[Frontiers in Bioscience S1, 108-116, June 1, 2009]

Natural killer T cell-mediated immunotherapy for malignant diseases

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1. ABSTRACT

Human invariant Valpha24 Natural Killer T (NKT) cells are unique lymphocyte subsets, characterized by an invariant T-cell receptor Valpha24 chain paired with Vbeta11. Recent findings have highlighted the role of NKT cells in tumor immunity. Valpha24 NKT cells are activated by a specific glycolipid ligand, alpha-Galactosylceramide and rapidly produce high levels of cytokines upon stimulation, thereby modulating other immune cells such as NK cells antigen-specific $CD4^+$ and $CD8^+$ T cells and dendritic cells. Abnormalities in the numbers and functions of Valpha24 NKT cells have been observed in patients with various malignant diseases. Therefore, therapeutic strategies have recently focused on the reconstitution of an adequate number of functionally sufficient Valpha24 NKT cells which is thought to be logical and reasonable for cancer treatment. The quantitative alteration and functional impairment of circulating Valpha24 NKT cells are herein reviewed in various cancer-bearing patients and the progress to date in the clinical applications of NKT cell-based tumor immunotherapy is summarized.

2. INTRODUCTION

Natural killer T (NKT) cells co-express an invariant T-cell receptor (TCR), Valpha24Ja18 gene segment paired with Vbeta11 in human, together with NK markers and constitute a distinct lymphocyte subpopulation (1, 2). Unlike conventional $CD4^+$ and $CD8^+$ T cells that interact with peptide antigen presented on classical major histocompatibility complex (MHC) class I or II molecules, NKT cells respond to glycolipid antigen, alpha-Galactosylceramide (alpha-GalCer), in the context of the nonpolymorphic MHC class I-like antigen-presenting molecule CD1d (3-5). Alpha-GalCer (KRN7000) is a synthetic glycolipid, originally extracted from the marine sponge *Agelas mauritianus*. After activation with alpha-GalCer, NKT cells exhibit NK-like MHC-independent cytotoxic activity against various tumor cells through several cell-death-inducing mechanisms, including perforin/granzyme, Fas ligand, or TNF-related apoptosis-inducing ligand (TRAIL) (6-8). Furthermore, NKT cells contribute to a wide array of immune regulation due to a potent ability to produce a variety of cytokines.

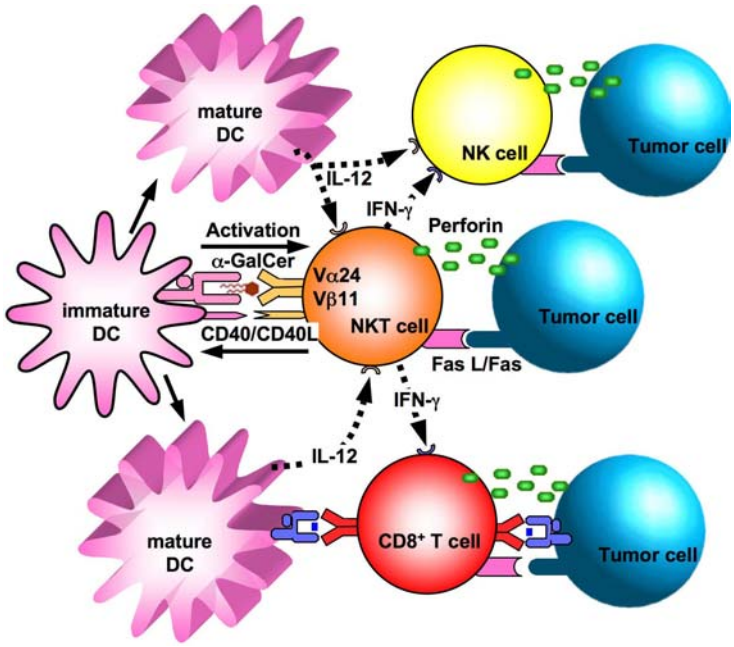

Figure 1. Direct antitumor effects and adjuvant effects of Valpha24 NKT cell in activation with alpha-GalCer. Invariant Valpha24Vbeta11 T cell receptor recognize glycolipid antigen, alpha-GalCer, in a CD1d dependent manner. After the recognition of alpha-GalCer, NKT cells activate and exert direct antitumor activity, including perforin/granzyme and FasL (Fas ligand). The upregulation of CD40L (CD40 lignad) on NKT cells stimulate CD40 on immature DC which induce mature DC that has a potent ability to produce IL-12. Large amounts of IFN-gamma secreted by NKT cells, induce NK cells and $CD8^+$ T cells to exert anti-tumor responses.

