberg FDR. Then, the 40 (MAS5) or 58 (RMA) differentially expressed genes (DEGs) in the presence versus absence of IFNB showing more than a 2-fold change were extracted. The genes shared between the present study and the previous study (Zhang et al. J Immunol 182: 3928 -3936, 2009) are marked by dots.

Table 1. Fifty-eight differentially expressed genes (RMA) in activated T-cells incubated in the presence or absence of IFNB

Entrez GeneID	Fold Change	Gene Symbol	Overlap with MAS5	INTERFEROME IRG(Type)	ISRE	STAT	IRF	NFkB
6373	8.096138	CXCL11	Y	Y(1>2)	Y	Y	Y	Y
3429	5.965393	IFI27		Y(1>2)	Y	Y	Y	
3434	3.8773782	IFIT1		Y(1>2)	Y	Y	Y	Y
200315	3.308835	APOBEC3A	Y					
710	3.1044571	SERPING1		Y(1>2)		Y		Y
3433	2.9863205	IFIT2		Y(1>2)				
11274	2.9688022	USP18		Y	Y	Y	Y	Y
629	2.9578424	CFB		Y		Y	Y	Y
730249	2.8871603	IRG1	Y					
5168	2.8765597	ENPP2						
91543	2.8394268	RSAD2	Y	Y(1>2)	Y	Y	Y	Y
27299	2.8118875	ADAMDEC1	Y					
4332	2.7625558	MNDA	Y	Y(1)		Y	Y	Y
4938	2.728571	OAS1	Y	Y(1>2)		Y	Y	Y
6614	2.610088	SIGLEC1						
8743	2.6076565	TNFSF10	Y	Y		Y	Y	Y
441168	2.6009846	FAM26F	Y	Y				
3433	2.5998137	IFIT2	Y	Y(1>2)				
3437	2.558451	IFIT3	Y	Y(1>2)	Y	Y	Y	Y
8638	2.5475945	OASL	Y	Y(1>2)	Y	Y	Y	Y
23586	2.4248114	DDX58	Y	Y(1>2)	Y	Y	Y	Y
91624	2.4191918	NEXN						
51191	2.4141314	HERC5	Y	Y(1>2)	Y	Y	Y	Y
9636	2.405012	ISG15	Y	Y(1>2)	Y	Y	Y	Y
4600	2.3609657	MX2	Y	Y(1>2)	Y	Y	Y	Y
952	2.3308659	CD38	Y	Y(1>2)		Y	Y	Y
129607	2.323656	CMPK2	Y	Y		Y	Y	Y
9332	2.30428	CD163						
7130	2.2603915	TNFAIP6	Y					
10288	2.257882	LILRB2		Y(1)		Y		Y
79993	2.224418	ELOVL7						
4940	2.2227726	OAS3	Y	Y(1>2)	Y	Y	Y	Y
4499	2.2209272	MT1M						
54453	2.2073474	RIN2		Y(1=2)				
51251	2.2031093	NT5C3	Y	Y(1)		Y		Y
3627	2.1876068	CXCL10	Y	Y	Y	Y	Y	Y
6363	2.1816716	CCL19						
7941	2.1754074	PLA2G7	Y					
4599	2.1654127	MX1	Y	Y(1>2)	Y	Y	Y	Y
23179	2.153466	RGL1	Y					
10673	2.1527755	TNFSF13B	Y	Y(1>2)		Y	Y	Y
2537	2.1354163	IFI6	Y	Y(1>2)	Y	Y	Y	Y
3759	2.126041	KCNJ2	Y					
10563	2.1205661	CXCL13	Y					
26010	2.1059296	LOC26010	Y	Y				
81030	2.0621655	ZBP1						
6355	2.0459619	CCL8	Y	Y(1>2)		Y	Y	Y
6374	4.93119	CXCL5	Y	Y(1)		Y	Y	Y
4360	3.1806405	MRC1						
9966	2.7580967	TNFSF15						
4318	2.4843144	MMP9	Y	Y(1)		Y		Y
23601	2.3458712	CLEC5A						
4312	2.3377943	MMP1	Y					
3552	2.3325033	IL1A		Y(1)		Y	Y	Y
3578	2.2278018	IL9						
6367	2.1467485	CCL22						
1116	2.1120112	CHI3L1						
2921	2.0152957	CXCL3		Y(1)		Y	Y	Y

The fourty-seven IFNB-upregulated genes are shadowed. Abbreviations: Y; yes.

