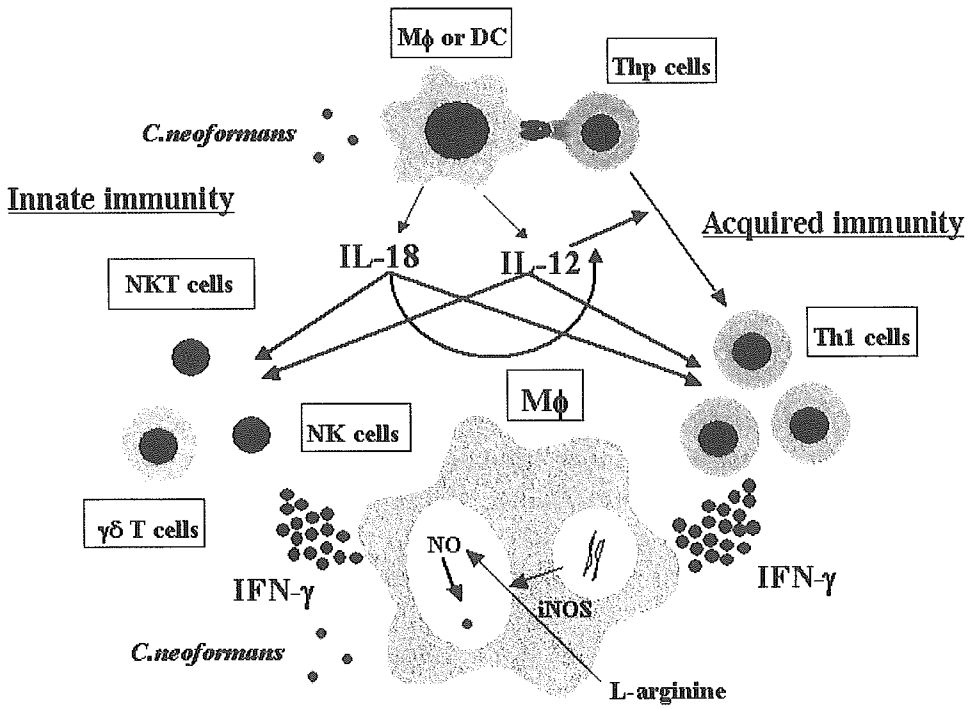
encoding caspase-1, which processes pro-IL-1β and pro-IL-18 into their biologically active forms [33]. This mutation did not affect the susceptibility to primary infection, but highly impaired Th1-mediated protection from re-infection with *C. albicans*, which was correlated with reduced production of IL-12 and IFN-γ. Administration of exogenous IL-18 restored the impaired host resistance by increasing IFN-γ synthesis. Based on these results, it was concluded that caspase-1-dependent production of IL-18 was important for the development of Th1-mediated host resistance to re-infection, but not to primary infection, with *C. albicans*.

Cryptococcus neoformans, a yeast-type fungal pathogen which has recently been found to reside within macrophages [34], causes life-threatening meningo-encephalitis in severely immunocompromised hosts such as patients with AIDS. Host defense to cryptococcal infection is mediated mainly by cellular immunity [32]. CD4+ T cells play a central role in eradicating this fungal pathogen from the lung and preventing its disseminated infection to the central nervous system [35-37]. Th1-type cytokines, such as IFN-γ, TNF-α and IL-12 [38-40], are essential for protection against *C. neoformans*, and administration of exogenous cytokines makes hosts resistant to infection [41-43]. In contrast, Th2 cytokines, such as IL-4 and IL-10, are considered to exert antagonistic effects on Th1-mediated host resistance against infection with *C. neoformans* [40,44].

In our recent investigation [45], we compared host resistance against infection with *C. neoformans* and the delayed-type hypersensitivity (DTH) response to this pathogen, between wild-type (WT) and IL-18 deficient (IL-18-/-) mice. Lung clearance of the microorganism was impaired in the latter, compared to the former group, although there was no significant difference in disseminated infection to the brain between the two groups. The DTH response, as evaluated by hind footpad swelling, was also diminished in IL-18-/- mice compared to WT mice. Serum levels of IL-12 and IFN-γ were significantly lower in IL-18-/- mice than in control mice. Spleen cells obtained from infected IL-18-/- mice produced a smaller amount of IFN-γ upon stimulation with *C. neoformans* than similarly-stimulated spleen cells from infected WT mice. Administration of IL-18 almost completely restored this response in IL-18-/- mice, while IL-12 exhibited a marginal effect. Thus, IL-18 plays an important role in eliminating *C. neoformans* from the lungs by potentiating IFN-γ production.

