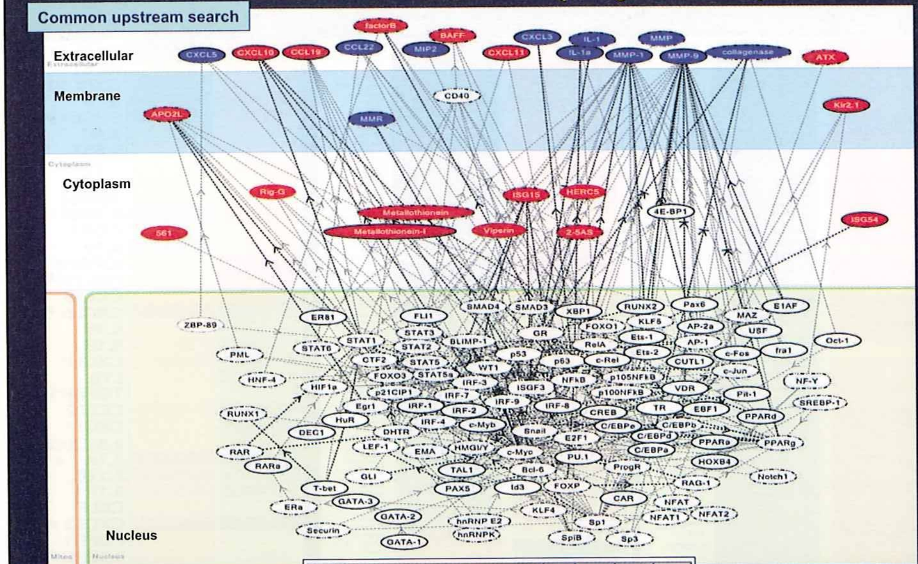
Molecular Network (KeyMolnet)

Common upstream search



rank	name	score	score(p)	score(v)	score(c)
1	IRFによる発現調節 (Transcriptional regulation by IRF)	55.415	2.082E-017	0.033	0.118
2	NFkBによる発現調節 (Transcriptional regulation by NFkB)	50.502	6.272E-016	0.031	0.111
3	BLIMP-1による発現調節 (Transcriptional regulation by BLIMP-1)	30.673	5.841E-010	0.014	0.222

IFNB-Responsive Downregulated Genes

Entrez GeneID	Fold Change	Gene Symbol	Overlap with MAS5	Gene Name	INTERFEROME IRG(Type)	ISRE	STAT	IRF	NFkB
6374	4.93119	CXCL5	Y	chemokine (C-X-C motif) ligand 5	Y(1)		Y	Y	Y
4360	3.1806405	MRC1		mannose receptor, C type 1					
9966	2.7580967	TNFSF15		tumor necrosis factor (ligand) superfamily, member 15					
4318	2.4843144	MMP9	Y	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	Y(1)		Y		Y
23601	2.3458712	CLEC5A		C-type lectin domain family 5, member A					
4312	2.3377943	MMP1	Y	matrix metallopeptidase 1 (interstitial collagenase)					
3552	2.3325033	IL1A		interleukin 1, alpha	Y(1)		Y	Y	Y
3578	2.2278018	IL9		interleukin 9					
6367	2.1467485	CCL22		chemokine (C-C motif) ligand 22					
1116	2.1120112	CHI3L1		chitinase 3-like 1 (cartilage glycoprotein-39)					
2921	2.0152957	CXCL3		chemokine (C-X-C motif) ligand 3	Y(1)		Y	Y	Y

11 Down-IRGs contain ...

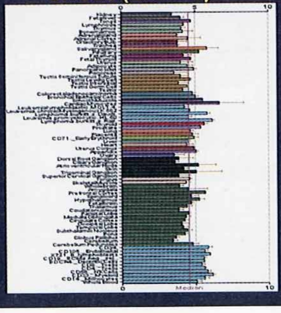
#1. No ISRE

#2. Two MMPs

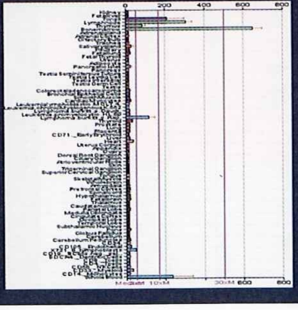
#3. Three Chemokines

#4. Three Cytokines

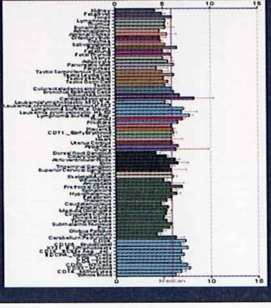
CXCL5 (ENA-78)

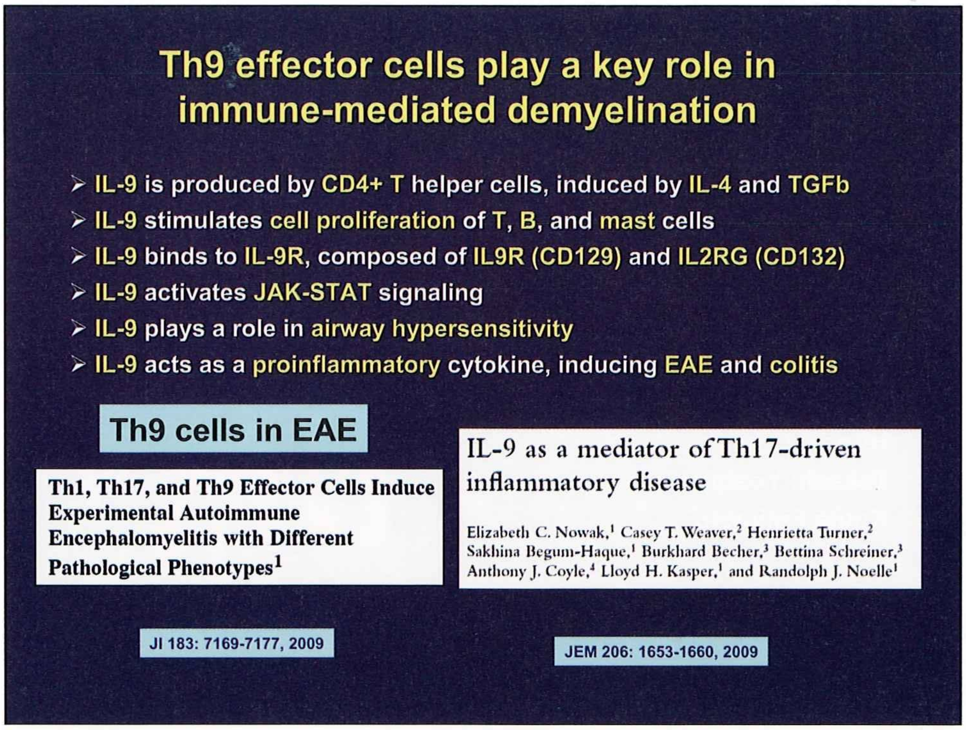


MMP9



IL9





Th9 effector cells play a key role in immune-mediated demyelination

- ➢ IL-9 is produced by CD4+ T helper cells, induced by IL-4 and TGFb
- ➢ IL-9 stimulates cell proliferation of T, B, and mast cells
- ➢ IL-9 binds to IL-9R, composed of IL9R (CD129) and IL2RG (CD132)
- ➢ IL-9 activates JAK-STAT signaling
- ➢ IL-9 plays a role in airway hypersensitivity
- ➢ IL-9 acts as a proinflammatory cytokine, inducing EAE and colitis