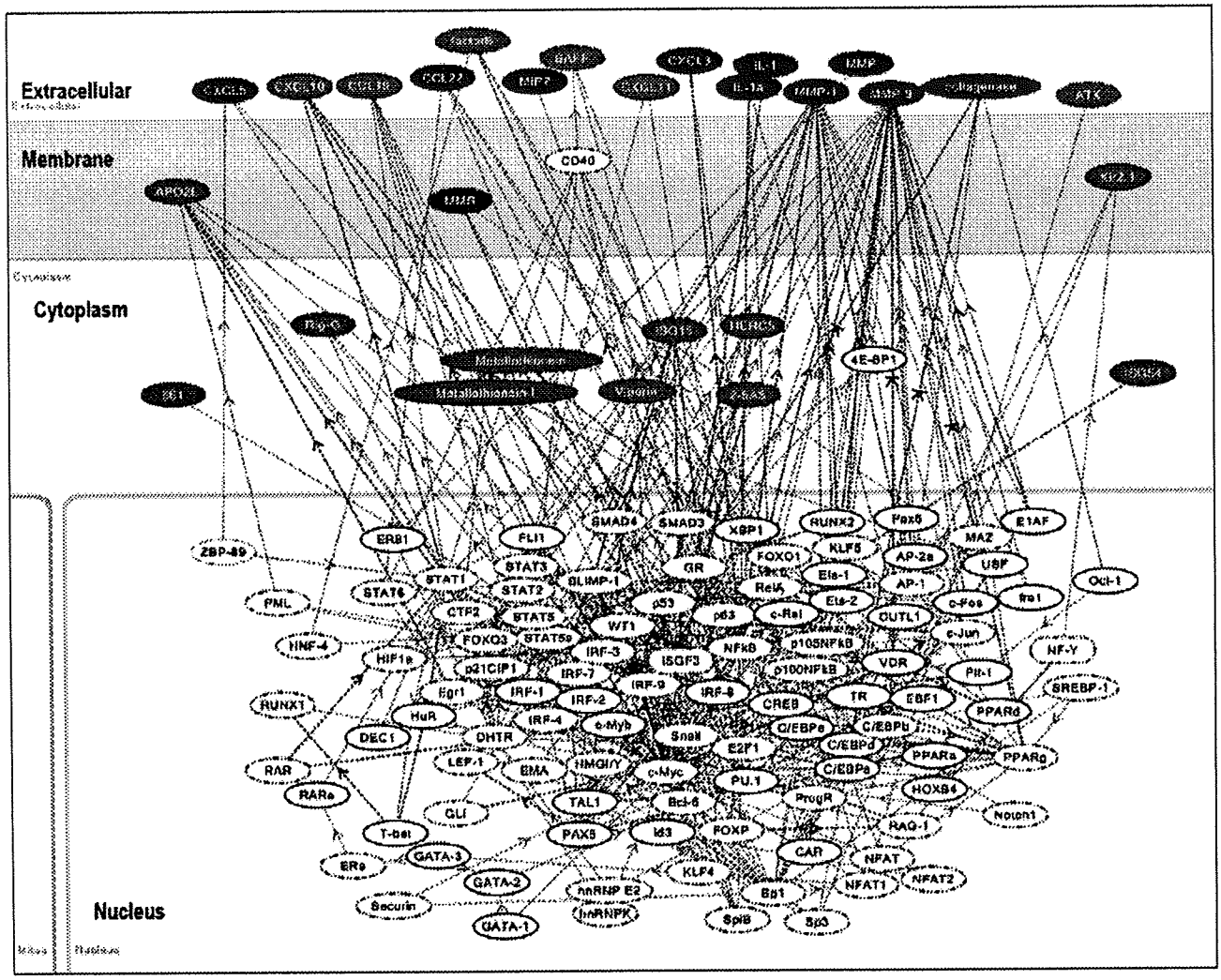

Fig. 2. The common upstream search of 58 differentially expressed genes (RMA) in activated T-cells incubated in the presence or absence of IFNB. The 58 DEGs in activated T-cells imported into KeyMolnet extracted 75 genes directly linked to 58 DEG. The "common upstream" search of 75 genes generated a molecular network composed of 140 nodes arranged according to the subcellular distribution. The network has the most relevant relationship with gene regulation by IRF (p = 2.082E-17) and NF-kappaB (p = 6.272E-16).

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・平成21年度

書籍

著者氏名	論文タイトル名	書籍全体の編集者名 書籍名	出版社名 出版地	出版年 ページ
荒浪利昌　、山村隆	多発性硬化症	松島綱治・西脇　徹 炎症・再生医学事典	朝倉書店 東京	2009 216-218

研究成果の刊行に関する一覧・平成21年度

雑誌

発表者氏名	論文タイトル名	発表雑誌誌名	巻号	項	出版年
Christian Klemann., Benjamin JE Raveney, Shinji Oki, Takashi Yamamura	Retinoid signals and Th17-mediated pathology	Jpn. J. Clin. Immunol.	32	20-28	2009
Christian Klemann, Benjamin JE Raveney, Anna K Klemann, Tomoko Ozawa, Stephan von Hrsten, Koichi Shudo, Shinji Oki, Takashi Yamamura	Synthetic retinoid AM80 inhibits Th17 cells and ameliorates EAE	Am. J. Pathol.	174(6)	2234-45	2009
Michael-Mark Theil, Sachiko Miyake, Miho Mizuno, Chiharu Tomi, J. Ludovic Croxford, Hiroshi Hosoda, Julia Theil, Stephan von Horsten, Hiroaki Yokote , Asako Chiba, Youwei Lin, Shinji Oki, Takashi Akamizu, Kenji Kangawa, Takashi Yamamura	Suppression of experimental autoimmune encephalomyelitis by ghrelin	J. Immunol.	183	2859-66	2009
Fujita, M., T. Otsuka, M. Mizuno, C. Tomi, T. Yamamura, and S. Miyake	Carcinoembryonic antigen-related cell adhesion molecule 1 modulates experimental autoimmune encephalomyelitis via an iNKT cell-dependent mechanism	American Journal of Pathology	175(3)	1116-1123	2009
Satoh J, Obayashi S, Misawa T, Sumiyoshi K, Oosumi K, Tabunoki H.	Protein microarray analysis identifies human cellular prion protein interactors.	Neuropathology and Applied Neurobiology	35 (1)	16-35	2009
Satoh J.I, Tabunoki H, Yamamura T.	Molecular network of the comprehensive multiple sclerosis brain-lesion proteome.	Multiple Sclerosis	15	531-541	2009
Satoh J, Tabunoki H, Arima K.	Molecular network analysis suggests aberrant CREB-mediated gene regulation in the Alzheimer disease hippocampus.	Disease Markers	27 (5)	239-252	2009