In our next series of studies [46], we examined the contribution of IL-18 to the host defense against *C. neoformans* in mice with defective IL-12 production, to exclude the possible influence of the latter cytokine. Experiments were conducted in IL-12-/- mice, in which host resistance was impaired, as shown by the increased number of organisms in both lungs and brains, compared to control mice. Serum IFN-γ was still detectable in these mice at a considerable level (20-30% of that in control mice). The host resistance was moderately impaired in

IL-12-/- mice compared to IFN-γ-/- mice. Neutralizing anti-IFN-γ mAb further increased the lung burdens of *C. neoformans*. In addition, treatment with neutralizing anti-IL-18 Ab almost completely abrogated the production of IFN-γ and also impaired the host resistance. Host resistance in mice with targeted disruption of the genes for both IL-12 and IL-18 (IL-12-/-IL-18-/- mice) was more severely impaired than in IL-12-/- mice. Spleen cells obtained from infected IL-12-/- mice did not produce any IFN-γ upon restimulation with *C. neoformans*, while those from infected and IL-12-treated mice did produce IFN-γ. In contrast, IL-18 did not exert such an effect. Finally, depletion of NK cells by anti-asialo GM1 Ab largely abrogated the residual production of IFN-γ in IL-12-/- mice. Thus, IL-18 was demonstrated to contribute to the host resistance against cryptococcal infection by inducing IFN-γ production by NK cells and potentiating the development of Th1 cells caused by IL-12, as summarized in Figure.



Figur 1. Role of IL-18 in the host protective response to *C. neoformans* *C. neoformans*-infected macrophages (Mϕ) and dendritice cells release IL-12 and IL-18. Both cytokines act on the innate immune cells, such as NK cells, to induce IFN-γ at early phase of infection (innate immunity). Precursor Th (Thp) cells specific for cryptococcal antigens proliferate and differentiate into Th1 cells under microenvironment in which IL-12 is secreted. IL-18 strongly promotes this response. Th1 cells secrete a large amount of IFN-γ upon stimulation with IL-12 and IL-18 (acquired immunity). IFN-γ in turn increases macrophage fungicidal activity against *C. neoformans* via nitric oxide (NO)-dependent mechanism. iNOS: inducible nitric oxide synthase.

Protozoa

The role of IL-18 in host resistance to *Leishmania major* infection was elucidated using IL-18-/- mice in three independent studies. Two studies concurred on a positive role for this cytokine, while another study disagreed. Wei *et al.* [19] demonstrated increased susceptibility of IL-18-/- mice to *L. major* infection, which paralleled reduced IFN-γ synthesis and elevated production of IL-4. Similar results were reported by Ohkusu *et al.* [47] who also showed a synergistic effect of IL-18 with suboptimal IL-12, which was sufficient to cause expression of the IL-18 receptor, in inducing IFN-γ and nitric oxide and protecting mice from infection. In contrast, Monteforte and co-workers [48] did not observe a reduced Th1 response and host resistance to *L. major* infection in IL-18-/- mice, although the clearance of parasites was sometimes delayed. At present, this discrepancy has not been reasonably explained.

Early host resistance to *Toxoplasma gondii* infection requires IL-12, which stimulates IFN-γ production by NK cells [49]. Cai and co-workers [50] elucidated the role of IL-18 in this response by administering neutralizing anti-IL-18 Ab or recombinant IL-18 to *T. gondii*-infected SCID mice. Although anti-IL-18 Ab did not affect the resistance, administration of exogenous IL-18 resulted in increased IFN-γ production, reduced parasite burdens and extension of survival time. Such a protective effect was entirely dependent on IL-12 and IFN-γ. Thus, IL-18 was demonstrated to potentiate IL-12-mediated resistance to *T. gondii* infection, although endogenously synthesized IL-18 was likely to have only a limited role in innate host resistance.

In unpublished observations by Okamura *et al.* [2], higher levels of IL-18 were detected in mouse serum after infection with *Plasmodium berghei.* IL-18 is likely to be important in host resistance to this parasite, as neutralizing anti-IL-18 Ab shortened the survival time of infected mice. They further observed that IL-18 markedly diminished mortality of mice infected with *P. berghei* when administered with low doses of IL-12. Similar results were also reported by Singh *et al.* using IL-18-gene knockout mice [51]. These results suggest an important role for IL-18 in host protection against plasmodial infection.

Viruses

Fujioka and co-workers [52] reported that IL-18 treatment protected mice from acute viral infection with herpes simplex virus type 1 (HSV-1). The effect was observed both in nude and SCID mice, indicating the involvement of innate immunity. In their study, however, depletion of NK cells and nitric oxide synthesis did not have a major influence on IL-18-induced protection, which was strongly, but partially, abrogated by a genetic defect of IFN-γ synthesis. The

investigators therefore concluded that IL-18 promoted protection of mice from HSV-1 via both IFN-γ-dependent and -independent mechanisms.