Crosstalk with other cell types, including DCs, NK cells, conventional T cells is one of the important aspects of activated NKT cells (9-11). The presentation of alpha-GalCer on immature DCs to NKT cells induces the CD40L expression on NKT cells and ligation with CD40 on DCs, thus leading immature DCs to mature while also augmenting the IL-12 production by mature DCs (12). IL-12 and IFN-gamma activate NK cells, which augment the NK-cell cytolytic function against tumors and IFN-gamma production. The full maturation of DC achieved by activated NKT cells induces a strong adaptive T-cell immunity. Therefore, NKT cells show the adjuvant effect on the antitumor immune responses by activating other cytotoxic effector cells (Figure 1).

At first, the antitumor effect of direct alpha-GalCer administration was observed in a liver or lung metastasis model (13, 14). Next, alpha-GalCer-pulsed DCs activated murine Valpha14 NKT cells and eradicated established metastatic tumor foci in models of the mouse liver and lung metastasis, thereby suggesting that the administration of alpha-GalCer-pulsed DCs may exert a greater antitumor activity than the direct administration of alpha-GalCer (15). Alpha-GalCer was administered into mice by loading on DCs instead of free alpha-GalCer. Then a prolonged and marked production of IFN-gamma by NKT cells was observed, as well as a greater resistance to the metastases of B16 melanoma was induced (16). The intravenous injection of alpha-GalCer-pulsed DCs led to the activation and expansion of endogenous lung Valpha14 NKT cells (17). These findings in murine models suggest that similar antitumor effects after the activation of intrinsic Valpha24 NKT cells could thus be expected in cancer bearing condition when alpha-GalCer-pulsed DCs are administered intravenously. Based on these murine *in vivo* effects of alpha-GalCer-pulsed DCs, we designed a clinical study was designed using alpha-GalCer-pulsed autologous DCs aimed to activate endogenous Valpha24 NKT cells in patients with lung cancer.

This review summarizes the results of alteration of human Valpha24 NKT cells in cancer patients. In addition, the findings of recent immunotherapies targeted on the NKT cell immune system are discussed.

3. PROPERTIES OF HUMAN VALPHA24 NKT CELLS

3.1. Functional monitoring of Valpha24 NKT cells

In the peripheral blood, Valpha24 NKT cells constitute very small population in the mononuclear cells, which interrupt the precise analysis of freshly isolated peripheral blood Valpha24 NKT cell function. Since Valpha24 NKT cells can be expanded *in vitro* in the presence of alpha-GalCer and cytokine such as IL-2 (18), quantitation of Valpha24 NKT cell function was reported after short-term *in vitro* expansion, which might modify their original properties. To improve these aspects, a novel enzyme-linked immunospot (ELISPOT) assay was introduced to monitor NKT cell function in freshly isolated

human PBMCs (19). Valpha24 NKT cells in most healthy donors are capable of producing IFN-gamma, which can be detected in primary assays using PBMCs in the alpha-GalCer stimulation. It was clearly shown that the number of IFN-gamma spots correlates with the frequency of Valpha24 NKT cells and depends on $CD3^+$, $Valpha24^+$ and $Vbeta11^+$ cells. However, no IL-4 production is detected, thus indicating that freshly isolated Valpha24 NKT cells have a Th1 profile. In this context, this simple procedure was applied with a modification to monitoring of NKT cell immune therapy to quantify the frequency of functional Valpha24 NKT cells (20). IFN-gamma spot forming cells are comprised of both Valpha24 NKT cells and NK cells when endogenous NKT cell activation with alpha-GalCer-pulsed DCs or adoptive transfer of *in vitro* activated NKT cells are performed, since alpha-GalCer-activated Valpha24 NKT cells also subsequently stimulate NK cells to produce IFN-gamma (10, 11).