Obayashi S, Tabunoki H, Kim SU, Satoh J.	Gene expression profiling of human neural progenitor cells following the serum-induced astrocyte differentiation.	Cellular and Molecular Neurobiology	29 (3)	423-438	2009
Sumiyoshi K, Obasashi S, Tabunoki H, Arima K, Satoh J.	Protein microarray analysis identifies cyclic nucleotide phosphodiesterase as an interactor of Nogo-A.	Neuropathology		in press	2009
Satoh J, Obayashi S, Tabunoki H, Wakana T, Kim SU.	Stable expression of neurogenin 1 induces LGR5, a novel stem cell marker, in an immortalized human neural stem cell line HB1.F3.	Cellular and Molecular Neurobiology		in press	2009
Shiina Y, Arima K, Tabunoki H, Satoh J.	TDP-43 dimerizes in human cells in culture.	Cellular and Molecular Neurobiology		in press	2009
大木　伸司、山村　隆	多発性硬化症の病態解析から治療標的の同定へ	日本臨床免疫学会会誌	32	214-222	2009
大木　伸司、山村　隆	多発性硬化症の病態形成とオーファン核内受容体NR4A2	臨床免疫・アレルギー科	52	111-118	2009
大木　伸司	自己免疫疾患の診断と治療における核内受容体の可能性	日本国際医学協会誌	438	3-4	2009
佐藤準一	ゲノムワイド解析により同定された多発性硬化症のリスクアレル.	Medical Briefs in Brain & Nerve	17 (1)	10-11	2009
荒浪利昌 、山村隆	炎症とT細胞サブセット	治療学	印刷中		

Ⅳ. 研究成果の刊行物・別刷

1. 脳神経 — c. 多発性硬化症

a. 概念・定義

多発性硬化症（multiple sclerosis；MS）は中枢神経系（脳・脊髄・視神経）にリンパ球浸潤，抗体沈着，補体活性化などを伴う炎症病変が多発し，多彩な神経症状を呈する慢性疾患である．

b. 疫　学

欧米では若年成人を侵す神経疾患の中で最も多い（北欧や北米では人口10万人当たりの有病率は50～200人）．有病率は白人＞アジア人＞黒人の順である．わが国の有病率は従来3～5人/10万と報告されてきたが，近年増加傾向にあり，現在の患者数は12,000人以上と推定される．他の免疫・アレルギー疾患と同様，遺伝的要因と環境要因の両者の関与する疾患である．一般的には高緯度地域で有病率が高く，日照時間や衛生環境との関連が推測される．発症年齢は30歳前後で，3歳以前あるいは70歳以降に発症することはまれで，女性に多い傾向がある（男女比≒1：3）．

c. 病　因

原因は明らかでないが，自己抗原に対する免疫反応を本態とする自己免疫疾患であるという理解が一般的である．活動性病巣にはT細胞，マクロファージ，B細胞などの浸潤と脱髄（髄鞘崩壊）を認める．標的となる自己抗原として，ミエリン塩基性タンパク，プロテオリピッドタンパクなどが推定されている．自己免疫反応は患者ごとに均一ではなく，病期によっても異なる．

近年では大規模な遺伝子解析により，HLA-DRやIL-2受容体遺伝子などの多型とMS感受性の相関が報告されている．しかし，一卵性双生児のMS発症一致率は30％程度であり，遺伝的素因に加えて，環境因子や感染因子も重要な役割を果たすことは明白である．ウイルス感染の意義については否定的な意見もあるが，最近EBウイルスの関与が注目されている．これらの要因が複雑に絡み合ってMSが発症すると考えられる（図1）．