Th9 cells in EAE

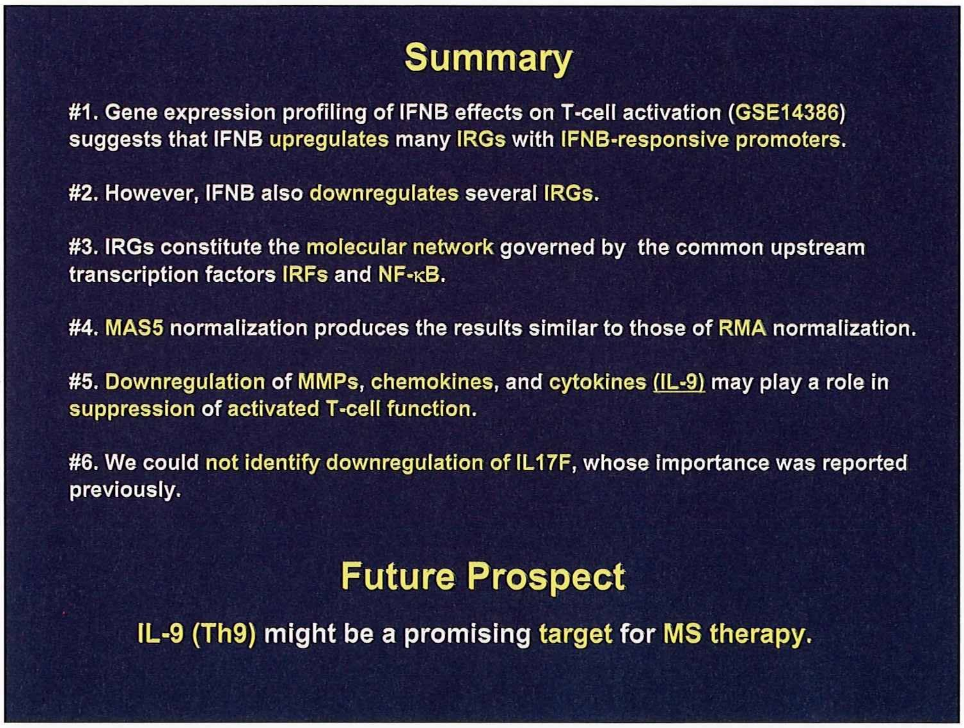
Th1, Th17, and Th9 Effector Cells Induce Experimental Autoimmune Encephalomyelitis with Different Pathological Phenotypes[1]

JI 183: 7169-7177, 2009

IL-9 as a mediator of Th17-driven inflammatory disease

Elizabeth C. Nowak,[1] Casey T. Weaver,[2] Henrietta Turner,[2]
Sakhina Begum-Haque,[1] Burkhard Becher,[3] Bettina Schreiner,[3]
Anthony J. Coyle,[4] Lloyd H. Kasper,[1] and Randolph J. Noelle[1]

JEM 206: 1653-1660, 2009



Summary

#1. Gene expression profiling of IFNB effects on T-cell activation (GSE14386) suggests that IFNB upregulates many IRGs with IFNB-responsive promoters.

#2. However, IFNB also downregulates several IRGs.

#3. IRGs constitute the molecular network governed by the common upstream transcription factors IRFs and NF-κB.

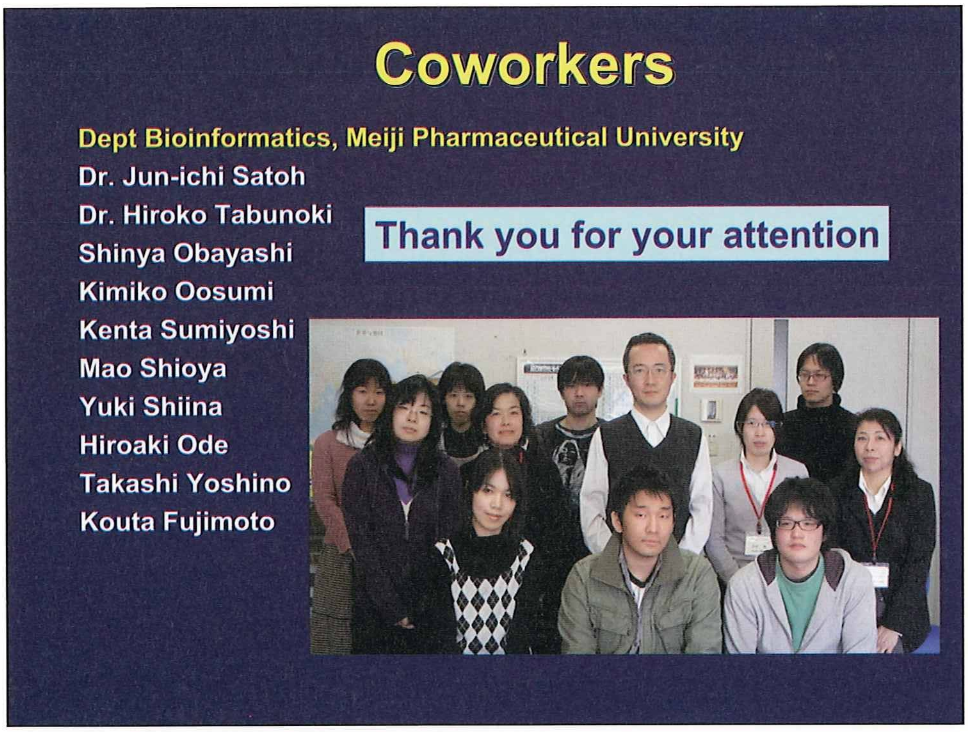
#4. MAS5 normalization produces the results similar to those of RMA normalization.

#5. Downregulation of MMPs, chemokines, and cytokines (IL-9) may play a role in suppression of activated T-cell function.

#6. We could not identify downregulation of IL17F, whose importance was reported previously.

Future Prospect

IL-9 (Th9) might be a promising target for MS therapy.