Shimizu *et al*. reported a method to investigate the function of human Valpha24 NKT cells using a xenogenic combination of mouse DCs, because dysfunction of antigen presenting cells in often exists in patients (21). The CD1d molecule is highly conserved in mammalian, human Valpha24 NKT cells can respond to mouse CD1d and murine Valpha14 NKT cells to respond to human CD1d (6, 22). This method should therefore greatly benefit studies for evaluating the function of Valpha24 NKT cells in patients with different types of cancer.

3.2. Valpha24 NKT cells in cancer bearing condition

Various studies have demonstrated a deficiency of Valpha24 NKT cells in cancer. In patients with primary lung cancer (n=60), the number of circulating Valpha24 NKT cells significantly decreases independent of tumor progression (22). In addition, the proportion of IFN-gamma producing NKT cells in the PBMCs is preserved. Molling *et al.* documented that in a total of 120 patients with various types of malignant neoplasms, the number of circulating Valpha24 NKT cells significantly decreased, and this decrease was not recovered after tumor removal (23). The number of alpha-GalCer-reactive IFN-gamma producing cell detected by ELISPOT assay did not decrease, and these findings were compatible with the previous results. Therefore due to a reduced number of circulating NKT cells, cancer-bearing patients have decreased absolute number of IFN-gamma-producing NKT cells, even though the population of IFN-gamma-secreting NKT cell was normal.

In patients with glioma (n=9), the number of circulating Valpha24 NKT cells and the IFN-gamma-producing function are both preserved (24). The preservation of alpha-GalCer-reactive IFN-gamma production capacity in fresh PBMCs is detected by an ELISPOT assay, and the expanded NKT cells with autologous alpha-GalCer-loaded mature DCs were also capable of secreting IFN-gamma. In patients with advanced prostate cancer (n=6), peripheral blood NKT cells are conspicuously observed to decrease. In addition to the decreased proliferative response of alpha-GalCer-reactive NKT cells, IFN-gamma production by *in vitro* cultured NKT cells after stimulation with alpha-GalCer is also observed to remarkably decrease (25). Yanagisawa et al. reported that the proportions of circulating NKT cells from different types of advanced cancer patients (n=21) are preserved, NKT cells failed to proliferate after alpha-GalCer stimulation, which partially restores the granulocyte colony-stimulating factor (G-CSF), though the amelioration effect is very limited (26). Konishi et al. demonstrated that circulating Valpha24 NKT cells significantly decrease in recurrent lung cancer patients (n=55) (27). They also displayed a poor expansion rate when cultured with alpha-GalCer, which is in part improved by the addition of G-CSF in the culture medium.

The findings in patients with myelodysplastic syndromes (MDS) demonstrate a quantitative deficiency in Valpha24 NKT cells, but not in either NK cells or CD4+ or CD8+ T cells (28). The paucity of IFN-gamma-producing cells in response to alpha-GalCer and poor expansion rate of NKT cells using alpha-GalCer-loaded autologous DCs is seen in both the peripheral blood and bone marrow. Valpha24 NKT cells from patients with progressive multiple myeloma (MM), but not non-progressive MM or premalignant gammopathy, have a marked deficiency in their alpha-GalCer-dependent IFN-gamma production, even though the frequency of Valpha24 NKT cells are not decreased with disease progression (29).

Valpha24 NKT cells in cancer patients have some numerical and functional modifications and the potential mechanism underlying the quantitative and functional changes in Valpha24 NKT cells are unclear at present. Therapeutic strategies, however, focus on the reconstitution of an sufficient number of functional Valpha24 NKT cells by the adoptive transfer of *in vitro* activated NKT cells, since an increased number of peripheral blood or intratumor NKT cells have been shown to correlate with a better survival (30, 31).

4. CLINICAL STUDY OF NKT CELL-BASED IMMUNOTHERAPY

Murine studies showed that not only NKT cell activation but also IFN-gamma production and enhanced NK cell cytotoxicity following NKT cell activation were important for antitumor actions. Therefore, to demonstrate the induction of this immunological cascade in addition to proof of safety and feasibility, several clinical trials were performed with NKT cell-based tumor immunotherapy in patients with malignant diseases.