A recent investigation by Xing *et al.* [53] compared the contribution of two IFN-γ-inducing cytokines, IL-12 and IL-18, to the development of the Th1 response in mice that were infected intranasally with adenovirus. Neither IL-12 nor IL-18 was essential for induction of virus-specific Th1 cells in draining lymph nodes and spleen, implicating other cytokines in this process. In contrast, IL-18, but not IL-12, was required for optimal IFN-γ release in primary infected organs. These results suggest that IL-18 may contribute to IFN-γ-mediated host resistance against adenovirus infection.

In a recent study by Pien *et al.* [54] using gene-disrupted mice, IL-18 was found to have no major contribution to IFN-γ production in the liver or host protection from infection with mouse cytomegalovirus. This was in a sharp contrast to IL-12, although IL-18 was essential for IFN-γ production in the serum and spleen. Thus, the role of IL-18 in host protective response to this virus is likely limited.

Potential use of an immunotherapy with IL-12 and IL-18 against intractable infectious diseases

Possible therapeutic effects of IL-18 in infectious diseases

In animal studies, many investigators have demonstrated the effectiveness of IFN-γ, TNF-α and IL-12 in enhancing the host defense activity against a variety of infectious pathogens. As with IL-18, only a few studies have described the usefulness of this cytokine in the treatment of infectious diseases [50-52].

Our findings that IL-18 is important in the host defense to *C. neoformans* [45, 46] suggests its potential use clinically against this infection. In this regard, we examined the effects of IL-18 in a mouse model of pulmonary and disseminated infection with a highly virulent strain of *C. neoformans* [55]. Administration of murine recombinant IL-18, similarly as IL-12, enhanced elimination of live microorganisms from the lungs, prevented fungal dissemination to the brain and increased the survival rate of infected mice, although its effectiveness was apparently less than that of the latter cytokine [43]. Histological examination of lung sections of infected and untreated mice revealed a poor cellular inflammatory reaction and a large number of multiplying yeast cells in alveolar spaces. In contrast, massive infiltration of inflammatory cells, consisting mainly of mononuclear cells, characterized sections of lungs of infected mice treated with IL-18. Treatment with IL-18 also increased the level of serum IFN-γ. In addition, the protective effect of IL-18 against cryptococcal infection was abrogated by administration of neutralizing

anti-IFN-γ mAb [55]. Thus, our results support the possible application of IL-18 for clinical treatment of immunocompromised patients with intractable infectious diseases caused by *C. neoformans.*

IL-12 and IL-18

In vitro studies by Okamura et al. [1] and Micallef *et al.* [56] demonstrated a synergistic effect for IL-12 and IL-18 in inducing the production of IFN-γ by both murine and human T cells. Yoshimoto *et al.* [8] found that murine splenic B cells produced a high level of IFN-γ following stimulation with IL-12 plus IL-18 and in vivo administration of these cytokines inhibited the production of IgE by inducing IFN-γ production probably by B cells. A recent study by Munder *et al.* [9] demonstrated that murine bone marrow macrophages also produced biologically active IFN-γ upon combined stimulation with these two cytokines. Although the mechanisms of these synergistic actions of IL-12 and IL-18 are not yet completely understood, one possible explanation could be the increased expression of IL-18 receptor on the responding cells caused by IL-12 [57]. In other studies, the signal transduction pathways were found to be quite different between IL-12 and IL-18, which are mediated by Stat3 and Stat4 and NFκB followed by IRAK [5,58-60], respectively. Finally, these two cytokines are likely to act on different binding sites of the promoter region of IFN-γ through different DNA binding proteins [61]. These observations may at least in part explain the synergism of IL-12 and IL-18.

In a series of studies, we have also demonstrated that IL-12 and IL-18 synergistically induced the production of IFN-γ by cultured NK cells and augmented the fungicidal activity of peritoneal exudate cells against *C. neoformans* [6]. Furthermore, in our in vivo study [62], such combined treatment was therapeutically effective against a fatal form of murine infection caused by *C. neoformans*, as indicated by reduced fungal burdens in the lungs and brains and prolonged survival of infected mice, while the use of either IL-12 or IL-18 alone did not result in such effect. The in vivo effect was mediated by the local production of IFN-γ not only by NK but also by γδ T cells in the lungs. The latter cells are also known to produce IFN-γ in response to stimulation by IL-12 [62]. Thus, treatment of mice with experimentally-induced pulmonary cryptococcosis using IL-12 and IL-18 synergistically induced the production of IFN-γ by NK and γδ T cells and improved the course of infection. Similar findings were reported in animal models of other infectious diseases by Okamura and coworkers [2,47]. Combined treatment using these two IFN-γ-inducing cytokines diminished the mortality of mice infected with *L. major* or *P. berghei.*

Because in our latter study, subtherapeutic doses of IL-12 and IL-18 were also effective when used in combination, the use of reduced dose of each

cytokine may produce a protective effect. On the other hand, the use of higher doses increased the risk of adverse effects such as the development of wasting disease [63]. In this regard, although the combined administration of IL-12 and IL-18 may be clinically useful in the treatment of immunocompromised patients with severe disseminated cryptococcosis, the undesired effects of these two cytokines must be controlled before clinical application.