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Thank you for your attention

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
1	Sskuishi,K.,S.Miyake and Yamamura .T :	Role of NK cells and invariant NKT cells in multiple sclerosis	Results probl Cell Differ	Springer-Verlag Berlin Heiderberg	2009 全21ページ （Epub ahead of print)
2					
3					
4					
5					
6					
7					

研究成果の刊行に関する一覧表（平成21年度）

雑誌

	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
1	Ishizu T,Kira JI,Osoegawa M,Fukazawa T,Kikuchi S, Fujihara K,Matsui M,Kohriyama T,Sobue G,Yamamura T,Itoyama Y, Saida T,Sakata K	Heterogeneity and continuum of multiple sclerosis phenotypes in Japanese according to the results of the fourth nationwide survey	J.Neurol .Sci	8	22-28	2009
2	Satoh J.I, Tabunoki H, Yamamura T.	Molecular network of the comprehensive multiple sclerosis brain-lesion proteome.	Multiple Sclerosis	15	531-541	2009
3	Christian Klemann, Benjamin J.E.Raveney, Anna K. Klemann, Tomoko Ozawa, Stephan von Horsten, Koichi Shudo, Shinji Oki, Takashi Yamamura	Synthetic retinoid AM80 inhibits Th17 cells and ameliorates EAE	Am. J. Pathol.	174(6)	2234-45	2009
4	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
5	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
6	Croxford,J.L.,and T.Yamamura:	Back to the future for multiple sclerosis therapy:focus on current and emerging disease-modifying therapeutic strategies.	Immunotherapy	1 (3)	403-423	2009
7	Miyake.S.,and T.Yamamura	Ghrelin : Friend or Foe for Neuroinflammation	Discov Med	8 (41)	64-67	2009
8						
9						

Ⅳ．研究成果の刊行物・別刷

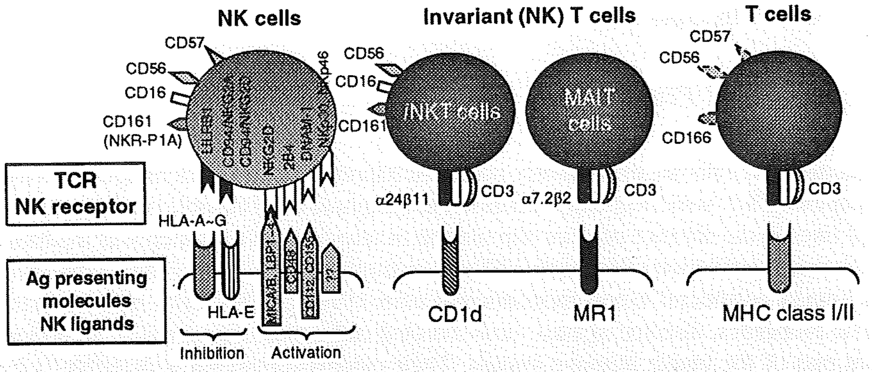
2 NK Cells and MS

2.1 *General Properties of NK Cells*

Natural killer cells are evolutionary primitive lymphocytes that lack antigen-specific receptors. They were originally identified as lymphoid cells capable of lysing tumor cell lines in the absence of prior stimulation *in vivo* or *in vitro*, which was the basis of their denomination (Trinchieri 1989). Constituting about 10% of the lymphocyte in human peripheral blood mononuclear cells (PBMC), NK cells possess cytotoxic properties, directed against virus-infected cells, thus considered as an important part of the innate immune system. Their cytotoxic reaction is determined by collective signaling of an array of inhibitory and stimulatory receptors expressed on their surface (Kirwan and Burshtyn 2007) (Fig. 1). Inhibitory receptors, commonly referred to as killer inhibitory Ig-like receptors (KIRs), interact with shared allelic determinants of classical and non classical MHC class I. Hence, NK cells are kept in an inactivated state through contact with self MHC class I molecule expressed on healthy cells. For example, CD94/NKG2A heterodimer expressed on NK cell surface recognize HLA-class Ib molecule, HLA-E (Borrego et al. 2006; Lopez-Botet et al. 1997). On the contrary, stimulatory receptors on NK cell surface bind to NK stimulatory receptor ligand up-regulated on other cells upon undergoing cellular stress. The main activating receptors constitutively found on all NK cells in peripheral blood are NKG2D, 2B4, and the two of the three natural cytotoxicity receptors (NCRs), NKp30, and NKp46. One example of NK stimulatory receptor ligand is the protein encoded by retinoic acid early inducible gene (RAE-I), which was isolated from tumor lines. RAE-1 is also expressed on virus-infected cells (Backstrom et al. 2007), and binds to the stimulatory receptor expressed on NK cells, NKG2D (Diefenbach et al. 2000; Smyth et al. 2005). As an overall effect, NK cells would lyse target cells that have lost or express low amounts of MHC class I molecules, including tumor cells or cells infected by viruses such as certain Herpes viruses or Adenoviruses.

Once activated, NK cells display cytotoxic functions which is mediated by direct cell-to-cell contact as well as secretion of cytokines and chemokines. The cell contact pathways include perforin/granzyme (Warren and Smyth 1999), Fas/Fas-ligand (Screpanti et al. 2005), and TRAIL/TRAIL ligand interaction (Takeda et al. 2001). They also produce inflammatory cytokines such as IFN-γ, TGF-β, and GM-CSF. Despite these cytotoxic actions against tumor cells and virus infected cells, it is now well conceived that some NK cells could act as modulator of adaptive immunity and have the potential to eliminate self-reactive T cells.

Although the diversity of NK cells remained to be ambiguous some time ago, recent works have greatly contributed to clarifying their heterogeneity in phenotypes and functions. The majority of human NK cells in PBMC belong to $CD56^{dim}CD16^{+}$ cytolytic NK subset. These cells express homing markers for inflamed peripheral sites and carry perforin to rapidly mediate cytotoxicity. $CD56^{bright}$ $CD16^{-}$ cells constitute a minor NK subset that lacks perforin but secrete large amounts of IFN-γ and



	Natural Killer cells	Invariant T cells		Conventional T cells
		*i*NKT cells	Vα7.2 *i*T cells	
TCR-Ag presenting molecules	None	α24β11- CD1d	α7.2β2/13- MR1	αβ- CD8:MHC class I CD4:MHC class II
NK marker	CD161 (NKR-P1) CD16 CD56 CD57 CD122	CD161 (NKR-P1) CD16 CD56	CD161 (NKR-P1)? CD16 ? CD56 ?	CD161, CD56, CD57 + in some subsets
NK receptor-ligands	Inhibition (KIR): CD94/NKG2A-HLA-E CD94/NKG2C-HLA-E LILRB1-HLA-A~G Activation: NKG2D-MICA/B ULBP1~4 NKp30- ??	CD94/NKG2A -HLA-E NKG2D-MICA/B ULBP1~4	??	Some cells pos. by induction
Memory phenotype		Majority CD69+	Majority CD69+	+ (Memory T cells)
Cytokine production	NK1: IFN-γ, TNF-α NK2: IL-5	DN: IFN-γ,TNF-α CD4: IL-4, IL-5, IL-13 (IL-17, IL-21)	? ? IFN-γ Th2 cytokine	CD8: IFN-γ CD4: Th1cell: IFN-γ Th2cell: IL-4, IL-5 Th17cell: IL-17
Perforine activation	+ (mainly NK1)	+ (mainly DN cells)	??	+ (mainly CD8 cells)
Frequency in PBMC	10 %	0.1 - 0.5 %	??	30-40 %

Fig. 1 Comparative features of human NK cells, invariant *i*NKT cells, and conventional T cells

TNF-α upon activation. They are superior to CD56^{dim} cells in the regulatory functions that are mediated by these cytokines (Moretta et al. 2001). Moreover, they express surface markers such as CCR7 and CD62L that allow their homing to the lymph nodes, which results in the predominance of this NK cell subset in the secondary lymphoid organs.