4.1. Active immunotherapy

4.1.1. Direct administration of alpha-GalCer

Giaccone *et al.* reported clinical studies using soluble alpha-GalCer (KRN7000) aimed at endogenous NKT cell activation (32). From 1998 to 2000, a phase I dose escalation study of direct intravenous injection of KRN7000 was performed and total of 24 patients were enrolled. In this study, the intravenous injection of KRN7000 was found to be well tolerated in patients with advanced solid tumors (Table 1). Although no objective clinical responses including complete response (CR) or partial response (PR) were observed, 7 patients showed stable disease (SD) for a median duration of 123 days

Table 1. Adverse events, immunological responses and antitumor responses

Treatment	Number of patients	Adverse events Grade 1	Grade 2	>Grade 3	Immunological responses	Antitumor effects	reference
alpha-GalCer	24	flush (1), sneezing (1)		fever (1)	increase in serum GM-CSF and TNF-alpha	SD[10] (7)	32
alpha-GalCer-pulsed DC	11	hot flash (1), headache (1), elevation of Cre[3] (1) and Bil[4] (1)	hyperkalemia (2), cystitis (1), malaise (1)	none	NKT cell expansion, augmentation of IFN-gamma mRNA level	SD (3)	33
alpha-GalCer-pulsed DC[1]	12	tumor flare, fever, malaise, lethargy, headache		none	NKT cell expansion, up-regulation of CD69 expression on T and NK cells, increased NK cell cytotoxicity, increase in serum IL-12 and IFN-gamma	reduction of tumor markers (2), extensive necrosis of tumor (1)	35
alpha-GalCer-pulsed DC[2]	5	fever (1), rash (1), ANA[5], RF[6] positive (1), increase in APTT[7] (1)	none	none	NKT cell expansion, increase in memory CMV[8]-specific CD8+ T cells, increase in serum IL-12 and IP-10[9]	reduction of serum or urine M protein (3), SD (1)	37
alpha-GalCer-pulsed DC	9	None	anemia (2)	none	NKT cell expansion, augmentation of IFN-gamma spot forming cells	PR[11] (1), SD (7)	38
NKT cell	6	hot flash (1), headache (2), malaise (1), fever (1), liver dysfunction (3), arrhythmia (1)	facial paralysis (1)	none	NKT cell expansion, augmentation of IFN-gamma spot forming cells	SD (2)	20

Numbers of the patients are indicated in the parenthesis. [1]grading and total numbers were not described [2]treatment-related toxicity only 3creatinine 4bilirubin 5antinuclear antibody 6rheumatoid factor 7activated partial thromboplastin time [8]cytomegalovirus [9]IFN-gamma-inducible protein-10 [10]partial response [11]stable disease

(Table 1). As for immunomonitoring, this study showed that KRN7000 administration resulted in both the rapid depletion of circulating Valpha24 NKT cells and the transient decrease of NK cells. Increased serum GM-CSF and TNF-alpha levels were only observed in patients with relatively high Valpha24 NKT cell frequency (Table 1). These results suggest the importance of a sufficient pool of circulating Valpha24 NKT cells.

4.1.2. Dendritic cell therapy pulsed with alpha-GalCer

As mentioned above, alpha-GalCer-pulsed DCs enhanced the antitumor responses more effectively in comparison to the free alpha-GalCer in murine models. In addition, the administration of alpha-GalCer-pulsed DCs induces the expansion of NKT cells in the lung and the efficient inhibition of tumor growth *in vivo* in murine lung metastasis models. Based on these results, lung cancer was chosen as a target for NKT cell-based immunotherapy. From 2001 to 2002, a phase I dose escalation study was performed with alpha-GalCer-pulsed antigen presenting cells (APCs) including DCs in patients with advanced or recurrent non-small cell lung cancer (Figure 2A) (33). In clinical trials, whole PBMCs cultured with GM-CSF and IL-2 were used as APCs instead of the adherent fraction of PBMCs cultured with GM-CSF and IL-4. Ishikawa *et al.* demonstrated that IL-2/GM-CSF-cultured APCs are superior to monocyte-derived DCs (moDC) cultured with GM-CSF and IL-4 in their expansion ability of Valpha24 NKT cells and IFN-gamma production potential (34). $CD11c^+$ DCs in the IL-2/GM-CSF-cultured APCs showed mature phenotype without further stimulation, while also showing a strong activity on Valpha24 NKT cells stimulation, thus enabling them to produce IFN-gamma preferentially to an extent equivalent to mature moDCs stimulated with lipopolysaccharide (LPS) or a cytokine cocktail including TNF-alpha. In addition, $CD11c^-CD3^+$ T cells in the IL-2/GM-CSF-cultured APCs induced the maturation of moDCs through TNF-alpha, thereby yielding the efficient expansion and activation of Valpha24 NKT cells to produce IFN-gamma.