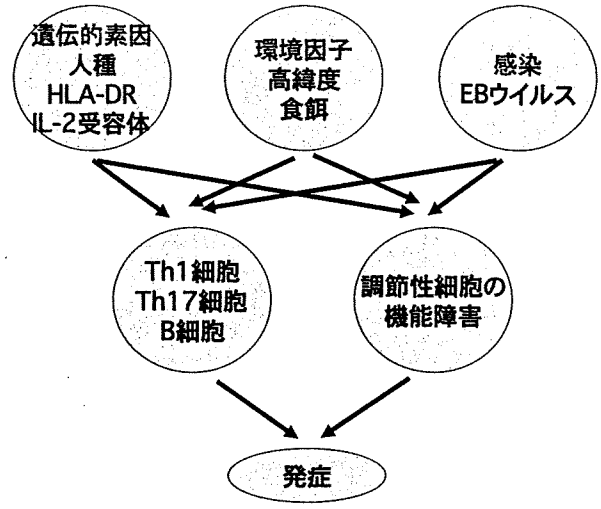

図1　MSの発症に至る道筋
遺伝的素因，環境因子，感染因子が重なることにより，病原性細胞（Th1細胞，Th17細胞，B細胞など）の活性化と調節性細胞の機能障害が誘導され，最終的に自己破壊的な免疫応答の活性化が起こる．

MSの動物モデルである実験的自己免疫性脳脊髄炎（experimental autoimmune encephalomyelitis；EAE）では，インターフェロンγ（interferon-γ；IFN-γ）を産生するTh1細胞およびインターロイキン17（interleukin-17；IL-17）を産生するTh17細胞が，脳実質内で炎症性サイトカインやケモカインを産生して炎症を誘導する（図2）．ヒトMSの病態ではTh1細胞の重要性が強調されているが，これはIFN-γやミエリン塩基性タンパクアナロ

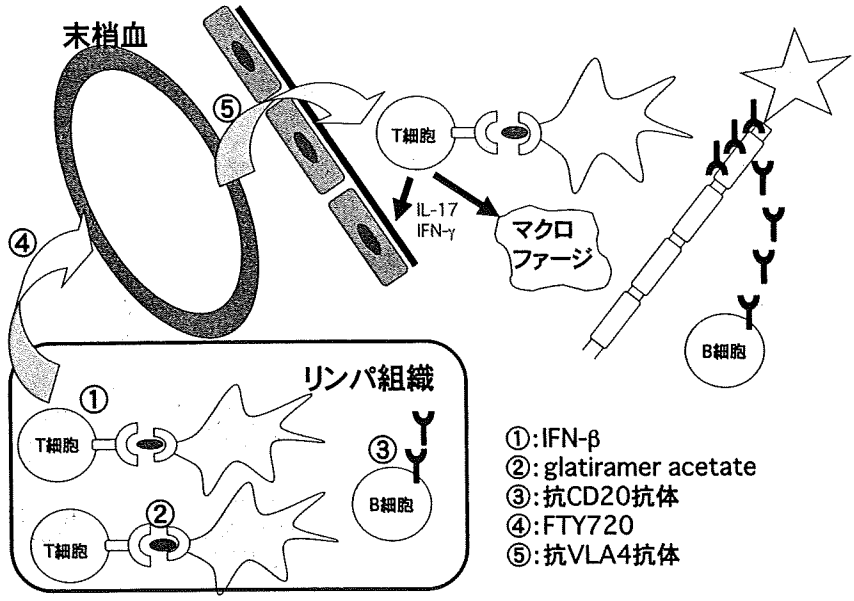

図2 MS治療薬の主な作用点

末梢リンパ組織において抗原刺激で活性化したT細胞は，末梢血を経由して血液脳関門を通過する．さらに中枢神経内で再活性化され，IFN-γ，IL-17などのサイトカインや炎症性ケモカインを産生して，炎症反応を惹起する．

① IFN-β；自己抗原提示の抑制，② glatiramer acetate；制御性T細胞の誘導，③抗CD20抗体；B細胞の殺傷，④FTY720；末梢リンパ組織からのT細胞移動阻害，⑤抗VLA4抗体；T細胞血管内皮接着抑制．

グ・ペプチドの試験的投与に引き続いて，Th1細胞活性化に伴うMSの増悪が認められたという事実に基づいた推論である．しかし，Th17細胞やCD8陽性T細胞の重要性を示唆する報告もあり，自己抗体やB細胞も病態の形成に影響を及ぼす．また，病原性T細胞を制御する調節性細胞（CD25陽性T細胞，NKT細胞など）の異常も報告されている．

d．MSの病型

1） 臨床経過による分類

およそ8割の患者が急性増悪と寛解を繰り返す．この病型を再発・寛解型MSという．再発・寛解型MSでも罹病期間が長くなると，途中から明瞭な寛解を示すことなく，徐々に病状が進行する例が出てくる．これが二次進行型MSである．また，発症時から持続的に進行するMSを一次進行型MSという．

2） 病変分布による分類

大脳，小脳，脳幹，視神経，脊髄など中枢神経内に病巣が広く分布するMSを，古典型MS（西洋型，通常型）と呼ぶ．欧米ではMSといえばこの病型を指す．これに対して，視神経脊髄型MSは，視神経炎と脊髄炎を繰り返して進行する病型で，日本に比較的多い．最近，この病型において，水チャンネルであるアクアポリン4に対する自己抗体が検出されることが明らかになった．病理像や治療に対する反応性が古典型MSとはかなり異なることから，MSとは区別してneuromyelitis optica（NMO）と呼ぶことが多い．

e． 臨床症状，検査所見

病変部位に応じて，視力障害，複視，小脳失調，運動麻痺，感覚障害，膀胱直腸障害等などが出現する．精神症状，高次脳機能低下などが問題になることもある．髄液検査では，総タンパクおよびIgG比が上昇し，オリゴクローナルバンドが陽性になる（陽性率は欧米で90％以上．わが国では50～60％）．病変はMRIの

