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).

K. Sakuishi, S. Miyake, and T. Yamamura (✉)
Department of Immunology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4–1–1 Ogawahigashi, KodairaTokyo, 187–8502, Japan
e-mail: yamamura@ncnp.go.jp

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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.

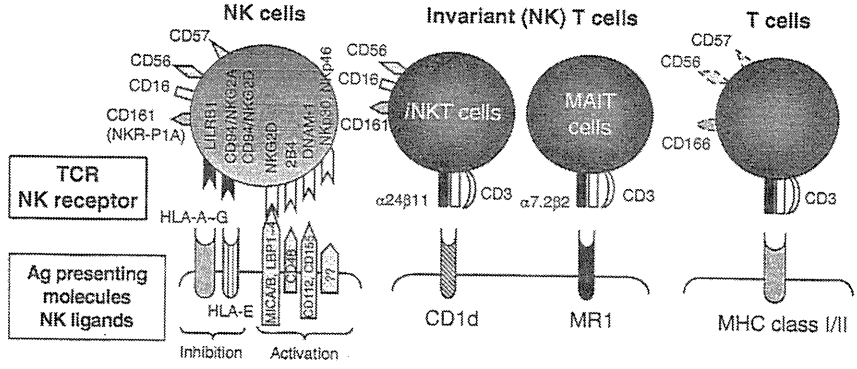
2 NK Cells and MS

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 /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.

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

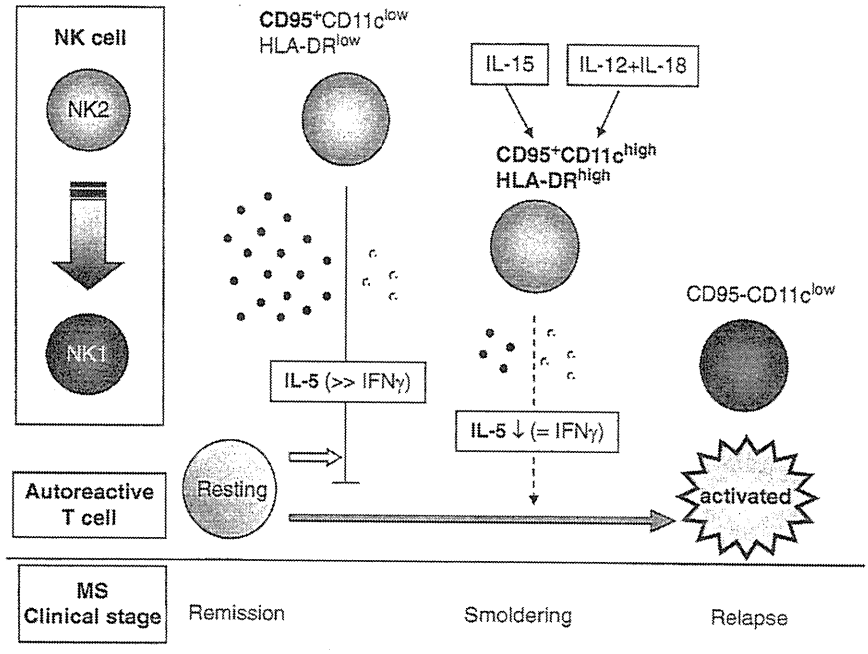


Fig. 2 Regulatory role of CD95+ NK 2 cell in MS remission

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 $CD56^{bright}$ 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 $CD56^{bright}$ 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 $CD56^{bright}$ 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 $CD56^{high}$, $CD95^{+}$, 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

