Review

Discovery of NKT cells and development of NKT cell-targeted anti-tumor immunotherapy

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Abstract: Natural Killer T (NKT) cells are unique lymphocytes characterized by their expression of a single invariant antigen receptor encoded by V14J18 in mice and V24J18 in humans, which recognizes glycolipid antigens in association with the monomorphic CD1d molecule. NKT cells mediate adjuvant activity to activate both CD8T cells to kill MHC-positive tumor cells and NK cells to eliminate MHC-negative tumor at the same time in patients, resulting in the complete eradication of tumors without relapse. Therefore, the NKT cell-targeted therapy can be applied to any type of tumor and also to anyone individual, regardless of HLA type.

Phase IIa clinical trials on advanced lung cancers and head and neck tumors have been completed and showed significantly prolonged median survival times with only the primary treatment. Another potential treatment option for the future is to use induced pluripotent stem cell (iPS)-derived NKT cells, which induced adjuvant effects on anti-tumor responses, inhibiting in vivo tumor growth in a mouse model.

Keywords: NKT cells, adjuvant effects, α-galactosylceramide, NKT cell-targeted therapy, induced pluripotent stem cells

1. Discovery of NKT cells expressing a unique single invariant V14J18 antigen receptor in mice

Natural killer T (NKT) cells were discovered as a unique lymphocyte lineage characterized by the expression of a single invariant T cell receptor (TCR) α-chain encoded by V14J18 in mice and V24J18 in humans, which is predominantly associated with a limited repertoire of TCRβ-chains, mainly Vβ8.2 in mice and Vβ11 in humans.1)–3) The single invariant V14J18 receptor is exclusively used by NKT cells and, unexpectedly, not by conventional T cells even though both gene segments are present in a typical context within the TCRβ-chain gene locus. These findings were unambiguously confirmed when it was shown that only NKT cells, but not conventional T cells or NK cells, developed when the invariant V14J18 together with TCRβ8.2 genes were introduced into RAG-knockout (KO) mice,4) indicating that the rearrangement of V14 and J18 gene segments determines the commitment to NKT cell development.

In 1986, the discovery of murine invariant V14J18 NKT cell antigen receptor gene was made by evaluation of the TCRβ-chain from 13 hybridomas with regulatory functions.6)–7) Southern blot analysis of TCRβ-chain gene usage by these 13 independently established hybridomas showed exactly the same DNA restriction fragment length polymorphism (RFLP) pattern, even after using three different enzymes, EcoRI, BamHI and HindIII. These results suggested the possibility that all the hybridomas used the same TCRβ-chain gene sequence and found that these could be classified into 4 types, all of which were, however, expressing the same V14 and the same J18 gene segments with only a one-nucleotide addition at the N-region being

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different in each clone, an C, A, T, or G nucleotide. Since this particular site of the N-region is in the third base of a glycine codon, any nucleotide addition at this position still encodes glycine, and thus the V$\alpha_{14}$J$\alpha_{18}$ antigen receptor is invariant at the amino acid level.\(^7\) To exclude the possibility that our surprising results were due to an unknown bias in the hybridoma cloning, we carried out RNase protection assays in which C57BL/6 (B6) type $^{32}$P-labelled antisense V$\alpha_{14}$J$\alpha_{18}$ mRNA derived from one of the hybridomas was hybridized with total mRNAs (sense) from normal B6 thymocytes.\(^8\) In this assay, the samples are then treated with RNase to digest the unhybridized single stranded portion of the $^{32}$P-labelled probe, thus “protecting” that portion of the probe that had hybridized with thymocyte mRNAs, leaving it intact (Fig. 1A). Using this assay, we detected a single 630 bp protected $^{32}$P-labelled antisense band in B6, a single 400 bp band in BALB/c, and 630/400 bp double bands of B6 and BALB/c types in DBA/2 mice (Fig. 1B). Remarkably, the protected band(s) represented 2–4% of the total TCR$\alpha$ mRNA in the thymus of unmanipulated mice. The frequency of expression of any one particular TCR$\alpha$-chain is theoretically estimated to be $1/10^6$, because there are 100 V$\alpha$ genes in the TCR$\alpha$ locus among a total repertoire of $10^8$ TCR$\alpha$-chains.\(^8\) Therefore, the frequency of V$\alpha_{14}$J$\alpha_{18}$ expression that we detected in unmanipulated mice with different genetic backgrounds was more than $10^4$ times higher than expected. This suggested that NKT cells were clonally expanded under physiological conditions without any external antigen stimulation.