Patients with histologically diagnosed lung cancer for which no standard treatment was available, were eligible and eleven patients were enrolled in the study. Four times of alpha-GalCer-pulsed APCs (level 1: 5 x $10^7/m^2$; level 2: 2.5 x $10^8/m^2$; and level 3: 1 x $10^9/m^2$) were administered, and no severe adverse events were observed in any patients (Table 1). After the first and second injection of alpha-GalCer-pulsed APCs, a marked increase in the peripheral blood Valpha24 NKT cell number was observed in one case and moderate increase in two cases receiving the level 3 dose. Simultaneously, increased IFN-gamma mRNA from sorted and purified circulating Valpha24 NKT cells was confirmed after the first and second injections of alpha-GalCer-pulsed APCs in one case in which the NKT cell number changed strikingly. Although no objective clinical response was found, one case who showed apparent NKT cell specific immune responses demonstrated a stable intra-thoracic lesion for more than two and half years with a good quality of life and she was able to survive for more than 4 years (unpublished observation). In summary, this clinical trial of alpha-GalCer-pulsed APCs including DCs was well tolerated and it could be done safely even in patients with advanced stages. Alpha-GalCer-pulsed APCs (at 1 x $10^9/m^2$) have been extended to a phase I-II clinical trial to test the immunological responses, safety, and the clinical responses. About 20 patients with advanced or recurrent lung cancer fulfilled the criteria to participate in the study protocol. This study essentially confirmed the observation in the previous phase I study regarding the immunological and clinical responses without any severe adverse event.