Although the dominant role of CD4$^+$ T cells in MS has long been emphasized (Hafler 2004), more recent works indicate that CD8$^+$ T cells (Huseby et al. 2001; Skulina et al. 2004) and B cells also play a critical role in the disease development, and actually comprise a proportion of the CNS infiltrating cells. CD8$^+$ cells are reported to be predominant in the CNS lesions of MS, although compositions of cellular infiltrates vary greatly, depending on types and stages of this disease (Sospedra and Martin 2005). Now, the key question in MS lies in what disrupts the T cell and B cell immunological tolerance against the CNS antigens that are usually kept well secluded from the systemic immune system (Goodnow et al. 2005; Kyewski and Derbinski 2004; Walker and Abbas 2002). The relevance of this question is obvious because better understanding of the mechanism for the disruption of self-tolerance will lead to development of various new approaches to prevent the onset of MS and to control its further progression.

One of the distinctive and intriguing aspects of MS is that individual patients show various patterns in the longitudinal changes of its disease activity. While a large majority of the patients exhibit a relapsing and remitting course, some patients develop into or even start out as a progressive chronic illness (Sospedra and Martin 2005; Steinman 2001). Despite the vigorous efforts to control the activity of MS, currently available therapeutics to do not halt the progression of disease in a majority of cases, although some patients do not exhibit any sign of worsening for a long period of time even without treatment.

To clarify the regulation of autoimmune responses, much efforts have been dedicated to investigate the role of specialized adaptive regulatory T cells, including CD4$^+$ T cells expressing transcription factor Foxp3 (Miyara and Sakaguchi 2007), IL-10 producing T regulatory 1 (Tr1) cells (Roncarolo et al. 2006), and TGF-β producing Th3 cells (Awasthi et al. 2007; Baecher-Allan and Hafler 2006). However, recent publications provide evidence that cells of the innate immune system also have an unexpected potential to inhibit autoreactive CD4$^+$ T cells from mediating autoimmune disease and to protect tissues from collateral damage by T cells reactive to exogenous pathogens (Carrol and Prodeus 1998; Fearon and Locksley 1996; Medzhitov and Janeway 1997; Shi et al. 2001). Natural killer (NK) cells and invariant natural killer T (*i*NKT) cells, the main focus of this review, are also now recognized as innate cells with immunoregulatory potentials. Although they sense external ligands with different receptors (TCR for *i*NKT cells and NK receptor for NK cells), they behave like innate cells when they need to rapidly respond to stimuli. Therefore, it was believed previously that both cell types would primarily function within the innate arms of immunity. However, recent works have provided evidence that they would actively regulate T cell responses, thereby influencing the adaptive immune system (Bendelac et al. 1997; Carrol and Prodeus 1998; Fearon and Locksley 1996; Medzhitov and Janeway 1997; Shi et al. 2001; Shi and Van Kaer 2006).

In summary, NK cells and *i*NKT cells are now considered as multipotent cells that work at the border of innate and adaptive immunity, to prevent the induction, propagation, and activation of autoimmune T cells. Here, we review the latest advances in the research of the regulatory NK and *i*NKT lymphocytes with regard to the pathogenesis of MS and discuss the possibilities that they may serve as an effective target for MS therapy.

Role of NK Cells and Invariant NKT Cells in Multiple Sclerosis

Kaori Sakuishi, Sachiko Miyake, and Takashi Yamamura

Abstract Natural killer (NK) cells and invariant natural killer T (*i*NKT) cells are two distinctive lymphocyte populations, each possessing its own unique features. Although NK cells are innate lymphocytes with cytotoxic property, they play an immunoregulatory role in the pathogenesis of autoimmune diseases. NKT cells are T cells expressing invariant TCR α-chains, which are known to bridge innate and adaptive arms of the immune system. Accumulating data now support active involvement of these cells in multiple sclerosis (MS). However, unlike professionally committed regulatory cells such as Foxp3$^+$ regulatory T cells, NK, and *i*NKT cells have dual potential of acting as either protective or pathogenic lymphocytes depending on the disease setting, adding complexity to the interpretation of data obtained from human and rodent studies. They are potential therapeutic targets in MS, and further in-depth understanding of these cells will lead to designing new strategies to overcome the disabling disease MS.

1 Introduction

Over the past years, a growing number of evidence has indicated that multiple sclerosis (MS) is as an autoimmune disease mediated by T cell immunity (Sospedra and Martin 2005). As described in detail in other chapters, pathogenesis of MS would actually involve autoreactive T cells that recognize the central nervous system (CNS) antigens. The target antigens include myelin basic protein (MBP) (Bielekova et al. 2000; Martin et al. 1991; Ota et al. 1990; Pette et al. 1990; Richert et al. 1989), myelin proteolipid protein (PLP) (Correale et al. 1995; Illes et al. 1999; Kondo et al. 1996; Ohashi et al. 1995; Pelfrey et al. 1993), and myelin oligodendrocyte glycoprotein (MOG) (Iglesias et al. 2001; Koehler et al. 2002; Mendel et al. 1995).

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Results Probl Cell Differ, DOI 10.1007/400_2009_11
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Recent studies have shown that human NK cells are able to polarize *in vitro* into two functionally distinct subsets NK type 1 (NK1) or NK2 cells, analogous to T cell subsets Th1 or Th2. NK cells cultured in a condition favoring Th1 deviation (cultured with IL-12) would differentiate into NK1 cells producing IFN-γ and IL-10, whereas NK cells grown in a Th2 condition (cultured with IL-4) differentiate into NK2 cells producing IL-5 and IL-13 (Peritt et al. 1998). Although it was ambiguous whether the polarization actually occurs *in vivo*, an expansion of NK2 like cells producing IL-5 and IL-13 was observed in IFN-γ knockout mice (Hoshino et al. 1999), indicating that NK cells could functionally polarize into NK2-like cells in vivo.

Phenotypical analysis of NK cells in rodents has also identified a distinct population of NK cells that express CD11c, a prototypical dendritic cell (DC) marker. As the CD11c NK cells were shown to exhibit both NK and DC function, they are often referred to as "bitypic NK/DC cells" (Homann et al. 2002; Pillarisetty et al. 2005). CD11c molecule is known to be associated with integrin CD18 and form CD11c/CD18 complex. Although the precise function is not clear, CD11c is reportedly involved in binding of iC3b (Bilisland et al. 1994), adhesion to stimulated endothelium (Stacker and Springer 1991), and phagocytosis of apoptotic cells (Morelli et al. 2003). Bearing in mind that we have only very little knowledge of how these NK cell subsets are correlated to each other, we will next discuss on the recent progress which correlates the regulatory aspects of NK cells with the pathogenesis of MS.