Potential complications with cytokine therapy

With the discovery of newer cytokines in recent years, the potential use of cytokines for clinical therapy of infectious diseases is now considered by many investigators. The important issue that interferes with the development of such novel therapy is related to the side effects. For examples, IL-12 is reported to induce bone marrow suppression, hepatotoxicity, skeletal muscle necrosis and fluid leak with pleural effusion and ascites in mice, although it gives many beneficial effects on infectious diseases [64]. As discussed above, the combined use of IL-12 and IL-18 is likely to produce a potent effect on host defense against infection, because these cytokines synergistically induce the production of IFN-γ when used in combination [1, 2, 4, 6-10]. Furthermore, these studies suggest that the dose of both cytokines can be reduced when used together, compared to that used in a single treatment. This may help to reduce the development of serious side effects. In preliminary studies in our laboratory, we have identified, however, that administration of high doses of these cytokines in animals is associated with the development of serious side effects including wasting disease, and even death of some mice. Okamura and coworkers [65] injected high doses of IL-12 and IL-18 in mice and reported the development of serious pathological changes or manifestations, such as severe diarrhea and rapid weight loss. Histopathological examination of the affected tissues showed hemorrhagic erosion in the intestine and colon, fatty degeneration of the liver, necrotic change in the pancreas and marked atrophy of the thymus. These changes are thought to be due to Fas-independent apoptosis [65]. Thus, these problems definitely need to be resolved before the cytokine combination therapy can be clinically used.

Concluding remarks

Major efforts for overcoming infectious diseases have been made to develop stronger chemical "weapons" to defeat resistant pathogenic microorganisms. The history of development of these drugs indicates that antimicrobial agents do not always overcome the offending microbes because of the emerging of drug-resistant microorganisms. Moreover, the recent increase in the number of immunocompromised hosts has largely changed the aspects of infectious diseases. On the other hand, recent progresses in immunology and genetic

engineering have allowed the discovery of a number of new cytokines. Several reports using animal models have confirmed the effectiveness of these cytokines against various infectious diseases. Thus, I believe that it is now the appropriate time to consider the development of new chemotherapeutic agents as well as novel immunotherapy to improve the depressed host defense mechanisms. In this regard, IL-18 could be a promising immunotherapeutic agent to overcome the intractable infectious diseases, although further investigations need to be conducted to resolve the issues of adverse effects, which have prevented the progress of the novel therapy with immunomodulating agents.

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6 α-Galactosylceramide: NKT cell-based immunotherapy in intractable infectious diseases

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Introduction

Natural killer T (NKT) cells comprise a unique population of lymphocytes characterized by co-expression of both T cell antigen receptor and NK markers, including CD161 (NK1.1) and CD122 (IL-2Rβ) [1-4]. Specific features of this cell type include expression of a single invariant a chain of antigen receptor encoded by a rearranged Vα14-Jα281 (or Jα18) gene segment coupled with a highly skewed β chain, such as Vβ8.2, Vβ7 or Vβ2 in mice [1-4]. These cells are found in large numbers in the liver, thymus and bone marrow, and in low numbers in the spleen and lung [2]. NKT cells play an important role

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in various aspects of the regulation of allergic and autoimmune diseases [5-24], prevention of tumor metastasis [25-34], and protection against microbial infection [35-57]. α-Galactosylceramide (α-GalCer), a synthetic glycolipid originally isolated from a marine sponge, is recognized in a specific manner by Vα14+ NKT cells, which results in the prompt and pronounced synthesis of both gamma interferon (IFN-γ) and interleukin-4 (IL-4) [1-4, 58-60].

In this review, we introduce the characteristics and specific ligands of NKT cells and discuss their roles in host defenses against neoplastic cells and infectious microbial pathogens, and in the development of autoimmune diseases. We will also consider the therapeutic effects of α-GalCer treatment and its possible clinical application in these pathogenic conditions.

Ligands of NKT cells

CD1d

In 1995, Bendelac and co-workers [62] demonstrated that Vα14+ NKT cells recognized CD1, a family of non-polymorphic cell surface glycoproteins [61]. CD1 proteins are divided into two groups; group I (CD1a, -b, -c) and group II (CD1d). However, group I CD1 proteins are not expressed in mice and rats. Human and mouse CD1d share significant structural homology. Although the crystal structure is similar to that of MHC class I, which is dimerized by the α chain and β2 microglobulin, the antigen-binding groove of CD1d is narrow and deep, compared to MHC class I, and its bottom surface is comprised largely of hydrophobic amino acids [63]. This structure may be convenient for binding to lipid antigens. Most NKT cells are thought to be positively selected by the CD1d molecule, because the development of NKT cells is suppressed in CD1d-deficient [64-66] or β2 microglobulin-deficient mice [67-69].