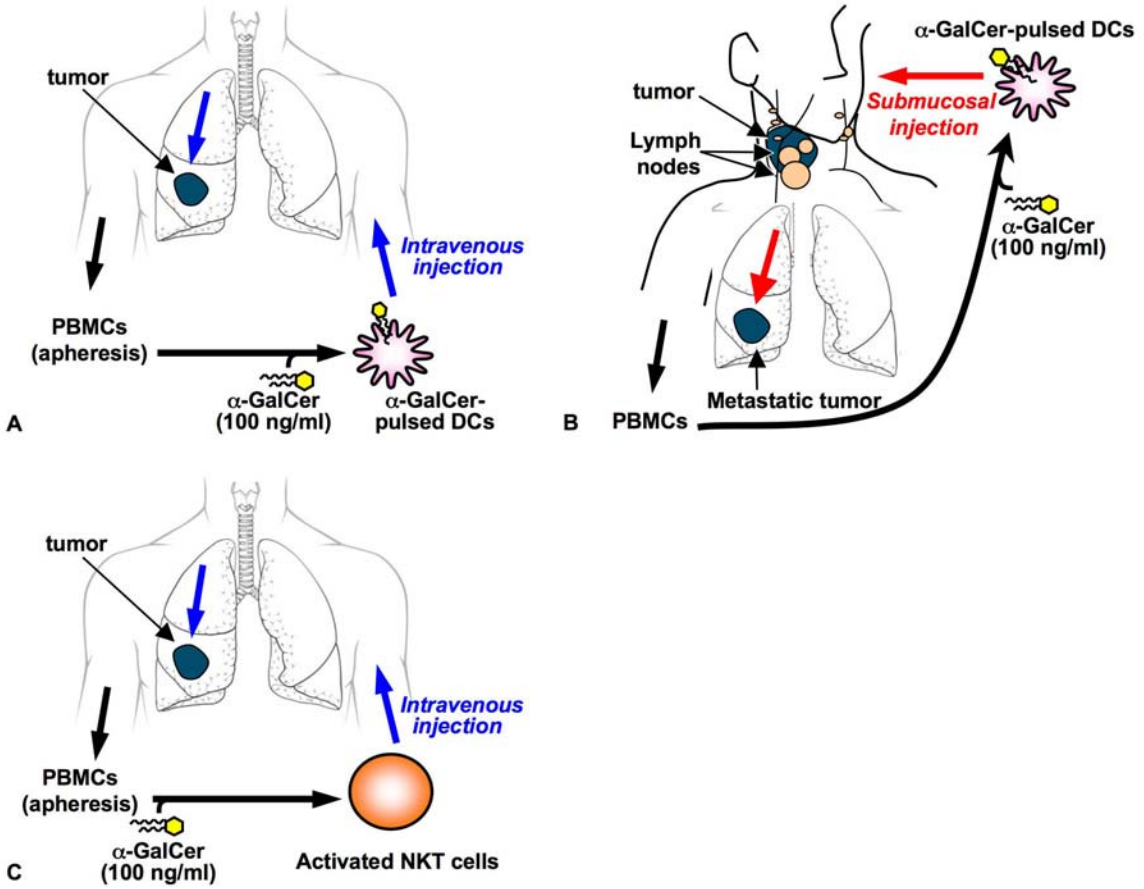

Figure 2. Clinical Study design of NKT cell-based immunotherapy. a) Active immunotherapy in patients with lung cancer: PBMCs were cultured with GM-CSF (granulocyte-macrophage colony-stimulating factor) and IL-2 for 1 or 2 weeks and alpha-GalCer was pulsed the day before administration. Alpha-GalCer-pulsed APCs including DCs were harvested and administered intravenously. b) Active immunotherapy in patients with head and neck cancer: PBMCs were cultured with GM-CSF and IL-2 for 1 week and 1 $x10^8$ alpha-GalCer-pulsed APCs including DCs were injected into the ipsilateral nasal submucosa. c) Adoptive transfer of *in vitro* activated NKT cells in patients with lung cancer: PBMCs were cultured with alpha-GalCer and IL-2 for 2 or 3 weeks, and then the alpha-GalCer-activated cells were administered intravenously.

Nieda *et al.* have also reported the results of a clinical trial with alpha-GalCer-pulsed monocyte-derived immature DCs (imDCs) in 12 patients with advanced malignancies including breast cancer, colon cancer, hepatocellular carcinoma, melanoma, peritoneal adenocarcinoma, renal cell carcinoma, prostate carcinoma and lung cancer (35, 36). In the study, the patients received two infusions of 5 $x10^6$ alpha-GalCer-pulsed imDCs intravenously, and two infusions intradermally. Several symptoms were reproducibly but transiently observed after alpha-GalCer-pulsed imDC treatment. Minor systemic side effects that were related to the treatment occurred in 9 of 12 patients (Table 1). Circulating Valpha24 NKT cells increased modestly and transiently after alpha-GalCer-pulsed imDC injection, and then activated T cells and NK cells increased after an augmentation of serum IFN-gamma (Table 1). The serum IL-12 levels also increased that might be a result of the interaction between activated NKT cells and immature DCs. Although no clear tumor reduction was detected, the tumor markers significantly decreased in two patients with colon cancer and peritoneal adenocarcinoma. One renal cell carcinoma showed extensive necrosis of tumor (Table 1). These findings may therefore indicate the antitumor effects of alpha-GalCer-pulsed DCs.

Chang *et al.* demonstrated the efficacy of alpha-GalCer-loaded mature DC immunotherapy (37). Adherent monocytes were cultured with GM-CSF and IL-4 and immature DCs were pulsed with alpha-GalCer and induced to mature using an inflammatory cytokine cocktail including IL-1b, IL-6, TNF-alpha and PGE_2. Five patients, including 3 with multiple myeloma, 1 with anal cancer, 1 with renal cell cancer received two times of 5 $x10^6$ alpha-GalCer-pulsed mature DCs administration intravenously. The adverse events related to the DC-injection are summarized in Table 1. In all 5 cases, the administration of alpha-GalCer-pulsed mature DCs resulted in an >100-fold increase in number of circulating Valpha24 NKT cells, despite the fact that no circulating Valpha24 NKT cells