We further sequenced V$\alpha_{14}$ genes from a total of 26 independent inbred laboratory mouse strains and classified into these 3 types according to the V$\alpha_{14}$ polymorphisms (B6 V$\alpha_{14}$ type: C57BL/10, B10(A), B10A (3R), B10A(4R), B10A(5R), C57Br; BALB/c V$\alpha_{14}$ type: A/J, AKR, A/WySn, CE/J, CDA/J, C3H, 129/J, I/LnJ, NZB, RIILs/J, PL/J, WB/ReJ, RFM/MsNrs, SM/J SWM, SJL; DBA V$\alpha_{14}$ type: DBA/1, DBA/2), demonstrating that the V$\alpha_{14}$ gene is unique and very highly conserved in all laboratory

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**Fig. 1.** Detection of invariant V$\alpha_{14}$J$\alpha_{18}$ gene expression by RNase protection assay. A) The RNase protection assay. Outline of the procedure and expected sizes of the invariant V$\alpha_{14}$ bands are depicted. B) Detection of the protected bands by the RNase protection assay. The intensities of the bands circled in red were compared with the C$\alpha$ bands, which represent the total TCR$\alpha$ transcripts in the population. Adopted from our original paper.\(^9\)
mouse strains. The above mentioned paper on the discovery of invariant NKT cells published in 1990 was selected by The American Association of Immunologists as one of the “The Pillars of Immunology” for its enormous impact on the field.

Based on the above results, we concluded that the expression of Vα14Jo18 in mice and Vα24Jo18 in humans is a defining signature of NKT cells that clearly distinguishes them from conventional T cells. Other differences between NKT and conventional T cells have since been recognized; NKT cells belong to either DN or CD4+ populations, express CD69, CD5\textsuperscript{high}, CD44 and NK1.1, are present in the thymus, liver, spleen and BM, and produce both Th1 and Th2 cytokines. Moreover, NKT precursor cells express the granulocyte macrophage colony stimulating factor receptor (GM-CSFR) and undergo Vα14 gene rearrangement after receiving GM-CSF signals. More surprisingly, the circular DNA created by the Vα14 and Jo18 gene rearrangement event was detected at an early stage in embryogenesis.

### 2. Positive Darwinian selection operated in the evolution of the Vα14 gene family

To understand the biological significance of NKT cells in the immune system, we analyzed genetic polymorphisms in Vα14-related (“Vα14”) genes and investigated the ratio of nonsynonymous vs synonymous substitutions in the “Vα14” genes of wild type mice, such as M. musculus (M. m. molossinus, M. m. domesticus, M. m. musculus, M. m. castaneous) and Mus species (M. platythyrix, M. caroli, M. leggada, and M. spretus), as well as Apodemus speciosus (intermediate between Rattus and Mus), Rattus, Hamster and Human. All of these mammalian species possessed “Vα14” genes similar but not identical to Vα14 subfamilies in B6, BALB/c and DBA laboratory strains. Most nucleotide substitutions in these “Vα14” genes were found at the first and the second nucleotide positions of codons, suggesting a higher frequency of non-synonymous nucleotide mutations in the “Vα14” sequences in the different species. As shown in Table 1, at the time of species divergence the frequencies of non-synonymous mutations were higher than those of synonymous mutations (Apodemus vs. M. musculus or other Mus species, and Rattus vs. Hamster). In fact, the ratio of non-synonymous vs synonymous substitutions was more than 1.0 when “Vα14” genes in Apodemus were compared with those in M. musculus or Mus species. In general, amino acid substitutions in most conventional TCR V regions other than Vα14 gene (e.g., Vα1) are neutral during evolution as suggested by the theory proposed by Kimura. In fact, the ratio of non-synonymous vs synonymous nucleotide substitutions in conventional T cell αβ receptor V region genes is generally 0.58 at the first, 0.47 at the second and 0.89 at the third nucleotide positions, respectively, as reported by Lu and Nei. Therefore, these observations suggest that positive selection of the “Vα14” genes occurred at the time of the divergence of Apodemus to M. musculus or Mus species (Table 1). Similarly, higher non-synonymous/synonymous ratios were observed at the species divergence between Hamster and Rattus (0.953), supporting the idea that positive Darwinian selection is operating in the different species. These results suggest that the “Vα14” gene family, unlike the conventional TCRVα genes, is selectively affected by environmental factors, indicating that NKT cells bearing “Vα14” antigen receptors are essential for the survival of species as they adapt to environmental changes during evolution.