T2 強調像および FLAIR 像で高頻度に検出できる．また，急性期の病巣はガドリニウムで増強される．2001 年に発表された McDonald の国際診断基準は，MRI 検査を重視している．広く用いられているが，わが国においては，この診断基準に当てはまらない症例も多い．

f. 治　療

急性期の治療としては，ステロイド大量点滴静注療法（パルス療法）が一般的である．慢性期には，再発を防止するためにインターフェロン β（IFN-β）製剤が処方されるが，IFN-β の有効な患者は全体の 30 % といわれる．IFN-β の副作用の強い例や無効例では，免疫グロブリン大量点滴静注療法（IVIg），定期的ステロイドパルス療法，免疫吸着・血漿交換療法，ミトキサントロンなどが試みられる．日本では未だ使用不可能であるが，ミエリン塩基性タンパクに多く含まれる 4 種類のアミノ酸のランダムなポリマーである glatiramer acetate（copolymer-1）と，リンパ球の血管内皮への接着を阻害する抗 VLA4（α4 インテグリン）抗体が，欧米では広く使われている．また B 細胞を除去する抗 CD20 抗体の有効性が報告されている．

g. 将来的な治療

現在，さまざまな治療法が臨床試験の段階である．FTY720 は sphingosine-1-phosphate(S1P) 受容体のアゴニストで，T 細胞が二次リンパ組織から出るのを阻害する．その他，抗 CD52 抗体などが検討されている．

［荒浪利昌，山村　隆］

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Retinoid signals and Th17-mediated pathology

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summary

For many years, CD4+ effector T cells were categorized into two subsets: T helper type 1 (Th1) and type 2 (Th2) cells. More recent research has refined this model, delineating further subsets; in particular, Th17 cells, activated CD4+ T cells characterised by the production of the cytokine IL-17. Autoantigen-specific Th17 cells are associated with pathology in a number of animal models of organ-specific autoimmune disease and evidence is mounting that Th17 cells are also critical in human autoimmunity.

Retinoids, a family of compounds that bind to and activate retinoic acid receptors (RARs and RXRs), are able to alter CD4+ T cell differentiation *in vitro* though agonism and antagonism of a range of retinoid receptors. For example, *all-trans* retinoic acid (ATRA) inhibits Th17 differentiation and instead promotes the upregulation of Foxp3, a key transcription factor in regulatory T cells. Importantly, treatment with retinoids can modulate Th17-mediated autoimmunity: experimental autoimmune encephalomyelitis (EAE), the murine model of multiple sclerosis (MS), is ameliorated by ATRA administration due to suppression of both the differentiation and the function of Th17 cells. In this review, we discuss the unveiled molecular mechanism and the possible clinical application of retinoids for the treatment of human Th17-mediated autoimmune diseases.

Key words——Retinoids; AM80; ATRA; EAE; Th17; IL-17; RORγt; Treg; Foxp3; IL-10

Introduction

During an immune response, CD4+ T cells can become activated in an antigen-specific manner and direct the nature of the response by activating and counter-regulating other leukocyte populations. Upon activation, naive CD4+ T cells can differentiate into a range of cell types, which elaborate a tailored response depending on the nature of the immune insult. These differentiated cell types can be effector CD4+ T cells, including Th1, Th2, and Th17 types, or CD4+ regulatory T cells (Treg) that deviate the function of other immune cells, including Tr3, Th10, iTreg types. Many factors in the microenvironment during CD4+ T cell activation tune these differentiation processes, including signals from the antigen-presenting cell and the cytokine milieu. In addition, retinoids can act to drive the generation of Treg and inhibit the differentiation of pro-inflammatory Th17 cells. In this review, we will discuss the potential of retinoids to influence Treg and Th17 responses, in order to treat autoimmune diseases.

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Discovery of Th17 cells

The dichotomous classification of effector CD4+ T cells based on their function, into Th1 and Th2 cells was first reported in 1986[1]. It was demonstrated that naïve Th cells differentiate to two functional classes of cell during an immune response, Th1 cells, which produce interferon (IFN)-γ and are involved in cell-mediated immunity and organ-specific autoimmunity, and Th2 cells, which secrete interleukin (IL)-4 and are involved in extracellular immunity and pathogenesis of asthma and allergy. Furthermore, a key finding was that these Th subsets were able to negatively regulate each other, explaining how a single Th-type response is established following a particular immune insult. This pioneering work fuelled the understanding of the immune system for many years and remained largely unchallenged.