Glycolipids

The microbial antigen for NKT cells remains to be identifined. Applying analyses, Joyce and colleagues [70] suggested that glycosylphosphatidylinositol (GPI) was an endogenous molecule that coupled with mouse CD1d. In addition, Schofield *et al.* [71] suggested that Vα14+ NKT cells recognized GPI-anchored protozoan antigens, with subsequent promotion of antibody formation by B cells against these antigens. However, contradictory findings were then reported by Molano and co-workers [72]. Recently, Gumperz *et al.* [73] demonstrated that NKT cells could recognize phospholipids as potential self-antigens presented by CD1d.

α-Galactosylceramide

Kawano and co-workers [58] demonstrated that Vα14+ NKT cells recognized α-galactosylceramide (α-GalCer), a synthetic glycolipid originally

isolated from a marine sponge, in context with CD1d. α-GalCer contains an α-anomeric sugar moiety with a long fatty acyl chain and sphingosine base [1,58]. Antigen receptor-mediated recognition of α-GalCer is followed by prompt secretion of large amounts of both IFN-γ and IL-4 and induction of cytolytic activity against tumor cells. They also demonstrated that α-GalCer activation of NKT cells requires co-stimulatory signals through B7-CD28 and CD40-CD40L interactions. IL-12 from dendritic cells (DCs) is also required for NKT cells to produce IFN-γ in response to α-GalCer [74]. In addition, direct contact between NKT cells and DCs through CD40/CD40 ligand interactions is necessary. Moreover, α-GalCer strongly upregulates the expression of IL-12 receptor on NKT cells. Human Vα24+ NKT cells also recognize the same glycolipid presented by CD1d and express similar responses [75,76].

Effect of NKT cells on the Th1-Th2 balance

Based on their ability to produce a large amount of IFN-γ and IL-4 by TCR engagement [1-4], Vα14 NKT cells have been suggested to have a regulatory role in Th1-Th2 differentiation. In earlier studies [77], Yoshimoto *et al.* [77] suggested the involvement of NKT cells as possible producers of IL-4 during Th2 cell differentiation. Consistent with this, they found that β2 microglobulin-deficient mice were unable to produce IgE in response to anti-IgD treatment. However, several groups have reported data that conflict with this supposition: NKT cell development and early IL-4 production were impaired, but antigen-specific Th2 responses were generated in CD1-deficient [65-67] and β2 microglobulin-deficient mice [78-80]. Thus, Vα14 NKT cells are likely to be dispensable for Th2 differentiation.

Which response does α-GalCer cause: Th1 or Th2? Some studies showed that repeated treatment with α-GalCer *in vivo* led to an enhanced Th2 response [81,82]. In contrast, Cui *et al.* [83,84] demonstrated that Vα14 NKT cells inhibited Th2 cell differentiation and IgE production by producing a large amount of IFN-γ after treatment with α-GalCer. In our recent study [29], activation of Vα14 NKT cells by α-GalCer resulted in the development of a Th1 response in mice infected with *Cryptococcus neoformans*. Jahng and colleagues [20] reported interesting results using a mouse model of experimental autoimmune encephalomyelitis (EAE). Co-immunization with α-GalCer and myelin basic protein (MBP) promoted a Th1 response, whereas prior treatment with α-GalCer resulted in decreased IFN-γ production and attenuation of EAE. At present, the explanation for these discrepant results in association with different schedules of α-GalCer treatment remains obscure.

Regulatory roles of NKT cells in autoimmune diseases

Collagen diseases

Sumida and colleagues [5] demonstrated that invariant Vα24JαQ (or Jα18) TCR expression was selectively reduced in systemic sclerosis (SSc) patients compared to healthy controls. Vα24JαQ+CD4$^-$CD8$^-$ double-negative (DN) T cells were absent in these patients, although such unconventional T cells expanded in healthy individuals. Based on these results, they speculated that the selective reduction of Vα24JαQ+ NKT cells might be associated with the development of autoimmune disease. Similarly, DN NKT cells were reported to be significantly decreased in the peripheral blood or rheumatoid synovial fluid from patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [6-8]. Recently, Kojo *et al.* [9] reported the mechanism of the selective reduction of Vα24+DN NKT cells in patients with autoimmune diseases. They observed that the number of Vα24Vβ11+DN NKT cells was significantly reduced in peripheral blood of patients with RA, SLE, SSc, and Sjögren syndrome (SS) compared with that in healthy controls. Because about half of these patients responded to α-GalCer, they suggested that the amount of natural ligands might be insufficient and that this might be the reason for the decreased number of NKT cells. Thus, they considered that α-GalCer might be an attractive tool for treating autoimmune disease.