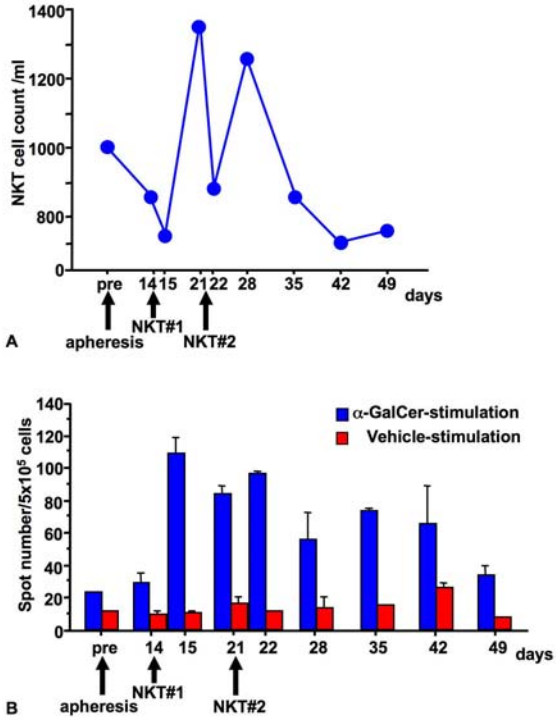

Figure 3. Representative data of NKT cell specific immune responses after in vitro activated NKT cell treatment. a) Absolute number of circulating Valpha24 NKT cell during the course of in vitro activated NKT cell treatment. The percentage of Valpha24+Vbeta11+ NKT cell was evaluated by a flow cytometry and the absolute number of NKT cell was calculated. Circulating Valpha24 NKT cells apparently increased after the first and second injection of in vitro activated NKT cells (NKT#1 and NKT#2). b) Detection of IFN-gamma-producing cells during the course of in vitro activated NKT cell treatment. Cryopreserved PBMCs were thawed and restimulated with alpha-GalCer for 16 hours. IFN-gamma producing cells were quantified by an ELISPOT (enzyme-linked immunospot) assay. After the first injection of activated NKT cells (NKT#1), the IFN-gamma producing cell number increased and thereafter gradually decreased, but still remained at higher levels than the baseline level until the end of the study period.

were detectable at baseline, and the expansion lasted for several months, which was superior to immature DC or alpha-GalCer alone. As for the functional analysis of Valpha24 NKT cells after alpha-GalCer-pulsed mature DC treatment, a very small degree of IFN-gamma production from NKT cells was detected. This impairment of IFN-gamma secretion from NKT cells was also detected after an adequate expansion of post vaccinated NKT cells stimulated with alpha-GalCer-pulsed DCs *in vitro*. The activation of NKT cells might have correlation with an increase in antigen-specific memory T cells, thus suggesting the adjuvant effects of NKT cells might therefore be expected from the murine models (Table 1). The best clinical responses included a decreased urine or serum M protein in 3 cases of myeloma and one stable disease in a patient with renal cell cancer (Table 1).

The submucosal injection of alpha-GalCer-pulsed APCs was observed in patients with head and neck squamous cell carcinoma (38). In this study, the administration route was unique, since the APCs were injected into the nasal submucosa. These quickly migrate into the ipsilateral regional neck lymph nodes (39). Consequently, alpha-GalCer-pulsed APCs were administered 2 times into the nasal submucosa in 9 cases of unresectable or recurrent head and neck cancer (Figure 2B). In comparison to intravenous administration, significantly smaller numbers of APCs ($1x10^8$ cells) were able to induce significant immune responses without any severe adverse events (Table 1). One patient revealed PR and 5 patients SD, with an obvious tumor regression being observed (Table 1). New design protocols were established to obtain more conclusive findings.

4.2. Adoptive immunotherapy

The cancer-bearing patients were shown to possess severe defects in the Valpha24 NKT cell pool and/or function and direct reconstitution of NKT cells might be favorable, if the adequate number of functionally sufficient NKT cells could be induced *in vitro*. Although the frequency of circulating Valpha24 NKT cells was very low, Valpha24 NKT cells were expanded by the repeated stimulation of peripheral blood mononuclear cells with alpha-GalCer *in vitro (*18). The *in vitro* expanded Valpha24 NKT cell lines showed potent cytotoxicity against tumor cell lines. Based on these results, the adoptive transfer of *in vitro* activated NKT cells were performed in 6 patients with lung cancer who were refractory to the standard treatment (20). PBMCs were stimulated with alpha-GalCer and IL-2 for 2 or 3 weeks, the cultured cells were intravenous injected twice with a cohort of level 1 ($1x10^7/m^2$) and level 2 ($5x10^7/m^2$) to test the safety, immunological and clinical responses (Figure 2C).