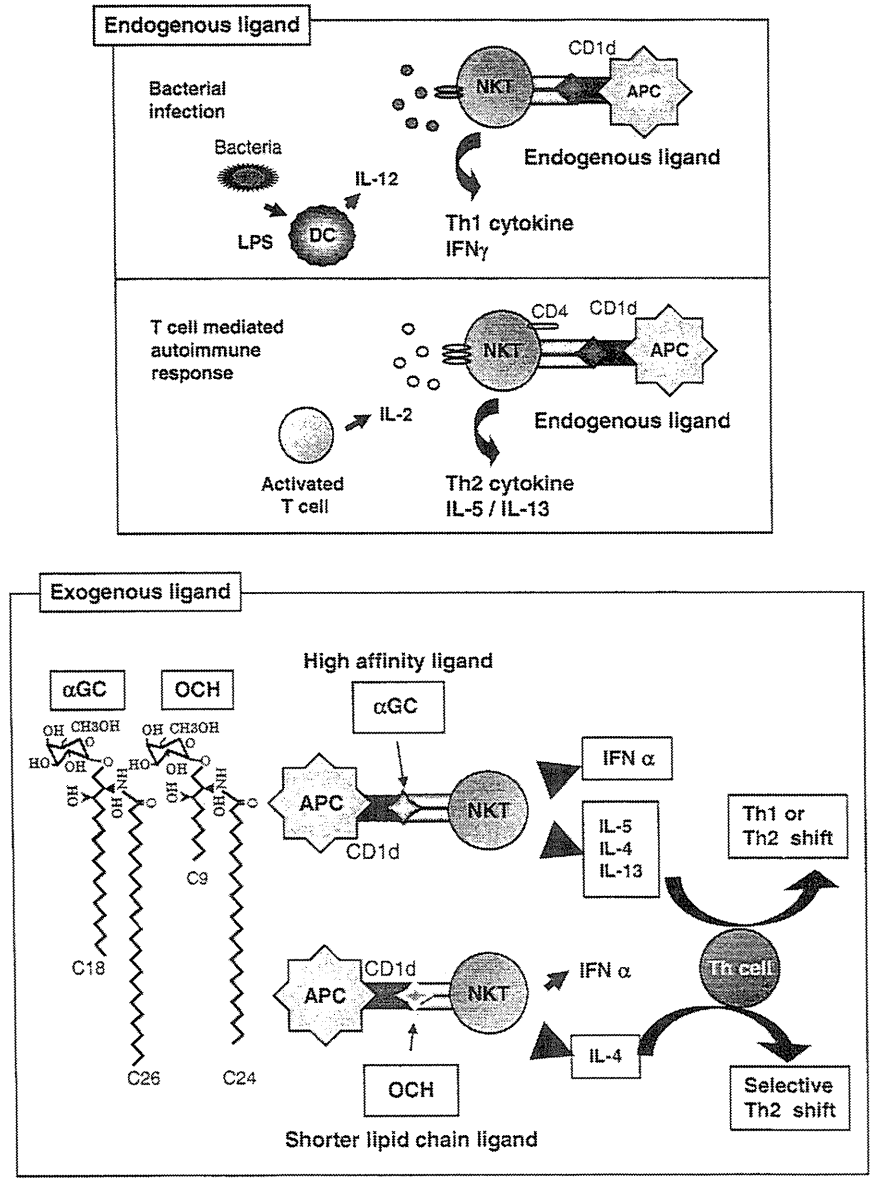


Fig. 3 Effects of lipid chain lengths in alpha-galactosylceramides on cyotokine release by natural killer T cells

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),

allowing us to speculate that the *i*NKT cell defects may account for the autoimmune susceptible nature. On the contrary, transgenic overexpression of the invariant TCR of *i*NKT cells was found to protect NOD strain of mice from development of EAE. This EAE protection was associated with an inhibition of antigen-specific IFN-γ production but was independent of IL-4 (Mars et al. 2002). These results indicate an inverse correlation of *i*NKT cell numbers/functions with the susceptibility to EAE, raising a simple idea that expanding *i*NKT cells may be beneficial for treating patients with MS.

After α-GC was identified as a potent ligand for *i*NKT cells, several laboratories have examined whether in vivo injection of α-GC may modify the clinical course of EAE by stimulating *i*NKT cells. A study by Singh et al. showed that α-GC is capable of down-modulating EAE, by inducing Th2 bias of *i*NKT cells (Singh et al. 2001). Furlan et al. also showed an efficacy of α-GC in EAE, but they did not reveal a Th2 bias but rather showed an enhanced IFNγ production by the liver *i*NKT cells (Furlan et al. 2003). In an independent study by Jahng et al., injection of α-GC with aim to suppress EAE resulted in diverse outcome, which depends on the administration route, timing of injection, and dose of this glycolipid (Jahng et al. 2001). Although the reason for these discrepancies remain unclear, it is possible that source of the mice, quality of the animal facilities, or even gut flora might have influenced the results.