Multiple sclerosis (MS), the human autoimmune inflammatory disease of the central nervous system (CNS), is characterised by perivascular infiltrates in the brain that display hallmarks of delayed-type hypersensitivity (DTH). This DTH response was ascribed to Th1 cells[2] and, following this research, MS and other autoimmune diseases were thus thought to be mediated by Th1 cells[3]. Experimental autoim-

mune encephalomyelitis (EAE), a well-established model of cell-mediated autoimmunity[4,5], is also thought to be generated by the action of Th1 cells, which was supported by the fact that autoreactive Th1 cells are able to transfer the disease. Furthermore, IL-12p40-deficient mice, which are unable to mount Th1 responses, were resistant to induction of EAE, supporting the hypothesis that the disease is a Th1-mediated disorder. Thus, it was predicted that the administratration of IFN-γ, the key effector cytokine produced by Th1 cells, should generate more severe EAE and conversely the inhibition of the effect of IFN-γ should reduce EAE. In fact, the opposite is the case: IFN-γ administration ameliorates disease, whilst neutralization of IFN-γ with blocking antibodies leads to worsen clinical outcome[6,7]. Furthermore, mice deficient in either IFN-γ, or IFN-γ receptor, which lack Th1 responses are also susceptible to more severe EAE[8]. Similar observations have also been made in other models of autoimmunity such as adjuvant-induced arthritis[9].

Despite such contradicting data, the Th1/Th2 paradigm was upheld for more than two decades until new data regarding the role of IL-23 allowed the formulation of a improved hypothesis of Th differentiation. IL-12 and IL-23 are both heterodimeric cytokines consisting of a shared p40 subunit, but unlike in IL-12 where this molecule combines with a p35 subunit, IL-23 possesses a unique p19 subunit[10]. The related structure of these cytokines was able to solve a long-standing puzzle: why mice deficient for p40, part of both IL-12 and IL-23, were protected from EAE induction, whilst IL-12p35 deficient mice develop worse disease. Thus, when IL-23 p19 deficient mice were generated, and were found to be protected from EAE induction, it was concluded that the protective effect in p40 mice was unrelated to IL-12[11]. Furthermore, anti-IL-23 treatment also leads to protection from EAE[12]. Despite this similar structural make up of these two cytokines, their biological activities differ greatly: IL-12 controls the differentiation of Th1 cells, whilst IL-23 does not. Instead, IL-23 was found to be associated with effector T cells that produced large amounts of IL-17A, IL-17F, IL-21, and IL-22[10,13]. The pivotal roles of IL-23-associate T cells were unveiled by a series of experiments that showed that the adoptive transfer of these T cells caused severe EAE[14,15], neutralization of IL-17 via mAb treatment ameliorated EAE, and IL-17-deficient mice developed less severe EAE with a delayed onset[16,17]. Other autoimmune diseases have also been shown to be Th17-mediated, such as rheumatoid arthritis[18], and in animal models of this disease severity is reduced in IL-17-deficient mice and by blockade of IL-17 signalling[19,20].

Earlier work demonstrated that addition of IL-23 to CD4$^+$ T cell cultures led to IL-17 production by T cells that were initially referred to as Th-IL-17 cells[21], but later this became abbreviated to Th17 cells[22]. At first, IL-23 was assumed to be a factor important for the *de novo* generation of Th17 cells. It soon became clear that this was not the case, since naive T cells do not express the IL-23 receptor[13,23]. Instead, a combination of the immune suppressive cytokine TGF-β and pro-inflammatory IL-6 has been identified as the differentiating factors of Th17 cells in mice[13,23,24]. Additionally, there may be a requirement for IL-1β in the differentiation of human Th17 cells. Interestingly, the differentiation of naïve CD4$^+$ T cells activated in the presence of TGF-β alone induces the generation of induced Tregs, which express the transcription factor Foxp3[25]. At this point of time, IL-23 is believed to play a crucial role in the expansion and maintenance of Th17 cells.

Th17 cells are characterized by the expression of the transcription factor retinoid acid-related orphan nuclear receptor-γt (RORγt)[26], RORα[27], and signal transducer and activator of transcription-3 (STAT3)[28,29]. When EAE is induced in RORγt-deficient mice, disease has a delayed onset and is of a milder form than in wildtype mice[26].

In humans, there is a growing body of evidence that implicates Th17 cells in autoimmune processes. There are increased levels of transcripts for IL-17 and IL-6 in the CNS lesions of patients with MS[30], and in such lesions, as well as in cerebrospinal fluid from MS patients, IL-17-secreting lymphocytes have been detected[31,32]. Th17 responses have also been associated with the human autoimmune disorders such as psoriasis[33,34], rheumatoid arthritis[35], and Crohn's disease and ulcerative colitis[36].

Vitamin A and its metabolites

Vitamin A (retinyl ester) plays essential roles in a number of physiological functions throughout the body, including vision, embryonic development, bone and blood metabolism, gene transcription, and immune functions[37~39]. The recommended daily allowances of vitamin A range from 300 μg/day in children to 1200 μg/day for lactating women[40]. It is important to note that vitamin A uptake deficiency compromises normal immune responses. Retinol, the

most common metabolite of dietary vitamin A, can be either absorbed by the gut following ingestion, or generated from provitamins such as betacarotene[37~39]. Retinol is then processed into retinal and retinoic acid (RA)[41]. All-trans-retinoic acid (ATRA), 9-cis RA, and 11-cis retinal are the most active metabolites found in the body[38,42]. 11-cis retinal is required for the synthesis of rhodopsin and thus is essential for vision. ATRA and 9-cis RA bind to retinoic acid receptors (RARs) and retinoid X receptors (RXRs) and via these interactions control transcription of a variety of genes, both activation and repression[37~39,43]. ATRA preferably binds to the RARs, whilst 9-cis RA can bind to both receptor classes[37,38]. As RARs and RXRs both have three isotypes, α, β, and γ, which can combine to a form many different heterodimers and homodimers, the range of retionid affinity for this range of receptors can lead to a great number of possible effects.