### 3. Identification of α-galactosylceramide as an NKT cell ligand

For several years, the identity of NKT cell ligand(s) was an enigma. Then, in 1997, we discovered a ligand for NKT cells as a glycolipid, α-galactosylceramide (α-GalCer), which is presented by the monomorphic MHC class I-like CD1d molecule. The idea that an NKT cell ligand might be a glycolipid was suggested by experiments using mice lacking the transporter associated with antigen processing (TAP-KO). TAP is essential for translocation of cytoplasmic peptides into the endoplasmic

| Species                  | Ratio of non-synonymous/synonymous mutations in CDR1/CDR2 |
|--------------------------|----------------------------------------------------------|
| Apodemus speciosus vs.   |                                                           |
| M. musculus              | 1.117 0.408                                              |
| M. platythyrix           | 1.442 0.408                                              |
| M. leggada               | 1.063 0.348                                              |
| Rattus vs.               |                                                           |
| Hamster                  | 0.953 ND                                                 |

*The ratio of non-synonymous vs synonymous nucleotide substitutions at the 1st (0.58) and 2nd (0.47) positions in most eukaryotic and prokaryotic genes, indicating that TCR Vα genes except for Vα14 are evolutionally neutral.
In RNase protection assays we could detect signs of sphingosine as an NKT cell ligand by screening TCRv with a glycolipid, lipid or carbohydrate that associated. These results strongly suggested that the NKT cell ligand was definitely not a peptide, but likely to be a glycolipid, lipid or carbohydrate that associated with a β2M-associated MHC class I-like molecule. At the time of the discovery of α-GalCer as the NKT cell ligand, the relevant MHC class I-like molecule was discovered to be the non-polymorphic CD1d, which has two large hydrophobic A’ and F’ pockets potentially allowing it to accommodate lipids with a carbon (C):25 chain length in the A’ and a C:20 chain length in the F’ pockets. Thus, we speculated that an NKT cell ligand should have both hydrophilic and hydrophobic properties, because of its requirement for binding with both hydrophobic CD1d pockets and the hydrophilic Vo14 antigen receptor. Based on these parameters, we identified α-GalCer with C:25 fatty acyl chain and C:18 sphingosine as an NKT cell ligand by screening various synthetic glycolipids and determined the important positions critical for the recognition by NKT cells based on the structure-function relationships. The structure also revealed that the 4 amino acids (Asp94, Arg95, Gly96 and Ser97) of Jα18, which are conserved in mouse and human (Fig. 2C), are essential for binding with both CD1d and α-GalCer (Fig. 2B). The Asp94 in Jα18 binds with Arg79 of CD1d, Arg95 in Jα18 with Arg79/Ser76/Asp80 of CD1d and the 3-OH on the sphingosine, Gly96 in Jα18 with the 2-OH on the galactose, and Ser97 in Jα18 with Gln150 of CD1d (Fig. 2B). Interestingly, the Glu83 of CD1d, although it makes no direct contribution to binding the ligand, is important for binding with the TCRβ-chain to make a stable complex with CD1d. Moreover, the CD1d amino acids Ser76, Arg79 and Asp80 (Fig. 2B), which are responsible for binding with either α-GalCer or Jα18, are also conserved among species. In fact, α-GalCer was first identified as a murine NKT cell ligand, but was subsequently shown to activate human NKT cells and thus can be used for human studies.