It was subsequently found that CD28-B7 costimulatory signals play a critical role in stimulating *i*NKT cells with α-GC. When *i*NKT cells were stimulated with α-GC in the presence of anti-B7 (CD80) antibody *in vitro*, they selectively produced Th2 cytokines (Pal et al. 2001). *In vivo* stimulation of *i*NKT cells along with blocking CD28-B7 interactions was found to suppress the onset of EAE (Pal et al. 2001). These results collectively indicated that proper stimulation of *i*NKT cells might lead to suppression of pathogenic Th1 responses. We have then explored whether a Th2 polarizing ligand could be identified among α-GC analogs. As discussed briefly in Sect. 3.1.2, we have found that an analog of α-GC, called OCH, bearing a shorter sphingosine chain could selectively induce production of IL-4 but not of IFN-γ and could modulate disease process of EAE when injected *in vivo* (Miyamoto et al. 2001). This protective effect against the development of EAE was abrogated by a simultaneous injection of anti-IL-4 antibody. Moreover, the protective effect of OCH could not be seen in IL-4 knockout mice, indicating that IL-4 produced from *i*NKT cells is involved in the disease suppression.

The molecular mechanism for the selective IL-4 production by OCH has been intensively studied in our laboratory. Owing to the truncation of sphingosine chain, OCH binds to CD1d molecule less stably compared to α-GC. We are proposing that the unstable OCH-CD1d interaction, which does not allow continuous TCR stimulation, is a key to understanding the Th2 polarizing character of OCH (Oki et al. 2004). When *i*NKT cells are stimulated by a-GC, IL-4 is produced within a few hours, which is then followed by production of a large quantity of IFN-γ (Pal et al. 2001). Of note is that de novo protein synthesis is required for the *i*NKT cell production of IFN-γ but not of IL-4 (Oki et al. 2004). Subsequent analysis has revealed that c-Rel protein is selectively induced, when *i*NKT cells are simulated by α-GC. Inhibiting c-Rel expression in *i*NKT cells has led to a selective IL-4 induction as a result of

suppressed production of IFN-γ, as seen with OCH stimulation. Taken together, it can be postulated that unstable binding of OCH with CD1d leads to disrupted TCR signaling, which does not induce expression of c-Rel and of its down-stream molecule IFN-γ. Compared with α-GC, which is capable of fully inducing c-Rel and IFN-γ, OCH would exhibit a unique Th2 polarizing effect on *i*NKT cells *in vitro* and *in vivo*. Intriguingly, in vivo injection of OCH induces defective IFN-γ production not only by NKT cells but also by NK cells (Oki et al. 2005). Mechanistic analysis has revealed that an injection of OCH induces an insufficient induction of CD40L in addition to lower primary IFN-γ production by the NKT cells, leading to a marginal IL-12 production by DCs. A combination of these differences between OCH and α-GC stimulation would account for the lower secondary IFN-γ production by NKT and NK cells by OCH. Of note, McCarthy et al. have recently confirmed that shortening of the phytosphingosine chain increased the rate of lipid dissociation from CD1d molecule and induced less sustained TCR signals (McCarthy et al. 2007). In this study, they have also demonstrated the decreased affinity of TCR to OCH bound-CD1d.

Other lipid chain truncated analogs of α-GC have been reported to display a similar skewing of cytokine profile towards Th2 but the mechanism seems to differ from that found in OCH (Goff et al. 2004; Yu et al. 2005). Taken together, altered glycolipid provides attractive means for *i*NKT cells mediated intervention of inflammatory autoimmune disease such as EAE and human MS.