Diabetes mellitus

Non-obese diabetic (NOD) mice are used as an animal model for type 1 diabetes (T1D) [10]. These mice have both numerical and functional defects in NKT cells [10-13]. This deficiency may lead to T1D, because overexpression of transgenes for Vα14 and Vβ8.2 or transfer of NKT cells protects mice from diabetes through production of IL-4 and/or IL-10 [14,15]. Recently, three groups demonstrated that α-GalCer treatment protected NOD mice from T1D in a CD1d-dependent manner [16-18]. The mechanism of prevention of the disease by α-GalCer is considered to be the suppression of IFN-γ (but not IL-4) production by NKT cells. Since Wilson and colleagues [19] reported that Vα24JαQ+ NKT cells showed an extremely biased Th1 response in T1D patients, α-GalCer may be applicable for clinical therapy of human T1D.

Experimental autoimmune encephalomyelitis

EAE is considered to be an instructive model for human multiple sclerosis (MS). Two groups demonstrated that α-GalCer treatment resulted in reduction of IFN-γ production and prevention of development of EAE [20,21]. This protection was dependent upon the secretion of IL-4 and IL-10. However, in a study by Jahng *et al.* [20], co-administration of α-GalCer and myelin basic

protein (MBP) led to enhancement of the Th1 response and exacerbation of EAE. Based on these discrepant results, it remains to be substantiated whether NKT cell-based therapy with α-GalCer is beneficial in this disease. In this regard, an interesting study was reported by Miyamoto *et al.* [22], who succeeded in making an analog of α-GalCer, named OCH, that induced predominant production of IL-4 by NKT cells and suppressed the development of EAE. The number of Vα24+ NKT cells is greatly reduced in the peripheral blood of MS patients, compared with that in other autoimmune neurological disorders, such as chronic inflammatory demyelinating polyneuropathy [23]. Therefore, NKT cell activating agents might provide useful and clinically effective treatments for MS patients.

Inflammatory bowel diseases

Saubermann and co-workers [24] examined the effect of α-GalCer on dextran sodium sulfate (DSS)-induced colitis to investigate the role of NKT cells in the development of inflammatory bowel diseases. They demonstrated that α-GalCer injection or adoptive transfer of NKT cells preactivated by α-GalCer led to significant improvement of clinical symptoms and survival time in DSS-induced colitis. These data suggested that NKT cells functioned as critical regulators in inflammatory bowel conditions.

NKT cells and tumor rejection

Role of NKT cells in IL-12-mediated tumor rejection

Systemic administration of IL-12 activates hepatic NKT cells and inhibits the metastasis of colon tumors to the liver and lung [25,26]. Cui and co-workers [27] elegantly demonstrated that Vα14+ NKT cells are required for IL-12-mediated tumor rejection. They used mice with a disruption of Jα281 gene segment, which were also deficient for Vα14+ NKTcells, and mice with a deletion of the RAG-1 gene and overexpression of transgenes for Vα14 and Vβ8.2 ($RAG^{-/-}V\alpha14^{tg}V\beta8.2^{tg}$), which lacked antigen receptor αβ-bearing T cells (except for Vα14+ NKT cells). They found that IL-12 treatment inhibited tumor growth and metastasis in the liver of wild-type and $RAG^{-/-}V\alpha14^{tg}V\beta8.2^{tg}$ mice, but not in the Jα281-/- mice. Thus, Vα14+NKTcells were found to be an essential target of IL-12 for tumor rejection.

Rejection by NKT cells activated by α-GalCer

Taniguchi and coworkers [28] reported that α-GalCer selectively activated Vα14+ NKT cells, resulting in prevention of tumor metastasis. α-GalCer-activated Vα14+ NK T cells mediated their cytotoxicity in a perforin-dependent manner. The same group further investigated whether α-GalCer-pulsed DCs

were effective in eliminating the established metastatic tumor foci. Injection of α-GalCer-pulsed DCs effectively diminished the tumor, even after multiple metastatic nodules were already formed [29]. Nakagawa *et al.* [30] demonstrated that NK cells were the principal effectors for the antimetastatic action of α-GalCer in the liver. α-GalCer-activated NKT cells increased both the innate anti-tumor cytotoxicity of NK cells and the adaptive anti-tumor response of $CD8^+$ T cells through the production of IFN-γ, resulting in inhibition of hepatic tumor metastasis. Recently, Smyth *et al.* [31] also reported that IL-12, IFN-γ and NK cells were necessary for the anti-metastatic effect of α-GalCer treatment in the lungs and the liver. Whereas IL-18 was also required for optimal serum IFN-γ production and played a role in the control of lung metastasis by α-GalCer, this cytokine seemed to be unnecessary for α-GalCer-induced suppresion of liver metastasis.