5. NKT cell-mediated adjuvant activities augment anti-tumor immune responses

For the development of anti-cancer immunotherapy, the following three issues have to generally be considered. The first is that, for an optimal therapy, both MHC-positive and -negative tumor cells should be eliminated simultaneously to avoid tumor relapse, because tumors in general contain both cell types. The second important point is that tumor cells do not usually contain any endogenous adjuvant materials because, unlike pathogens, they are derived from autologous cells, so that it is difficult to mount efficient anti-tumor immune responses in patients without adjuvant. The third point is that it is essential to convert DCs from an immature to mature state in the tumor patients to induce efficient protective responses, because most advanced cancer patients are immune deficient. Activation of NKT cells with α-GalCer promotes production of IFNγ, which induces NKT cell-mediated adjuvant activity, activating both NK and CD8T cells and bridging innate and acquired immune systems and thus overcoming the problem.
of the tumor-relapse because it induces clonal expansion of antigen-specific CD8T cells and activation of NK cells as well as maturation of immature DCs in patients (Fig. 3A).29),30) Therefore, NKT cell-targeted therapy should be useful for treatment of advanced cancer patients, whose specific CD8T cells, even though they are present in patients, fail to expand, because DCs are usually immature due to the immune suppressive cytokines, such as interleukin (IL)-10 or TGFβ, produced by tumor cells.31)

Unlike other lymphocytes, only NKT cells have the ability to interact with immature DCs in the presence of α-GalCer, leading to the induction of full maturation of DCs through CD40-CD40L interactions,32),33) resulting in their robust production of IL-12 to further activate NKT cell production of IFN-γ. The end result of these cellular interactions is the activation of both CD8 cytotoxic T cells and NK cells to eliminate MHC-positive and -negative tumor cells, respectively, resulting in tumor eradication without relapse (Fig. 3A). In fact, it was experimentally demonstrated the strong adjuvant activity mediated by NKT cells is observed in a tumor model using OVA as an artificial tumor antigen30),32) (Fig. 3B). In this experiment, mice were immunized with OVA-loaded TAP-KO spleen cells together with α-GalCer; TAP-KO mice were used because they are unable to present peptide antigen to conventional CD8T cells. For the detection of IFN-γ production, we stimulated spleen cells with OVA peptide in vitro and observed significant production of IFN-γ by NK cells and CD8T cells (Fig. 3C), as well as a robust increase in the number of OVA-specific IFN-γ producing CD8T cells in vivo (Fig. 3D) and, importantly, suppression of the OVA-expressing EL4 (EG7) tumor growth (Fig. 3E).
Therefore, NKT cell-targeted anti-tumor immunotherapy overcomes the major problem of tumor relapse usually observed with current anti-cancer immunotherapies, which target only one type of anti-tumor immune effector cells. For example, in the immunotherapy using tumor antigen peptides or antibodies against PD-1 or CTLA4, the target is a CD8 T cell, which kills MHC-positive but not -negative tumor cells, resulting in the recurrence of an MHC-negative tumor. Similarly, in the artificial cells recently developed by the forced expression of NK receptor ligands, such as Rae1/H60/Mult-1 (NKG2D-L), or TNF ligand (CD70-L), the target cell is an NK cell, which eliminates only MHC-negative tumor cells, resulting in relapse with MHC-positive tumor cells. Only NKT cells, and not other effector cell types, simultaneously activate both CD8 T and NK cells, resulting in the simultaneous elimination of both MHC-positive and -negative tumor cells. Therefore, NKT cell-targeted therapy is a promising treatment option for tumor treatment to overcome tumor relapse.

6. Clinical trials of NKT cell-targeted therapy in cancer patients

We carried out preclinical studies using the liver metastasis model of the murine B16 melanoma to investigate the clinical efficacy of NKT cell-targeted therapy using α-GalCer-DCs. Mice bearing metastatic melanoma nodules 1–2 mm in diameter in the liver were treated by intravenous administration of α-GalCer-DCs; this therapy resulted in the
complete eradication of melanoma metastasis within 7 days.34)

Based on the dramatic effects of α-GalCer-DCs in the mouse melanoma model, we conducted clinical trials to evaluate clinical outcomes: 1) at Chiba University hospital, in collaboration with Profs. T. Nakayama and S. Motohashi, of NKT cell-targeted therapy using α-GalCer-DCs on patients with advanced lung cancer, and 2) with Prof. Y. Okamoto in patients with head and neck tumors (Fig. 4). No severe adverse events related to the NKT cell-targeted therapy were observed in the Phase I clinical trials.35),36) We then carried out Phase II clinical trials on 17 cases with advanced (IIIB, IV) or recurrent non-small cell lung cancer refractory to the standard treatments, including surgery, chemotherapy and radiation therapy. The patient’s peripheral blood mononuclear cells (PBMCs), which were cultured with GMP-grade GM-CSF and IL-2 and pulsed with α-GalCer (α-GalCer-DCs), were administered intravenously into autologous patients (1.6 × 10^6 cells/m²).37),38)