4 MR1- Restricted Invariant T Cells in MS

Another novel invariant NK cell receptor-positive T cell population besides *i*NKT cells has been described in mice and humans. They are preferentially located in the gut lamina propria and are generally termed mucosal-associated invariant T (MAIT) cells (Treiner et al. 2003). Of interest, they are absent in germ-free mice, which indicates the role of gut flora for generation and maintenance of this lymphocyte. The discovery of this population is dated back to 1993, when DN T cell population expressing an invariant TCR α-chain was described along with the identification of Vα24 *i*NKT cells (Porcelli et al. 1993). It is now established that the new invariant T cells are distinct from *i*NKT cells in the expression of another conserved CDR3α sequence (Vα7.2-Jα33 in humans and Vα19-Jα33 in mice) and restricted use of Vβ2 and Vβ13 in mice and humans. Unlike *i*NKT cells selected by CD1d, they are selected by another MHC class Ib molecule, MR1, that is also highly conserved among species (Treiner et al. 2003). The mouse MAIT cells were isolated from $NK1.1^+$ T cells in the liver of CD1d deficient mice lacking "conventional" *i*NKT cells, allowing us to call the cells "Vα19-Jα33 NKT cells." As seen with "conventional" NKT cells, human MAIT cells constitutively express memory phenotype and some NK cell markers other than CD57 (Treiner et al. 2005) (Fig. 1). Several lines of evidence suggest that MR1 presents lipid ligands such as α-mannocylceramide (Shimamura et al. 2007). Although the function of MAIT cells is unclear at the moment, their cardinal features such as the semiinvariant repertoire, restriction by

monomorphic class I-like molecule and the natural memory phenotype suggest that *i*NKT cells and MAIT cells may exhibit similar and/or complementary functions.

When expression of Vα7.2 invariant TCR for human MAIT cells was investigated in MS patient samples, there was a striking difference between the MAIT and *i*NKT cell invariant TCR in their expression. Expression of the invariant TCR chain for NKT cells was clearly reduced in the peripheral blood of MS patients (Illes et al. 2000), whereas invariant TCR for MAIT cells was clearly detected in the great majority of the patients (Illes et al. 2004). Parallel analysis of CNS lesions from MS patients showed that MAIT cells would infiltrate the majority of the lesions, whereas *i*NKT cells do not (Illes et al. 2000, 2004). The differential expression of the two invariant chains in samples from MS suggests that MAIT cells and NKT cells may complement each other and MAIT cells may substitute deficiency of *i*NKT cells in MS.

The protective role of MAIT cells is further delineated by the study of mouse EAE. We found that overexpression of the invariant Vα19-Jα33 TCR in B6 mice is protective against EAE induction and progression (Croxford et al. 2006). Consistently, EAE was exacerbated in MR1 deficient mice, which lack Vα19-Jα33 invariant T cells. The protective effect was found to accompany a reduced production of inflammatory mediators as well as an increased secretion of IL-10. We have also demonstrated that IL-10 production occurred in part through interactions between B cells and Vα19 MAIT cells involving ICOS costimulatory molecule.

5 Concluding Remarks

NK cells and *i*NKT cells are groups of innate lymphocytes with multi potential qualities. Recent advances in cell biology of these cells have brought our attention to their ability in regulating autoimmune inflammatory responses. Selective induction of their regulatory properties could be an effective means for modification of autoimmune disease affecting the CNS. It is also notable that NK cells and *i*NKT cells change their phenotypes, number, and gene expression profile during disease course of MS. They could be good targets also for those who attempt to identify useful biomarkers for MS.

References

Araki M, Kondo T, Gumperz J, Brenner M, Miyake S, Yamamura T (2003) T_h 2 bias of $CD4^+$ NKT cells derived from multiple sclerosis in remission. Int Immunol 15:279–288

Araki M, Miyake S, Yamamura T (2004) Continuous oral glucocorticoid therapy restores the NKT cell frequency in multiple sclerosis. Neuroimmunology 12:175–179

Aranami T, Miyake S, Takahashi K (2006) Differential expression of CD11c by peripheral blood NK cells reflects temporal activity of multiple slcerosis. J Immunol 177:5659–5667

Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8:1380–1389

Backstrom E, Ljunggren H, Kristensson K (2007) NK cell-mediated destruction of influenza A virus-infected peripheral but not central neurons. Scand J Immunol 65:353–361

Baecher-Allan C, Hafler D (2006) Human regulatory T cells and their role in autoimmune disease. Immunol Rev 212:203–216