2.2 *NK Cell in MS*

Despite the extensive studies in the past, there has been no simple uniform consensus regarding the role of NK cells in MS. Some of the earlier studies have found an inverse relationship between the number or the functional activity of circulating NK cells and the clinical or radiological activity of the patients with MS. NK cells isolated from MS patients were reported to be inefficient at cytotoxic killing and IFN-γ production (Benczur et al. 1980; Kastrukoff et al. 1998; Munschauer et al. 1995; Vranes et al. 1989). Furthermore, a longitudinal study showed that the functional activities of NK cells would decline during the relapse and then normalized during remission (Kastrukoff et al. 2003). On the contrary, several earlier studies failed to reveal any quantitative or qualitative difference between NK populations in MS patients versus controls (Hauser et al. 1981; Rauch et al. 1985; Rice et al. 1983; Santoli et al. 1981). The reason for these controversial findings remains to be unclear. However, it is of note that the criteria used to classify NK cells have been variable among the researchers and as a result the assays and protocols used to measure their functions and frequencies differ widely among the studies above mentioned. Moreover, because of difficulties in enrollment of patients, each of the studies might have examined the group of patients in different conditions. We also assume that they did not unify various confounding factors, some of which were not recognized when the study was conducted. Even duration of time between blood sampling and examination may affect the condition of NK cells (Takahashi et al. 2001).

In spite of the setbacks, the notion that NK cells have a significant role in reducing neuroinflammation and CNS injury stems from indirect evidences that were extracted from studies of an animal model experimental autoimmune encephalomyelitis (EAE) and from human clinical trials.

2.2.1 Protective Role of NK Cells in EAE

Monophasic EAE can be induced in C57BL/6 strain of mice (B6 mice) by immunizing the mice with an encephalitogenic myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}). When NK cells were depleted in vivo by antibody specific for NK1.1 molecule (CD161), mice developed an aggravated form of EAE in terms of onset and clinical severity (Zhang et al. 1997). Furthermore, NK cell depletion was found to increase proliferation and production of Th1 cytokines by memory $CD4^+$ T cells in the recall response to MOG. Similarly, NK cell depletion augmented the severity of EAE induced in β_2-microglobulin –/– mice. As the mice are lacking expression of CD1d molecule necessary for $NK1.1^+$ T cell development, it was assumed that NK cells would play a regulatory role in a manner independent of $NK1.1^+$ T cells. Furthermore, co-transfer of whole splenocytes, but not of NK cell-depleted splenocytes, ameliorated EAE that was induced by adoptive transfer of MOG-specific T cells into $Rag2^{-/-}$ hosts. Taken together, it was concluded that NK cells play a regulatory role in EAE. Involvement of NK cells was also demonstrated in Lewis rat EAE model which can be induced by sensitization to MBP (Matsumoto et al. 1998). When NK cells were depleted by antibody specific for either NKR-P1 (analogous to NK1.1) or asialo GM1, the rats developed an aggravated form of EAE, characterized by higher maximal clinical scores and increased mortality rates. Subsequently, Swanborg et al. have shown that rat bone marrow-derived NK cells would exhibit potent inhibitory effects on proliferation of auto-reactive T cells (Smeltz et al. 1999), further strengthening the postulate that NK cells play a regulatory role in the CNS autoimmunity.

More recently, Huang et al. have reported that mice deficient in CX3CR1 (the fractalkine receptor) develop a more severe form of EAE (Huang et al. 2006). Compared with their littermates, $CX3CR1^{-/-}$ mice immunized with MOG_{35-55} would exhibit a higher incidence of CNS hemorrhage, leading to a higher mortality rate. Moreover, the survived mice failed to recover neurological functions after they reached the peak of EAE. Although the $CX3CR1^{-/-}$ mice developed more serious manifestations of EAE, recall responses to MOG_{35-55} and generation of encephalogenic T cells in the peripheral lymphoid organs were not augmented in the mice. Notable differences were found in the CNS infiltrating cells. Namely, $NK1.1^+CD3^-$ cells were selectively depleted from mononuclear cells isolated from the spinal cord of the $CX3CR1^{-/-}$ mice, whereas they comprised 10–20% of the CNS infiltrates in wild-type mice and heterozygous $CX3CR1^{+/-}$ littermates. These findings led the authors to speculate that the exacerbated disease in $CX3CR1^{-/-}$ mice was due to a failure of regulatory NK cells to enter the target organ. In support of this, the majority of CNS-infiltrating NK cells in the littermate mice suffering from EAE expressed CX3CR1.

When NK cells were depleted in vivo by injecting anti-NK1.1 antibody, difference between CX3CR1$^{-/-}$ and the littermate CX3CR1$^{+/-}$ mice in the severity of EAE was no more evident. Of interest, soluble CX3CL1 was increased in the CNS of the EAE mice, and protein extracts from the CNS tissues showed a chemotactic activity for NK cells. It is of particular interest that a reduced number of circulating CX3CR1^{+} NK cells has recently been reported in patients with MS (Infante-Duarte et al. 2005), which would prompt further investigation to examine a possible correlate between EAE and MS with regard to NK cell-mediated immunoregulation.

2.2.2 Ex Vivo Analysis Revealed an Alteration of NK cells in MS

Given putative roles of NK cells in MS, one may ask if there is a significant correlation of NK cell functions and the disease activity of MS. By analyzing surface phenotypes and cytokine secretion profile of peripheral blood NK cells, we demonstrated in 2001 that NK cells from MS patients during clinical remission are characterized by a higher frequency of CD95^{+} cells as well as a higher expression level of IL-5, which represents a feature highly reminiscent of NK2 cells (Takahashi et al. 2001). The patients were selected from those who were not given any disease-modifying drugs, including corticosteroids. Remarkably, the NK2 cell-like feature, that is, a strong bias toward producing IL-5, was lost during the relapse of MS and regained after recovery. It was also found that NK2 cells induced *in vitro* from the peripheral blood of healthy subjects would inhibit the induction of Th1 cells, suggesting that the NK2 cells in vivo may also prohibit autoimmune effector T cells. Subsequently, we showed that when MS patients in remission are divided into two groups, according to the CD95^{+} NK cell frequency, memory T cells reactive to MBP are increased in patients who possess a higher number of CD95^{+} NK cells (Takahashi et al. 2004). Interestingly, NK cells from the “CD95 high patients” exhibited an ability to actively suppress the autoimmune T cells. These results allowed us to propose a model that CD95 low patients are enjoying very stable remission wherein an actual frequency of pathogenic autoimmune T cells is low, whereas CD95 high patients are in a more active state (which we call “smoldering state”) wherein a higher number of autoreactive T cells are counter-regulated by NK cells (Fig. 2).