Since IFN-γ produced by NKT cells serves as an adjuvant and thus could be a functional marker for patient NKT cells, we investigated the relationship between the ability of the patient PBMCs to produce IFN-γ and the clinical efficacy of the therapy. A dramatic increase (25–60 fold) in the number of IFN-γ-producing PBMCs assayed by ELISPOT was observed in 10 out of 17 (60%) patients.38) These 60% of patient group with high IFN-γ production had a significantly prolonged median survival time (MST) of 29.6 Mo compared with those with low IFN-γ production of a MST 9.7 Mo and the best supportive care group with a MST of 4.6 Mo. B) Clinical trials of the combination therapies of α-GalCer-DC and activated NKT cells on head and neck tumors: All 10 cases completed showed significant clinical efficacy (stable disease status, SD, in 5 cases in black and partial responses, PR, in 5 cases in red). The bar-graph shows the percent of the tumor size at 4 wks after the treatment compared with the initial size before treatment.

![Fig. 4. Clinical trials of the NKT cell-targeted adjuvant therapy in patients with advanced non-small cell lung cancers and head and neck tumors: A) Clinical trials of α-GalCer-DC therapy in advanced non-small cell lung cancer patients. Sixty percent of patients with high IFNγ production showed significantly prolonged median survival time (MST) of 29.6 Mo compared with those with low IFNγ production of a MST 9.7 Mo and the best supportive care group with a MST of 4.6 Mo. B) Clinical trials of the combination therapies of α-GalCer-DC and activated NKT cells on head and neck tumors: All 10 cases completed showed significant clinical efficacy (stable disease status, SD, in 5 cases in black and partial responses, PR, in 5 cases in red). The bar-graph shows the percent of the tumor size at 4 wks after the treatment compared with the initial size before treatment.](image-url)
cant NKT cell infiltration into the tumor in situ, similar to that observed in cases of advanced lung cancer.\textsuperscript{36,44} In the cases with PR, we observed a decrease in tumor size and necrosis at the center of the tumor.

Currently, we are conducting randomized Phase II clinical trials in collaboration with National Hospital Organizations (Drs. Ito, S., Saka, H. and Ichinose, Y.) using α-GalCer-DCs for treatment of stage II-A-III A non-small cell lung cancer patients after radical surgery. We have chosen this patient population because 70–75% of them will experience relapse after surgical resection of the primary tumor. Because of its significant effects on various tumor cell types, the NKT cell-targeted adjuvant cell therapy was approved by the Japanese government for the advanced medical care assessment system (B) on advanced non-small cell lung cancer in 2011, head and neck tumors in 2013, and Stage II A-III A non-small cell lung cancer after surgery in 2014. Since the clinical efficacy was not formally confirmed by the two-arm study, it is now being evaluated in the current clinical trials using the advanced medical care assessment system.

7. Generation of iPS cells from mature NKT cells

The clinical efficacy of NKT cell-targeted therapy looks promising, however, in the case of advanced cancer patients, two thirds of them are not eligible for this therapy due to their cancer-mediated immunodeficiency, which results in a limited number of NKT cells. It is also difficult to obtain enough NKT cells for clinical use with the current in vitro culture system. To overcome these problems, we established in vitro methods for the generation of unlimited numbers of functional NKT cells by using iPS technology.\textsuperscript{45} Such in vitro generated cells could then be transferred into the advanced cancer patients who have few of their own NKT cells.

In our previous studies using mouse embryonic stem (ES) cells, we replaced the ES cell nucleus with a nucleus derived from a mature NKT cell (NKT cloned ES) by nuclear transfer. Using this approach, we have successfully generated a large number of NKT cells in vitro, which could mediate adjuvant activity in vivo and regress tumor growth.\textsuperscript{46} The efficiency of reprogramming of lymphocytes by using iPS technology with Klf4, Sox2, Oct3/4 and c-Myc genes was originally reported to be very low.\textsuperscript{47} However, based on the frequency of establishing ES cells by nuclear transfer into enucleated unfertilized eggs, we discovered that NKT cells have a higher reprogramming efficiency (71%) compared to conventional T cells (12%).\textsuperscript{48} Therefore, we speculated that NKT cells would be suitable for reprogramming and generating iPS cells.