Ballas Z, Rasmussen W (1990) NK1.1+ thymocytes, adult murine $CD4^-CD8^-$ thymocytes contain an $NK1.1^+$, $CD3^+$, $CD5^+$, $CD44^+$, TCR-Vb 8^+ subset. J Immunol 145:1039–1045

Benczur M, Petranyl G, Palffy G, Varga M, Talas M, Kotsy B, et al (1980) Dysfunction of natural killer cells in multiple sclerosis: a possible pathogenetic factor. Clin Exp Immunol 39:657–662

Bendelac A, Fearon DT (1997) Innate pathways that control acquired immunity. Curr Opin Immunol 9:1–3

Bendelac A, Savage P, Teyton L (2007) The biology of NKT cells. Annu Rev Immunol 25:297–336

Bielekova B, Goodwin B, Richert J, Cortese I, Kondo T, Afshar G (2000) Encephalitogenic potential of the myelin basic protein peptite (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nat Med 6:1167–1175

Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann T, et al (2006) Regulatory CD56 (bright) natural killer cells mediate immunomodulatory effects of IL-2Rα-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci U S A 103:5941–5946

Bilisland C, Diamond M, Springer T (1994) The leukocyte integrin p150, 95 (CD11c/CD18) as a receptor for ic3b: activation by a heterologous β subunit and localization of a ligand recognition site to the I domain. J Immunol 152:4582–4589

Borg N, Wun K, Kjor-Nielson L, Wilce M, Pellicci D, Koh R, et al (2007) CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. Nature 448:44–49

Borrego F, Masilamani M, Marusima A, Tang X, Coligan J (2006) The CD94/NKG2 family of receptors from molecules and cells to clinical relevance. Immnol Res 35:263–294

Brigl M, Brenner M (2004) CD1: antigen presentation and T cell function. Annu Rev Immunol 22:817–890

Brossay L, Chioda M, Burdin N, Koezuka Y, Casorati G, Dellabona P, et al (1998) CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. J Exp Med 188:1521–1528

Carrol M, Prodeus A (1998) Linkages of innate and adaptive immunity. Curr Opin Immunol 10:36–40

Correale J, McMillan M, McCarthy K, Le T, Weiner L (1995) Isolation and characterization of autoreactive proteolipid protein-peptide specific T cell clones from multiple sclerosis patients. Neurology 45:1370–1378

Croxford J, Miyake S, Huang Y, Shimamura M, Yamamura T (2006) Invariant Va19i T cells regulate autoimmune inflammation. Nat Immunol 7:987–994

Dellabona P, Padovan E, Casorati G, Brockhaus M, Lanzavecchia A (1994) An invariant Vα24-J a Q/Vb11 T cell receptor is expressed in all individual by clonally expanded $CD4^-CD8^-$ T cells. J Exp Med 180:1171–1176

Diefenbach A, Jamieson A, Liu S, Shastri N, Raulet D (2000) Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol 1:119–126

Exley M, Garcia J, Balk S, Porcelli S (1997) Requirements for CD1d recognition by human invariant $Vb24^+$ $CD4^-CD8^-$ T cells. J Exp Med 186:109–120

Exley M, Garcia J, Wilson S, Spada F, Gerdes D, Tahir S, et al (2000) CD1d structure and regulation on human thymocytes, peripheral blood T cells, B cells and monocytes. Immunology 100:37–47

Fearon D, Locksley R (1996) The instructive role of innate immunity in the acquired immune response. Science 272:50–53

Fowlkes B, Kruisbeek A, Ton-That H, Weston M, Coligan J, Schwartz R, et al (1987) A novel population of T-cell receptor aβ-bearing thymocytes which predominantly express a single Vβ gene family. Nature 329:251–254

Furlan R, Bergami A, Cantarella D, Brambilla E, Taniguchi M, Dellabona P, et al (2003) Activation of invariant NKT cells by agalcer administration protects mice from MOG 35–55-induced EAE: critical roles for administration route and IFN-γ. Eur J Immunol 33:1830–1838

Gigli G, Caielli S, Cutuli D, Falcone M (2007) Innate immunity modulates autoimmunity: type 1 interferon-b treatment in multiple sclerosis promotes growth and function of regulatory invariant natural killer T cells through dendritic cell maturation. Immunology 122:409–417