In a separate study, we found that CD11c expression on peripheral NK cells tends to correlate with temporal disease activity of MS (Aranami et al. 2006). Our study has revealed that surface CD11c expression on NK cells is significantly up-regulated in a proportion of patients with MS in remission, compared with healthy subjects or the rest of the patients. In the group of patients whose NK cells express higher levels of CD11c (“CD11c high patients”), IL-5 production from NK cells was significantly down-regulated and conversely, HLA-DR class II molecule was up-regulated. Accordingly, NK cells from “CD11c low patients” are NK2-biased, whereas those from “CD11c high patients” are not. NK cells from human PBMC would up-regulate expression of both CD11c and HLA-DR molecules after culture with IL-15 or a combination of IL-12 and IL-18 inflammatory cytokines commonly found in MS. Remarkably, the “CD11c high patients” tended to relapse significantly

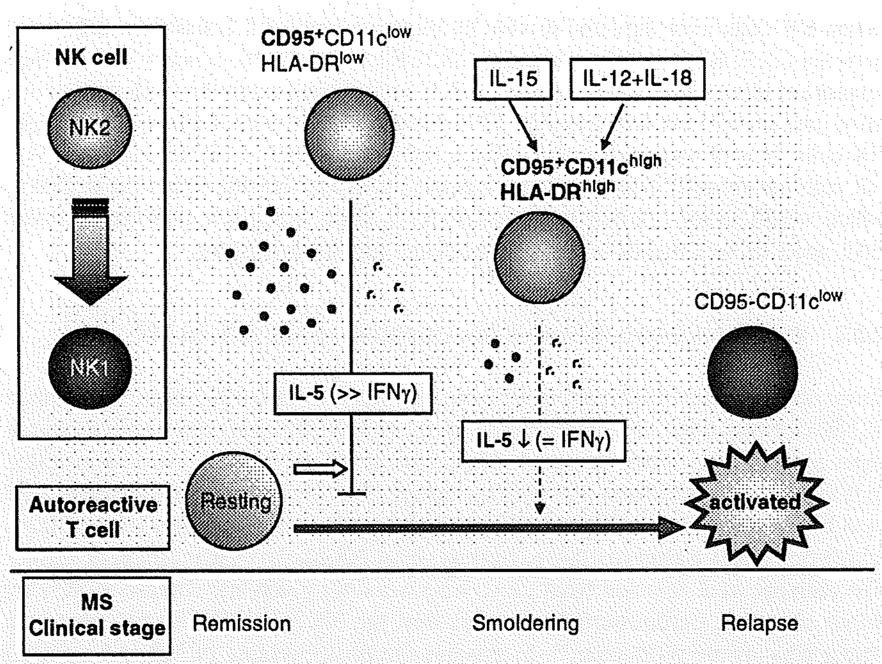

Fig. 2 Regulatory role of CD95$^+$ NK 2 cell in MS remission

earlier than "CD11c low patients," indicating that "CD11c high patients" are clinically more active. We, therefore, propose that expression levels of CD11c on NK cells may serve as a good indicator of the disease activity (Fig. 2).

Another evidence for the role of NK cells in MS was obtained in the clinical trial of a new humanized monoclonal antibody against IL-2 receptor α-chain. In a recent phase II trial with the antibody (daclizumab), Bielekova et al. have noticed that an expansion of CD56$^{\text{bright}}$ immunoregulatory NK cells and their increased perforin expression would highly correlate with the reduction of the disease activity (Bielekova et al. 2006). In fact, contrast enhanced lesion on brain MRI was significantly suppressed along with an expansion of circulating CD56$^{\text{bright}}$ NK cells. NK cells isolated from patients being given daclizumab were found to exhibit cytotoxity towards autologous activated T cells, even without prestimulating NK cells with IL-2. These results raise a possibility that induced regulatory NK cells may at least partly mediate daclizumab effects on MS. In another study, an increase of CD56$^{\text{bright}}$ NK cells was demonstrated in the blood of newly diagnosed patients with relapsing-remitting MS who were started on interferon-β treatment a few months ago (Saraste et al. 2007). This work also supports a role for induced regulatory NK cells in patients who respond to immunomodulatory therapy. Taking the available data together, we assume that NK cells harbor functional subpopulations that play a protective role in CNS autoimmunity. Regulatory NK cells could be CD56$^{\text{high}}$, CD95$^+$, or CX3CR1$^+$, although mutual relationship of the populations still remains unclear. Further attempts to find a way to selectively activate regulatory NK cells are warranted, because it will lead to developing a new treatment strategy for MS. It is known that NK cells show cytotoxic insults against CNS components in some *in vitro* conditions (Morse et al. 2001). To develop safe and effective drugs targeting NK cells, it is also important to know if regulatory NK cells could be selectively induced without augmenting cytotoxic NK cells that are potentially harmful for MS.

3 *i*NKT Cells in MS

3.1 What Is iNKT Cell?

3.1.1 General Properties of Invariant NKT (*i*NKT) Cells

Invariant NKT (*i*NKT) cells are a unique subset of lymphocytes that recognize a glycolipid antigen such as α-galactosylceramide (α-GC) (Kawano et al. 1997), that is bound to a monomorphic MHC class I-like molecule CD1d (Bendelac et al. 2007; Kronenberg 2005; Taniguchi et al. 2003). The term "NKT cells" was first introduced in mice to define a broader range of T cells that express the NK cell-associated marker NK1.1 (CD161) (Ballas and Rasmussen 1990; Fowlkes et al. 1987). The term "*i*NKT cells" defines a more limited population among NK1.1$^+$ T cells that express a single invariant α-chain (Vα14-Jα18 in mice and Vα24-Jα18 in humans) and respond to α-GC bound to CD1d (Dellabona et al. 1994; Exley et al. 1997; Koseki et al. 1991) (Fig. 1). The invariant α-chain is coupled with a noninvariant β-chain which selectively uses Vβ8.2, Vβ7, and Vβ2 gene segments in mice and Vβ11 (a molecule homologous to mice Vβ 8.2) in humans. It is currently known that mouse NK1.1$^+$ T cells (or NKT cells in the classic definition) are composed of *i*NKT cells, CD1d-restricted noninvariant T cells, conventional T cells that are not restricted by CD1d, and MAIT cells (see Sect. 4). On the other hand, there are a significant number of NK1.1-negative T cells that express the invariant Vα14-Jα18 TCR and react to α-GC/CD1d. In most of the current literatures, such T cells are also called *i*NKT cells.