The transferred Valpha24 NKT cells mostly expressed either double negative or $CD8^+$ NKT cells which were reported to produce Th1-type cytokines (7, 40). It was reported that murine $CD4^-$ NKT cell subset showed potent protective responses against tumor cells, whereas $CD4^+$ NKT cells exhibited impaired tumor rejection *in vivo* (41). At present, although it is difficult to utilize only desired NKT cell subset from the peripheral blood NKT cell pool, our *in vitro* expanded NKT cells ($CD4^-$) may be suitable for the use of treatment of cancer patients. In fact, our *in vitro* expanded NKT cells showed a potent antitumor activity on human tumor cell lines *in vivo* and *in vitro* (6). The circulating Valpha24 NKT cells increased in 2 of 3 cases that received a level 2 dose and increased numbers of IFN-gamma-producing cells were detected (Figure 3 and Table 1). These IFN-gamma-producing cells were shown to be $CD3^-CD56^+$ NK cells but not $CD3^+$ T cells, which might results from the stimulation of NK cells *in vivo* by the injected Valpha24 NKT cells. Two of three patients who received the level 2 dose revealed SD for 9 and 12 months after the study period (Table 1).

5. PERSPECTIVE

Chemotherapy is a standard treatment for either advanced or recurrent lung cancer. Refractory or relapsed cases after the first-line therapy are far more difficult. The patients with late-stage lung cancer are often symptomatic and standard chemotherapy frequently causes unfavorable complications, therefore, the establishment of a novel therapy with low-toxicity, such as immunotherapy is highly desirable. Recently, this new cell therapy was performed as a clinical trial of Valpha24 NKT cell-based immunotherapy against various cancers and, consequently, novel immunological results were thus precisely accumulated. Now, it is necessary to address the immunological analyses of the tumor site, since the direct interaction between the tumor and host immunity can be observed. More detailed investigations of the cellular and molecular mechanisms controlling the functions of NKT cells at the tumor site will help resolve the problems encountered with the present NKT cell-based therapeutic approaches and help establishing new treatment strategies for various human cancer. A now protocol is now being developed to explore the direct interaction at the tumor site.

Since a clinical response was rarely observed, continuous refinement to augment the cytolytic activity is needed, without losing the safety profile that is the marked advantage of cancer immunotherapy. A combination therapy consisting of *in vitro* activated NKT cell transfer followed by alpha-GalCer-pulsed DCs treatment thus seems worth considering in order to augment the activation levels of NKT cells. Other reagents which activate distinct cell populations and promote adaptive immunity, such as a tumor-associate antigen peptide vaccine might also be useful for activating tumor specific immunity in combination with NKT cell-based immunotherapy. A limited-dose radiation therapy to sensitize tumor cells to NKT cell-mediated cytotoxicity is thought to be another way to enhance the adaptive immunity. In any event, in order to allow the full induction of protective immunity by using adjuvant effects of NKT cells, new combination approaches should be considered. The NKT cell-based immunotherapy might be a potent new tool for modulating the immune responses against cancer, and the most viable application of this treatment appears to be for the eradication of small tumor foci such as micrometastases after either a complete surgical resection or complete remission induced by chemotherapy.

6. ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (Japan) (Grants-in-Aid: for Scientific Research on Priority Areas #17016010; Scientific Research (B) #17390139, Scientific Research (C) #18590466, #19590491 and #19591609; Exploratory Research #19659121 : Special Coordination Funds for Promoting Science and Technology, and : Cancer Translational Research Project), the Ministry of Health, Labor and Welfare (Japan), The Japan Health Science Foundation, Kanae Foundation, Uehara Memorial Foundation, Mochida Foundation, Yasuda Medical Foundation, Astellas Foundation and Sagawa Foundation. The GMP-grade alpha-GalCer was provided by Kirin Pharma, Co.

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Abbreviations: alpha-GalCer: alpha-galactosylceramide, IFN: interferon, NKT: natural killer T, IL: interleukin, PBMCs: peripheral blood mononuclear cells, APCs: antigen presenting cells, DCs: dendritic cells

Key Words: NKT cell, Dendritic Cell, Clinical Trial, Immunotherapy, Cancer, Review

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