Goff R, Gao Y, Mattner J, Zhou D, Yin N, Cantu C 3rd, et al (2004) Effects of lipid chain lengths in alpha-galactosylceramides on cytokine release by natural killer T cells. J Am Chem Soc 126:13602

Goodnow C, Sprent J, Fazekas de St. Groth B, Vinuesa C (2005) Cellular and genetic mechanism of self-tolerance and immunity. Nature 435:590–597

Gumperz J, Miyake S, Yamamura T, Brenner M (2002) Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J Exp Med 195:625–636

Hafler D (2004) Multiple sclerosis. J Clin Invest 113:788–794

Hauser S, Ault K, Levin M, Garovoy M, Weiner H (1981) Natural killer cell activity in multiple sclerosis. J Immunol 127:1114–1117

Homann D, Jahreis A, Wolfe T, Hughes A, Coon B, van Stipdonk M, et al (2002) CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. Immunity 16:403–415

Hong S, Wilson MT, Serizawa I, Wu L, Singh N, Naidenko OV, et al (2001) The natural killer T-cell ligand α-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. Nat Med 7:1052–1056

Hoshino T, WInkler-Pickett RT, Mason AT, Ortaldo JR, Young HA (1999) IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN-γ. J Immunol 162:51–59

Huang D, Shi F, Jung S, Pien G, Wang J, Salazar-Mather T, et al (2006) The neuronal chemokine CX3CR1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system. FASEB J 20:896–905

Huseby E, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman JA (2001) Pathogenic role for myelin-specific $CD8^+$ T cells in a model for multiple sclerosis. J Exp Med 194:669–676

Iglesias A, Bauer J, Litzenburger T, Schubart A, Linington C (2001) T-and B-Cell responses to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis and multiple sclerosis. Glia 36:220–234

Illes Z, Kondo T, Yokoyama K, Ohashi T, Tabira T, Yamamura T (1999) Identification of autoimmune T cells among in vivo expanded $CD25^+$ T cells in multiple sclerosis. J Immunol 162:1811–1817

Illes Z, Kondo T, Newcombe J, Oka N, Tabira T, Yamamura T (2000) Differential expression of NK Tcell Va24 JaQ invariant TCR chain in the lesion of multiple sclerosis and chornic inflammatory demyelinating polyneuropathy. J Immunol 164:4375–4381

Illes Z, Shimamura M, Newcombe J, Oka N, Yamamura T (2004) Accumulation of V α7.2- J α33 invariant T cells in human autoimmune inflammatory lesions in the nervous system. Int Immunol 16:223–230

Infante-Duarte C, Weber A, Kratschmar J, Prozorovski T, Pikol S, Hamann I, et al (2005) Frequency of blood CX3CR1-positive natural killer cells correlates with disease activity in multiple sclerosis. FASEB J 19:1902–1904

Jahng A, Maricic I, Pedersen B, Burdin N, Naidenko O, Kronenberg M, et al (2001) Activation of natural killer T cells potentiates or prevents experimental autoimmune encephlomyelitis. J Exp Med 194:1789–1799

Kastrukoff L, Morgan N, Zecchini D, White R, Petkau A, Satoh J, et al (1998) A role for natural killer cells in the immunopathogenesis of multiple sclerosis. J Neuroimmunol 86:123–133

Kastrukoff L, Lau A, Wee R, Zecchini D, White R, Paty D (2003) Clinical relapse of multiple sclerosis are associated with novel valleys in natural killer cell functional activity. J Neuroimmunol 145:103–114

Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, et al (1997) CD1d-restricted and TCR-mediated activation of Va14 NKT cells by glycosylcermides. Science 278:1626–1629

Kim C, Johnston B, Butcher E (2002) Trafficking machinery of NKT cells: shared and differential chemokine receptor expression among $Va24^+Vb11^+$ NKT cell subsets with distinct cytokine-producing capacity. Blood 100:11–16

Kinjo Y, Wu D, Kim G, Xing G, Poles M, Ho D, et al (2005) Recognition of bacterial glycosphingolipids by natural killer T cells. Nature 434:520–525

Kirwan S, Burshtyn D (2007) Regulation of natural killer cell activity. Curr Opin Immunol 19:46–54