Natural and synthetic retinoids in the clinic[44]

The term retinoids is applied to a family of compounds that bind to and activate retinoic acid receptors (RARs and RXRs), resulting in a range of possible biological responses. Some natural retinoids, such as ATRA (Tretinoin), 9-cis RA (Alitretinoin), and 13-cis RA (Isotretinoin), are currently already used in the clinic. As the clinical use of natural retinoids is limited by their pharmacological profile, including instability, poor bioavailability, and possible side effect due to the nonspecific receptor binding of those natural retinoids, a number synthetic retinoids have been generated. These include mono-aromatic synthetic retinoids (second generation), such as Acitretin and Etretinate, and poly-aromatic synthetic retinoids (third generation), such as Tamibarotene (AM80), Tazarotene, and Targretin (LGD1069) (See Table 1). The aromatic rings found in the second and third generation retinoids confer a higher stability and resistance to heat/oxidation, increased half-lives, a higher potency, and improved spectrum of action with receptor specificities.

The most common clinical use of retinoids to date is in the treatment of acute promyelocytic leukemia (APL) and Kaposi's sarcoma[45,46]. APL results from an abnormal fusion protein PML-RARα, formed due to a t(15; 17) (q22; q12) chromosomal translocation, inducing abnormal promyelocytic cell proliferation. The efficacy of retinoid treatment of APL has been reported to be due to the promotion of granulocytic differentiation and maturation. Importantly, the synthetic retinoids exhibit greater potency in APL treatment compared with their natural occurring counterparts: for example the third generation retinoid Tamibarotene (AM80) is effective in ATRA-unresponsive APL patients[47]. Further studies have also suggested that autoimmune disease are effectively targeted with these new generation of retinoids, such of the skin disease psoriasis[48].

Retinoids have been studied for over 20 years as potential therapeutic agents in a variety of autoimmune models, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, type I diabetes, and lupus[49~53]. However, many clinical trials examining the potential for retionids in treating such diseases have indicated a efficacy and intolerable side effects[54]. Previously, as ATRA was shown to effect T cell differentiation, both suppressing Th1 development and enhancing Th2 development[55], the amelioration of autoimmune disease by retinoid treatment was attributed to a deviation of immune from Th1 to Th2. More recently, with an enhanced understanding of Th differentiation outcomes, the effect of retinoid treatment on Th17 and Treg cell development and function has reawakened interested in use of retinoids to treat immune disorders. Furthermore, this research

Table 1. Retinoids in clinical use

Name	Receptors	Clinical use
ATRA (Tretinoin)	pan-RAR	APL, Acne
9-cis RA (Alitretinoin)	pan-RAR, pan-RXR	Kaposi sarcoma
13-cis RA (Isotretinoin)	—	Acne
Etretinate	—	Psoriasis
Acitretin	pan-RAR	Psoriasis
Tamibarotene (AM80)	RAR$\alpha/\beta \gg \gamma$	APL
Tazarotene	RAR$\beta/\gamma \gg \alpha$	Acne
Targretin (LGD1069)	pan-RXR	Cutaneous T lymphoma

has been facilitated with the availability of a new range of enhanced, synthetic, receptor-specific retinoid compounds.

Retinoids and Th17 cells

It is now well established that Th17 cells constitute a distinct subset of inflammatory T cells, which are characterized by the production of IL-17 and the expression of the transcription factors RORγt and RORα[56]. Th17 differentiation is enhanced in CD4$^+$ T cells that are induced to overexpress RORγt or RORα and overexpression of both these genes has an even greater effect[27]. Interference with either of these genes reduces the propensity of CD4$^+$ T cells to differentiate into Th17 cells and if both RORγt and RORα are knocked out, Th17 differentiation is prevented[27]. Retinoids strongly suppress the *in vitro* production of IL-17 by polyclonal TCR stimulation of naïve CD4$^+$ T cells in the presence of IL-6 and TGF-β[57~59]. This inhibition of Th17 cell function is accompanied by downregulation of RORγt and upregulation of Foxp3[57~59]. The suppressive effect of retinoids on Th17 cell differentiation has been shown to be mediated via RARα[57,59]. Therefore, it is highly conceivable that retinoids, particularly those with high affinity for RARα, may be of use in the clinic to treat Th17-mediated pathology. However, the ability of retinoids to inhibit Th17 differenition may be of limited use in alleviating in human autoimmune disease, as when patients present, T cell activation and differentiation is likely to be at an advance stage. It is therefore essential to consider the effect of retinoid on terminally differentiated T cells.