Kawano and colleagues [32] demonstrated that human Vα24+ NKT cells acquired a potent perforin-dependent cytotoxic activity against human tumor cell lines by *in vitro* stimulation with α-GalCer. Metelitsa *et al.* [33] investigated the mechanism of the cytotoxic property of human NKT cells. They showed that, upon α-GalCer recognition, NKT cells mediated antitumor cytotoxicity by recognizing target cell CD1d bound with α-GalCer or indirectly by producing IL-2 to activate NK cells. It has been reported that Vα24+ NKT cells and DCs from melanoma patients were functionally normal, irrespective of the tumor-bearing status [32]. Recently, another group reported that Vα24+ NKT cells in unfractionated PBMCs obtained from cancer patients had a lower proliferative response to α-GalCer *in vitro* [34]. However, they found that these cells could efficiently respond to α-GalCer when fractionated by sorting or by adding G-CSF to the culture of unfractionated PBMCs. Thus, some unknown immunosuppressive factor (for example, TGF-β) was speculated to suppress the function of NKT cells in cancer patients.

Role of NKT cells in host defense against microbial infection

Microbial infectious pathogens are categorized into three groups based on the contribution of NKT cells to the host defense [35]. The first group, in which NKT cells have a protective role against infection, includes *Leishmania major*, *Trypanosoma cruzi*, *Plasmodium yoelli* and *P. berghei*, *Borrelia burgdorferii*, Hepatitis B virus, diabetogenic encephalomyocarditis virus (EMCV-D), *C. neoformans* and *Pseudomonas aeruginosa* [36-45]. For the second group, including *Listeria monocytogenes* and *Toxoplasma gondii*, NKT cells have a suppressive role in the host defense against these pathogens [46-48]. *Mycobacterium tuberculosis*, *M. bovis* BCG and *Salmonella choleraesuis* are

classified into the third group, in which NKT cells show a minimal contribution [49-54]. Thus, the role of NKT cells seems to be different between pathogens. These relationships are summarized in Table.

Table. NKT cells and infection

Role of NKT in Host defense to infection	Microbes	α-GalCer	anti-CD1d, CD1KO or Jα281KO	Mechanism (key factor)	References
Protective	*L. major*	-	Exacerbated	HSP65 expression ↓	36
	B. burgdorferi	-	Exacerbated	Specific IgG2a production ↑	38
	T. cruzi	-	Exacerbated	-	37
		Protected	-	IFN-γ dependent	41
	P. yoelii (&P. berghei)	-	Exacerbated	-	39
		Protected	-	IFN-γ dependent	43
	EMCV-D	Protected	Exacerbated	-	40
	HBV	Protected	-	IFN-γ and IFN-α/β synthesis ↑	42
	P. aeruginosa	Protected	Exacerbated	IFN-γ and TNF-α synthesis ↑	45
	C. neoformans	-	Exacerbated	Th1 response ↓	57
		Protected	-	IFN-γ, IL-12 dependent ↓	55,56
Minimally contributed	*M. tuberculosis*	-	Not Exacerbated	Not elucidated	50,51
		-	Minimally Exacerbated	IFN-γ, TNF-α, IL-12 ↓ TGF-β synthesis ↓	52
	M. bovis BCG	-	Not Exacerbated	IFN-γ synthesis →	53
	S. choleraesuis	-	Not Exacerbated	Liver injury ↓	54
Suppressive	*L. monocytogenes*	-	Protected	TGF-β synthesis ↓ IFN-γ, TNF-α, IL-12synthesis ↑	46
	T. gondii	-	Protected	HSP65 induction ↑	47,48

(-; not examined, ↑; increased, →; no difference, ↓; decreased)

Protective role

L. major and *T. cruzi* infections were exacerbated in Vα14+ NKT cell-deficient mice compared to control mice [37,38], and CD1dKO mice were more susceptible to *T. cruzi*, *B. burgdorferii*, *P. yoelli* and EMCV-D infection than control mice [38-41]. EMCV-D-induced encephalitis, myocarditis and diabetes were all improved by injection of α-GalCer [41]. The same treatment rendered mice resistant to infection with *T. cruzi* [42]. In this case, the protective effect of α-GalCer was independent of IL-12, but dependent upon IFN-γ produced by NKT cells. Furthermore, although a single α-GalCer treatment was sufficient to potentiate the host resistance to *T. cruzi* infection, repeated doses caused poor recovery from weight loss in the infected mice after parasitemia had resolved. Kakimi *et al.* [43] showed that α-GalCer-activated NKT cells inhibited hepatitis B virus replication in the liver of HBV-transgenic mice through the production of IFN-γ and IFN-α and -β. Gonzalez-Aseguinolaza *et al.* [44] clearly indicated