Koehler N, Genain C, Giesser B, Hauser S (2002) The human T cell response to myelin oligodendrocyte glycoprotein: a multiple scleorsis family-based study. J Immunol 168:5920–5927

Kondo T, Yamamura T, Inobe J, Ohashi T, Takahashi K, Tabira T (1996) TCR repertoire to proteolipid protein (PLP) in multiple sclerosis (MS): homologies between PLP-specific T cells and MS-associated T cells in TCR junctional sequences. Int Immunol 8:123–130

Koseki H, Asano H, Inaba T, Miyashita N, Moriwaki K, Lindahl K, et al (1991) Dominant expression of a distinctive V14+ T-cell antigen receptor a chain in mice. Proc Natl Acad Sci U S A 88:7518–7522

Kronenberg M (2005) Toward understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 2005: 877–900

Kyewski B, Derbinski J (2004) Self-representation is the thymus: an extended view. Nat Rev Immunol 4:688–698

Lee P, Benlagha K, Teyton L, Bendelac A (2002) Distinct functional lineages of human Vα24 natural killer T cells. J Exp Med 195:637–641

Lopez-Botet M, Perez-Villar J, Carretero M, Rodriguez A, Melero I, Bellon T (1997) Structure and function of the CD94 C-type lectin receptor complex involved in the recognition of HLA class I molecules. Immunol Rev 155:165–174

Mars L, Laloux V, Goude K, Desbois S, Saoudi A, Van Kaer L, et al (2002) Cutting edge: Vα14-Jα 281 NKT cells naturally regulate experimental autoimmune encephalomyelitis in nonobese diabetic mice. J Immunol 168:6007–6011

Martin R, Howell M, Jaraquemada D, Flerlage M, Richert J, Brostoff S (1991) A myelin basic protein peptide is recognized by cytotoxic T cells in the context of four HLA-DR types associated with multiple sclerosis. J Exp Med 173:19–24

Matsumoto Y, Kohyama K, Aikawa Y, Shin T, Kawazoe Y, Suzuki Y, et al (1998) Role of natural killer cells and TCR γδ T cells in acute autoimmune encephalomyelitis. Eur J Immunol 28:1681–1688

Mattner J, Debord K, Ismail N, Goff R, Cantu 3rd C, Zhou D, et al (2005) Exogenous, and endogenous glycolipid antigens activate NKT cells during microbial infection. Nature 434:525–529

McCarthy C, Shepherd D, Floire S, Stronge V, Koch M, Illarionov P, et al (2007) The length of lipids bound to human CD1d molecules modulates the affinity of NKT cell TCR and the threshold of NKT cell activation. J Exp Med 204:1131–1144

Medzhitov R, Janeway JC (1997) Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 9:4–9

Mempel M, Ronet C, Suarez F, Gilleron M, Puzo G, Van Kaer L, et al (2002) Natural killer T cells restricted by the monomorphic MHC class 1b CD1d1 molecules behave like inflammatory cells. J Immunol 168:365–371

Mendel I, Kerlero de Rosbo N, Bennun AA (1995) Myeline oligodendrocyte glycoportein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2β mice: fine specificity and T cell receptor Vb expression of encephalitogenic T ells. Eur J Immunol 25:1951–1959

Miyamoto K, Miyake S, Yamamura T (2001) A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. Nature 413:531–534

Miyara M, Sakaguchi S (2007) Natural regulatory T cells: mechanisms of suppression. Trends Mol Med 13:108–116

Morelli A, Larregina A, Shufesky W, Zahorchak A, Logar A, Papworth G, et al (2003) Internalization of circulating apoptopic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. Blood 101:611–620

Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari M (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 19:197–223

Morse RH, Seguin R, McCrea EL, Antel JP (2001) NK cell-mediated lysis of autologous human oligodendrocytes. J Neuroimmunol 116:107–115

Munschauer F, Hartrich L, Stewart C, Jacobs L (1995) Circulating natural killer cells but not cytotoxic T lymphocytes are reduced in patients with active relapsing multiple slcerosis and little clinical disability as compared to controls. J Neuroimmunol 62:177–181