We have observed that the restimulation of *in vitro* differentiated Th17 cells and activated CD4$^+$ splenocytes from mice with active Th17-mediated autoimmune disease results in the production of high-levels of IL-17 secretion, which is reduced by the addition of the synthetic retinoid AM80. Additionally, AM80 treatment of these cells also downregulates RORγt expression. We demonstrated inhibition of Th17 cell function at AM80 doses as low as 0.1-10 nM, however maximal suppression was achieved with concentrations in the order of 10-100 nM (unpublished observation). It is important to note that higher doses of retinoid treatment have been shown to have a wide-ranging anti-proliferative and immunosuppressive effect on T cells (unpublished data and[60]). Therefore, for translation to the clinic at the applicable human dose of retinoid must be determined to ensure efficacy of retinoid treatment without undesirable pan-immunosuppression. Xiao *et al.* demonstrated the potential application of ATRA to the treatment of EAE, however, in this report ATRA administration also generated significant anti-proliferative effects[61]. We have determined that the therapeutic effect of the synthetic retinoid AM80 on EAE occurs at much lower doses than ATRA. This is achievable as the pharmacological profile of AM80 allows its administration via the oral route, which may also be desirable in treatment of human disease. Critically, we were able to demonstrate that although these doses strongly downregulated Th17 mediated pathology, no general immunosuppression was observed.

An interesting observation is that retinoid treatment may actually already be in current use for Th17-mediated diseases. For some time, retinoids have been utilised as a standard treatment for psoriasis[62]. More recently, psoriasis has been linked with Th17 responses[33,34], thus it is likely that retinoids can be applied to treat a wider range of immune pathologies, in particular those associated with Th17 dysfunction.

Retinoids and Foxp3$^+$ regulatory T cells

The majority of Treg cells, CD4$^+$ T cells that are able to counter-suppress populations of T cells, express the transcription factor Foxp3. The function of these cells is critical in the maintenance of self tolerance and defects in Foxp3 lead to widespread autoimmunity. This is observed in both mice (Scurfy mice) and men, (e.g. X-linked (IPEX) syndrome)[63~65]. Foxp3$^+$ CD4$^+$ Treg can be generated in the thymus (natural Treg) or be generated in the periphery following activation (induced Treg). *In vitro*, naïve T cells can also be induce to differentiate into Foxp3$^+$ Treg by activation in a particular cytokine environment. One such stimulus for Treg differentiation is TGF-β, a cytokine that is also required for Th17 differentiation. Interestingly, it has been reported that retinoids, including ATRA and AM80, are also able to alter T cell differentiation, inducing Foxp3$^+$ CD4$^+$ T cells ([57-59,66-69] and unpublished data). Such differentiation occurs in the presence of TGF-β1 despite the addition of IL-6, conditions that would normally promote Th17 differentiation. It has been suggested that retinoids mediate this effect by disrupting the signalling of IL-6 and IL-23 through receptor downregulation and by Smad3-dependent amplification of the TGF-β signalling[61].

Intriguingly, a subset of CD103$^+$ dendritic cells found in the lamina propria, and mesenteric lymph

nodes have the ability to supply ATRA to T cells during antigen-priming[69,70], conversely splenic dendritic cells do not produce significant amounts of ATRA[67~69]. The upregualtion of Foxp3 induced by ATRA is dependent on both TGF-β and retinoid signalling. Neutralizing antibody against TGF-β blocks the induction of Foxp3$^+$ CD4$^+$ T cells[69] and inhibition of the enzyme required for ATRA synthesis or blocking retinoid receptor signalling also prevents Foxp3 induction[58,68,69].

Despite the upregulation of Foxp3 generated by retinoids *in vitro*, treatment of EAE with retinoids suppresses Th17 cell function without promoting Foxp3 expression[58,61]. It has been suggested that a lack of TGF-β may be the limiting factor[58] or that the adjuvant-induced pro-inflammatory cytokine milieu, including IL-1, TNF-α and IL-6, may actively suppress Treg conversion[61]. Nevertheless, retinoid treatment is effective in suppressing the *de novo* differentiation of Th17 cells *in vivo* as well as *in vitro*. Therefore, we suggest re-evaluation of previous findings showing the beneficial effects of retinoid treatment in autoimmune diseases.

Conclusion

We have summarized the recent findings that Th17 cells are a major component in the pathology of many autoimmune diseases and that retinoids, especially synthetic one such as AM80 with a higher stability, increased half-life, a higher potency, and improved spectrum of actions with receptor specificities, are potent candidates for disease intervention. Recent study has pointed out the existence of Foxp3/RORγt double positive T cell subset, which produces IL-10 instead of IL-17[71]. In addition, treatment of activated T cells with TGF-β and IL-6 in the absence of terminal differentiation by IL-23 rendered them to produce IL-10, which are protective for EAE when transferred adoptively[72]. IL-10 has been shown to be another important regulatory component for autoimmune responses. Interestingly, our recent work suggested that long-term treatment with retinoids such as AM80 might cause downregulation of IL-10 production. Therefore, retinoids have multifunctional target on immune systems (downregulation of IL-6/IL-23 signals, Th17 function, and IL-10 production and upregulation of Foxp3, Treg function and TGF-β signals) and thus, clinical application of retinoids should be determined by the evaluation of each component to achieve the maximum effect of retinoids.

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