*i*NKT cells constitutively express memory/activated T cell phenotype and are capable of robustly producing pro and antiinflammatory cytokines within hours after TCR engagement. The cytokine burst following *i*NKT cell activation then triggers a maturation process of downstream cells, such as NK cells, DCs, B cells, and T cells, which leads to subsequent alteration of a broad range of adaptive immune responses. Although *i*NKT cells utilize TCR for sensing a specific antigen, the behavior of the cells in response to external stimuli resembles that of innate lymphocytes (Mempel et al. 2002). Owing to the swift responsiveness to external stimuli, it is thought that *i*NKT cells play an important role in bridging innate and adaptive arms of immune response.

Another striking property of *i*NKT cells is to produce diverse combinations of cytokines, depending on how they are stimulated. Mouse *i*NKT cells can produce IFN-γ, IL-2–5, -13, -17, -21, GM-CSF, TNF-α, and osteopontin after an optimal engagement of TCR (Yamamura et al. 2007). In fact, they can produce a broad range of pro- and anti-inflammatory cytokines upon stimulation with α-GC, a highly potent ligand for *i*NKT cells (Kawano et al. 1997). In contrast, cytokine production by *i*NKT cells is much more finely regulated under physiological environment, which could result in production of a set of Th2 cytokines (Sakuishi et al. 2007).

*i*NKT cells are segregated into $CD4^+CD8^-$ and $CD4^-CD8^-$ double negative (DN) subsets. It has been shown that each subset differs remarkably in their functional properties. In humans, about 40–60% of *i*NKT cells are $CD4^+$, and a large majority of the remaining cells are DN cells. Some *i*NKT cells express CD8α, but only very few cells co-express CD8β. The $CD4^+$ subset potently produces both Th1 and Th2 cytokines, whereas the DN population selectively produces the Th1 cytokines (IFN-γ and TNF-α) and preferentially up-regulates perforin in response to IL-2 or IL-12 (Gumperz et al. 2002; Lee et al. 2002). It is also known that the $CD4^+$ and DN *i*NKT cells differentially express chemokine receptors: CCR4 on $CD4^+$ cells and CCR1, CCR6, and CXCR6 on DN cells (Kim et al. 2002). These results suggest the presence of a functional dichotomy in *i*NKT cells.

3.1.2 iNKT Cells and Their Ligands

To evaluate the potential of *i*NKT cells to regulate autoimmune diseases, it is particularly important to understand how they recognize a glycolipid antigen bound to CD1d. The CD1d molecule, highly conserved among mammalian species (Exley et al. 2000), is primarily expressed on the cells of hematopoietic origin, including thymocytes, B cells, macrophages, and DCs, and could also be induced on T cells upon activation. The binding cleft of the CD1d molecule consists of two nonpolar lined grooves, which makes it ideal for the presentation of hydrophobic antigens such as glycolipids. In 1997, a marine sponge-derived glycosphingolipid, α-GC, was identified as a potent ligand for mouse *i*NKT cells (Kawano et al. 1997). It was subsequently found that α-GC is stimulatory for human *i*NKT cells as well (Brossay et al. 1998). Thereafter, a synthetic α-GC has been used extensively for research (Fig. 3). A widely supported view on the topology of TCR/ligand/CD1d is that the two lipid chains of α-GC would be inserted into the CD1d hydrophobic grooves and α-linked sugar moiety becomes accessible for the TCR of *i*NKT cells (McCarthy et al. 2007). More recently, crystal structure analysis has demonstrated that the invariant α-chain of the *i*NKT cells would selectively recognize the α-linked sugar of α-GC (Borg et al. 2007). It is of note that glycolipids with a-linked sugars such as α-GC could not be found in mammalian tissues, but are rather ubiquitously present in the environment. After LPS-negative α-proteobacteria extracts were found to contain glycosphingolipids stimulatory for *i*NKT cells, a growing number of bacterial lipid antigens has been shown to stimulate *i*NKT cells (Bendelac et al. 2007), including diacylglycerol glycolipid extracted from Borrelia burgdorferi (Kinjo et al. 2005). Given that the TCRs of *i*NKT cells recognize such pathogen-derived antigens, the lipid antigens may be an important initiator for triggering the immune response in bacterial and parasite infection. However, it has recently been demonstrated that *i*NKT cells are activated during infection without recognizing a bacteria component

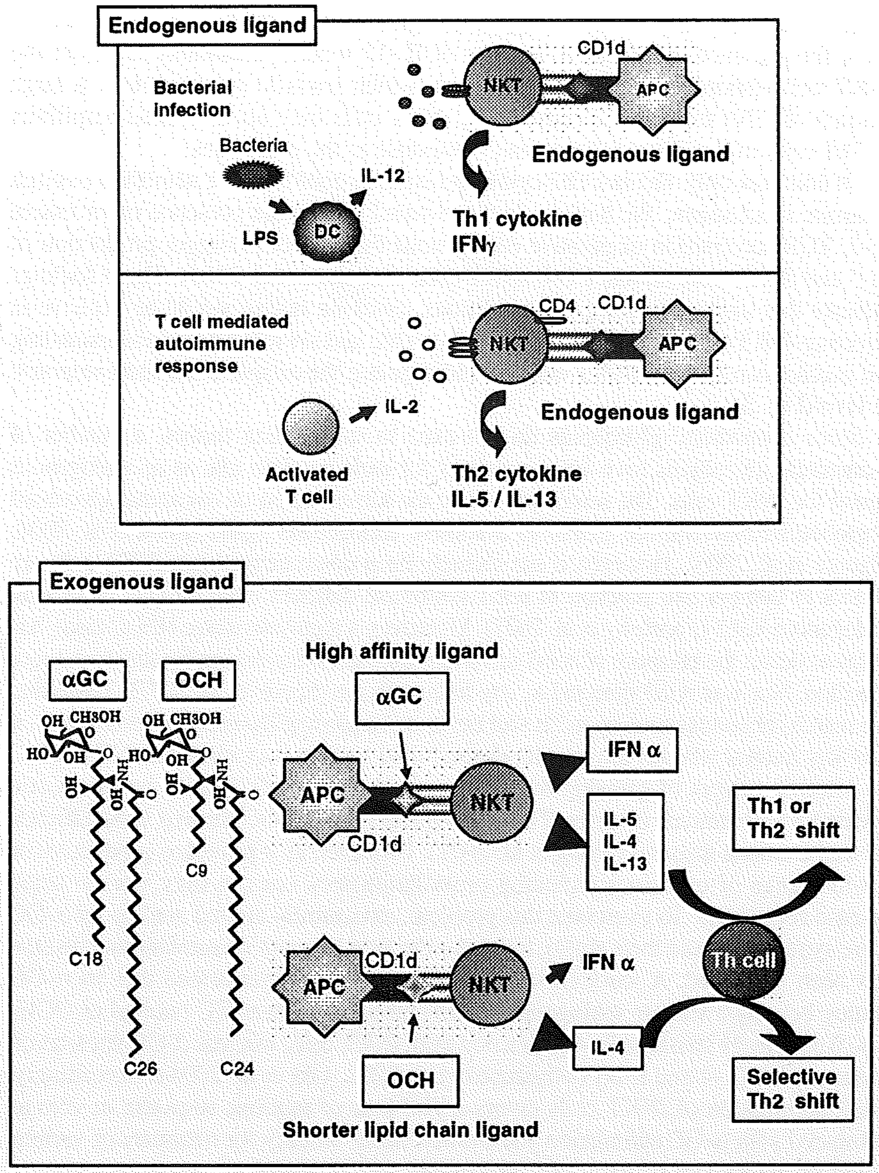

Fig. 3 Effects of lipid chain lengths in alpha-galactosylceramides on cyotokine release by natural killer T cells