Ohashi T, Yamamura T, J-i Inobe, Kondo T, Kunishita T, Tabira T (1990) Analysis of proteolipid protein (PLP)-specific T cells in multiple sclerosis: identification of PLP 95–116 as an HLA-DR2,w15-associated determinant. Int Immunol 7:1771–1778

Oki S, Chiba A, Yamamura T, Miyake S (2004) The clinical implication and molecular mechanism of preferential IL-4 production by modified glycolipid-stimulated NKT cells. J Clin Invest 113:1631–1640

Oki S, Tomi C, Yamamura T, Miyake S (2005) Preferential T_h 2 polarization by OCH is supported by incompetent NKT cell induction of CD40L and following production of inflammatory cytokine by bystander cells in vivo. Int Immunol 17:1619–1629

Ota K, Matsui M, Milford E, Mackin G, Weiner H, Hafler D (1990) T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 346:183–187

Pal E, Tabira T, Kawano T, Taniguchi M, Miyake S, Yamamura T (2001) Costimulation-dependent modulation of experimental autoimmune encephalomyelitis by ligand stimulation of Va14 NK T cells. J Immunol 166:662–668

Pelfrey C, Trotter J, Tranquill L, McFarland H (1993) Identification of a novel T cell epitope of human proteolipid protein (residues 40–60) recognized by proliferative and cytolytic $CD4^+$ T cells from multiple sclerosis patients. J Neuroimmunol 46:33–42

Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G (1998) Cutting edge. Differentiation of human NK cells into NK1 and NK2 subsets. J Immunol 161:5821–5824

Pette M, Fujita K, Wilkinson D, Altmann D, Trowsdale J, Giegerich G, Wekerle H (1990) Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple slcerosis patients and healthy donors. Proc Natl Acad Sci U S A 87:7968–7972

Pillarisetty V, Katz S, Bleier J, Shah A, Dematteo R (2005) Natural killer dendritic cells have both antigen presenting and lytic function and in response to CpG produce IFN-γ via autocrine IL-12. J Immunol 174:2612–2618

Porcelli S, Yockey C, Brenner M, Balk S (1993) Analysis of T cell antigen receptor (TCR) expression by human peripheral blood $CD4^-CD8^-$alph/β T cells demonstrates preferential use of several Vβ genes and an invariant TCRa chain. J Exp Med 178:1–16

Porubsky S, Speak A, Luckow B, Cerundolo V, Platt F, Grone H (2007) Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. Proc Natl Acad Sci U S A 104:5977–5982

Rauch H, Montgomery I, Kaplan J (1985) Natural killer cell activity in multiple sclerosis and myasthenia gravis. Immunol Invest 14:427–434

Rice G, Casali P, Merigan T, Oldstone M (1983) Natural killer cell activity in patients with multiple sclerosis given a interferon. Ann Neurol 1983: 333–338

Richert J, Robinson E, Deibler G, Martenson R, Dragovic L, Kies M (1989) Human cytotoxic T-cell recognition of a synthetic peptide of myelin basic protein. Ann Neurol 26:342–346

Roncarolo M, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings M (2006) Interleukin-10-secreting type I regulatory T cells in rodents and humans. Immunol Rev 212:28–50

Sakuishi K, Oki S, Araki M, Porcelli S, Miyake S, Yamamura T (2007) Invariant NKT cell biased for IL-5 production act as crucial regulators of inflammation. J Immunol 179:3452–3462

Santoli D, Hall W, Kastrukoff L, Lisak R, Perussia B, Trinchieri G, et al (1981) Cytotoxic activity and interferon production by lymphocytes from patients with multiple sclerosis. J Immunol 126:1274–1278

Saraste M, Irjala H, Airas L (2007) Expansion of $CD56^{bright}$ natural killer cells in the peripheral blood of multiple sclerosis patients treated with interferon-b. Neurol Sci 28:121–126

Screpanti V, Wallin R, Grandien A, Ljunggren H (2005) Impact of FASL-induced apoptosis in the elimination of tumor cells. Mol Immunol 42:495–499

Shi F, Van Kaer L (2006) Reciptocal regulation between natural killer cells and autoreactive T cells. Nat Rev Immunol 6:751–760