that the administration of α-GalCer induced strong anti-malarial activity, which inhibited the development of liver stage malaria caused by *P. yoelli* and *P. berghei*. The anti-malarial activity of α-GalCer was independent of IL-12, TNF-α, perforin and Fas/Fas ligand, but was dependent upon IFN-γ. In addition, NK, T and B cells were not required for the response. In the next series of experiments, the same group demonstrated that co-administration of α-GalCer with malaria vaccine potentiated the protective immunity by inducing IFN-γ-producing $CD8^+$ T cells in an antigen-specific manner [45]. Recently, it was reported that acute pneumonia caused by *P. aeruginosa* was exacerbated in CD1KO mice compared with control mice, associated with lower production of macrophage inflammatory protein-2 (MIP-2) and reduced numbers of neutrophils [46]. In this case, treatment of control mice with α-GalCer markedly enhanced the clearance of *P. aeruginosa* by increasing the production of IFN-γ and TNF-α in bronchoalveolar lavage fluid, and by increasing macrophage phagocytic activity.

Suppressive role

Treatment of mice with anti-CD1d mAb improved the clinical course of infection with *L. monocytogenes* through reduction of TGF-β production and increased synthesis of IFN-γ, TNF-α and IL-12 [47]. These data suggested a suppressive role for NKT cells in host protection against Listerial infection. Similar findings were observed by Nakano *et al.* [48,49] in *T. gondii* infection. Administration of anti-IL-2Rβ mAb enhanced the expression of heat shock protein 65 (HSP65) and IFN-γ mRNA, inhibited IL-4 mRNA expression, and ameliorated toxoplasmosis, while anti-asialo GM1 Ab treatment did not have any effect. The former treatment eliminated both NK and NKT cells, while only NK cells were depleted by the latter treatment. These observations led to a similar conclusion as for listerial infection.

Minimal contribution

Although it has been reported that NKT cells play an important role in the granulomatous response to mycobacterial cell wall components [50], host resistance to *M. tuberculosis* infection was not impaired in CD1KO mice compared to that in control mice [51,52], and was minimally affected by injection of anti-CD1d antibody [53]. In our study using mice lacking Vα14+ NKT cells, Th1-mediated responses and host protection to *M. bovis* BCG were not affected by deficiency of this particular lymphocyte subset [54]. Thus, we concluded that Vα14+ NKT cells played only a marginal role, if any, in host defense against *M. bovis* BCG infection. Ishigami *et al.* [55] demonstrated that Vα14+ NKT cells did not play a significant role in host protection against

infection with *S. choleraesuis*, although these cells may be a major effector population for liver injury in this infection.

Role of NKT cells in host defense against infection with *Cryptococcus neoformans*

Host defense mechanism against cryptococcal infection

C. neoformans, a yeast-like fungal pathogen with a thick polysaccharide capsule, causes granulomatous lesions in the lungs and hematogenously disseminates to the central nervous system, leading to fatal meningoencephalitis. Host resistance to this fungal pathogen is critically regulated by cellular immunity [85] and $CD4^+$ T cells play a central role in limiting infection [86-88], as predicted by the high morbidity rate observed in AIDS patients. The balance between Th1 and Th2 cytokines influences the outcome of infection [89]. Th1 cytokines, such as IFN-γ, TNF-α and IL-12, are essential for protection against *C. neoformans* infection [90-93], and administration of these cytokines increases host resistance [90,94-96]. In contrast, Th2 cytokines, such as IL-4 and IL-10, inhibit Th1-mediated host defense against this infection [93,97]. IL-12 is absolutely required for Th1 development and IL-18 potentiates this response [98]. We have demonstrated that both IL-12 and IL-18 play important roles in host defense and Th1 response to *C. neoformans* [99-102].

MCP-1-dependent accumulation of NKT cells in pulmonary cryptococcosis

In order to elucidate the role of NKT cells in host defense against cryptococcal infection, we first examined whether NKT cells ($CD3^+NK1.1^+$) accumulated in the lungs after infection with *C. neoformans* [55]. NKT cells constituted only 0.5-0.7% of lung lymphocytes before infection, but commenced increasing on day 1 (2.16%), reached a peak level on day 6-7 (3.48%) and thereafter decreased. NKT cells increased most profoundly at the infected sites, compared to other lymphocyte populations, T and NK cells, although these cells increased with similar kinetics as for NKT cells. Next, we examined the expression of Vα14 mRNA in the lungs and the proportion of α-GalCer-reactive cells, as detected using a α-GalCer-loaded CD1d tetramer, which has been reported to bind to Vα14+ NKT cells in a specific manner [103]. The expression of Vα14 mRNA and the proportion of α-GalCer-reactive cells increased in lungs. Thus, Vα14+ NKT cells were found to increase at the primary infected sites after pulmonary infection with *C. neoformans.*

How do NKT cells increase in the lungs after infection? Migration of inflammatory leukocytes is critically regulated by a variety of chemoattracting cytokines. NK cells are attracted by many chemokines, including MCP-1, -2, -3,