via TCR (Brigl and Brenner 2004; Mattner et al. 2005). The antigen recognized by the TCR of *i*NKT cells is thought to be an endogenous ligand bound with CD1d, but not an exogenous microbial ligand. These studies also showed that the role for the bacterial LPS is to trigger production of IL-12 from DCs. Although *i*NKT cells exhibit little response to the endogenous ligand/CD1d *i*NKT cells expressed by DCs, the presence of excessive amount of IL-12 would remarkably augment the *i*NKT cell response to endogenous ligand, which leads to production of a large amount of IFNγ from *i*NKT cells. Thus, *i*NKT cells may act as crucial amplifiers of Th1 cells in the initial inflammatory response to the pathogens.

Of note, not only Th1 but Th2 cytokine response could also be amplified through a similar mechanism. We have recently revealed that in the presence of excessive IL-2, TCR recognition of putative endogenous ligand would trigger production of IL-5 and IL-13 from human CD4$^+$ *i*NKT cells (Sakuishi et al. 2007). These findings indicate that under physiological conditions, cytokine milieu would be decisive in directing *i*NKT cell responses towards Th1 or Th2, and are relevant for understanding the mechanism of how *i*NKT cells would regulate the adaptive immune response in vivo (Fig. 3).

Since α-anomeric glycolipids do not exist in mammalian tissues, a number of β-anomeric glycolipids have been evaluated for their possible role as an endogenous ligand for *i*NKT cells. The search has led to the identification of lysosomal glycolipid isoglobotrihexosylceramide (iGb3) as a putative endogenous ligand (Zhou et al. 2004; Mattner et al. 2005). However, it has recently been demonstrated that *i*NKT cells are normal in number and function in iGb3 synthetase deficient mice, despite of lacking endogenous iGb3 (Porubsky et al. 2007). Moreover, a highly sensitive HPLC assay has failed to detect the presence of iGb3 in various mouse tissues except for the dorsal root ganglion. Nor was iGb3 detected in any human tissue (Speak et al. 2007). Therefore, the search for endogenous ligand is still not over. Regarding the pathogenesis of MS, it is of key interest whether any myelin-derived lipid antigen may stimulate *i*NKT cells.

Another subject of growing interest is to use *i*NKT cell ligands as therapeutic agents for autoimmune diseases. The prototypical ligand α-GC showed some efficacy for autoimmune diseases (Hong et al. 2001). However, as it provokes production of a wide range of cytokines including proinflammatory ones, it may worsen some disease conditions. To overcome this problem, structurally altered analogs of α-GC were synthesized and their ability to inhibit the development of autoimmune disease has been examined. A work from our laboratory has demonstrated that an α-GC analog bearing a shorter sphingosine chain compared with α-GC (named as OCH) would selectively stimulate IL-4 production from *i*NKT cells, whereas α-GC stimulation induces both IL-4 and IFNγ (Miyamoto et al. 2001; Oki et al. 2004). Accordingly, OCH stimulation of *i*NKT cells favors a Th2 bias of immune response in vivo as compared with α-GC stimulation and showed better efficacy for treatment of various autoimmune disease models (Fig. 3) (see Sect. 3.3 as well).

3.2 Studies of iNKT Cells in MS

Using single-strand conformation polymorphism (SSCP), a method for examining the TCR repertoire, we have previously analyzed blood samples from subjects with MS as well as other neurological diseases (Illes et al. 2000). Expression of the invariant Vα24-Jα18 rearrangement, the invariant TCR α-chain expressed by human *i*NKT cells, was greatly reduced in the blood lymphocytes of the patients with MS, compared with those from healthy subjects. The reduction was not observed in the patients with other autoimmune/inflammatory neurological diseases. Interestingly, the Vα24-Jα18 TCR was only rarely found in the CNS lesions of MS but was often detected in the biopsy samples from chronic inflammatory demyelinating polyneuropathy (CIDP).

More recently, we have reanalyzed the frequency of *i*NKT cells in the peripheral blood of MS by using flow cytometry. A striking reduction of the total number of *i*NKT cells was confirmed in the peripheral blood of the patients with MS in a drug-free remission state (Araki et al. 2003). Interestingly, when $CD4^+$ and DN *i*NKT cells were analyzed separately, a remarkable *i*NKT cell reduction was found to reflect a great reduction of DN *i*NKT cells, that are known to preferentially produce proinflammatory cytokines (Gumperz et al. 2002; Lee et al. 2002). Moreover, we found that the $CD4^+$ *i*NKT cell lines from MS patients were significantly biased for Th2: they produced much more IL-4 than those from healthy subjects, although the production of IFN-γ was not altered significantly (Araki et al. 2003). Collectively, the changes found in *i*NKT cells (a reduction of DN and Th2 bias of $CD4^+$ *i*NKT cells) are thought to be beneficial for maintaining the remission state of MS.

It is also worthwhile to mention that the currently available drugs may exert their actions through targeting *i*NKT cells. Although the drug-free remission state of MS was associated with a great reduction of *i*NKT cells in the peripheral blood (Araki et al. 2003), patients who were continuously given a low dose oral corticosteroid showed a normal frequency of *i*NKT cells in the blood, indicating that oral corticosteroid treatment may restore the frequency of *i*NKT cells (Araki et al. 2004). Interestingly, the cytokine profile of DN NKT cells from the corticosteroid-treated MS showed a trend for Th2 bias. This may represent one of the mechanisms of the corticosteroid effects in MS and other autoimmune diseases.

In a recent longitudinal study, IFN-β treatment significantly increased the number of *i*NKT cells in the peripheral blood mononuclear cell within same patients (Gigli et al. 2007). Furthermore, *i*NKT cells of IFN-β treated individuals showed a dramatically improved secretion of INF-γ, IL-4, and IL-5 in response to α-GC stimulation compared with those isolated from the same individuals before IFN-β treatment. The study also showed up-regulation of key costimulatory molecules expressed by DCs in the IFN-β treated patients. Thus, immune regulatory effect of IFN-β therapy in MS may possibly mediate *i*NKT cells.

3.3 iNKT Cells as a Therapeutic Target in MS/EAE

Results of EAE studies give us clues to understanding the role of *i*NKT cells in the pathogenesis of MS. It is well known that SJL/J mice are very susceptible to induction of EAE and other autoimmune diseases. In this strain of mice, *i*NKT cells are reduced in number and defective in IL-4 production (Yoshimoto et al. 1995),