In fact, we efficiently generated iPS cells from mature NKT cells.\textsuperscript{48} Isolated mature B6 NKT cells (10\textsuperscript{6}) were first stimulated with CD3/CD28 mAb and then cultured with IL-12/IL-2 for 7 days, followed by reprogramming according to the conventional protocol using Klf4, Sox2, Oct3/4 and c-Myc genes. We have established several iPS cell lines harboring a rearranged NKT cell-derived Va14Ja18 gene. In terms of gene expression profiles, we found that iPS cells established in this way are similar to ES cells but different from fibroblasts in terms of their expression of endogenous Klf4, Sox2, Oct3/4, Nanog, Rex1, Ecut1, Zfp296, and Gdf3 mRNA as well as in genomewide gene expression profiles. Moreover, Oct3/4 and Nanog genes are demethylated in NKT cell-derived iPS cells, similar to ES cells, while those are methylated in fibroblasts.

8. Development of functional NKT cells from iPS cells

The ability of the iPS cell lines to develop into functional NKT cells was investigated in a culture system using OP9/Dll-1 stromal cells in the presence of IL-7 and Flt-3L for 25 days.\textsuperscript{48} Using this culture system, we obtained 3 × 10\textsuperscript{7} NKT cells, a 300-fold expansion, from 10\textsuperscript{6} iPS cells. Upon either CD3/CD28 mAb or α-GalCer-DC stimulation, the NKT cells generated in vitro produced significant amounts of IFN-γ, essential for their adjuvant effect in anti-tumor responses, indicating that the iPS-derived NKT cells represent functional NKT cells. It is worthwhile mentioning that the NKT-derived iPS cells preferentially generated NKT cells and also a small number of CD11b\textsuperscript+ macrophages, but not γδT, αβT, NK or B cells in the in vitro 25 day-culture system.\textsuperscript{48} Moreover, iPS cells derived from fibroblasts failed to generate NKT cells under the same culture conditions, most likely because the frequency of the successful rearrangement of the required Va14 and Ja18 combination is estimated to be very low, less than 1/10\textsuperscript{8}. Therefore, the pre-rearranged Va14 gene in the natural chromosomal context predisposes the NKT cell fate.

9. In vivo function of iPS-derived NKT cells

The in vivo function of iPS-derived NKT cells was investigated by transfer into NKT-KO mice
A considerable number of iPS-derived NKT cells were detected in vivo, even 6 weeks after cell transfer. The transferred iPS-derived NKT cells produced large amounts of INF-γ upon intravenous administration of α-GalCer-DCs and expanded significantly in number. Moreover, we observed expansion of NK cells and their production of INF-γ as the result of NKT cell-mediated bystander adjuvant activity.

To investigate the adjuvant activity on the acquired immune system mediated by iPS-derived NKT cells, we again used the OVA model as an artificial tumor antigen. NKT-KO mice that had received iPS-derived NKT cells were immunized with OVA-loaded spleen cells as described. A significant increase (48 fold) in the number of OVA-specific IFN-γ producing CD8T cells was detected in the mice by the transfer of iPS-derived NKT cells, similar to WT mice (51 fold). B) Inhibition of the OVA-expressing EL4 (EG7) tumor growth by NKT cell-mediated adjuvant therapy using iPS-derived NKT cells in vivo in the OVA model. A significant suppression of tumor growth mediated by iPS-derived NKT cells in vivo (in red) was detected.

(Fig. 5). A considerable number of iPS-derived NKT cells were detected in vivo, even 6 weeks after cell transfer. The transferred iPS-derived NKT cells produced large amounts of INF-γ upon intravenous administration of α-GalCer-DCs and expanded significantly in number. Moreover, we observed expansion of NK cells and their production of INF-γ as the result of NKT cell-mediated bystander adjuvant activity.

To investigate the adjuvant activity on the acquired immune system mediated by iPS-derived NKT cells, we again used the OVA model as an artificial tumor antigen. NKT-KO mice that had received iPS-derived NKT cells were immunized with OVA-pulsed spleen cells followed by stimulation with α-GalCer. We observed a 48-fold increase in the number of OVA-specific IFN-γ producing CD8T cells, similar to that seen (51 fold) in WT mice (Fig. 5A). Under these conditions, we obtained significant growth suppression of the OVA-expressing EL4 (EG7) tumor (Fig. 5B).

Since NKT cells are highly conserved between mice and humans in terms of their function and α-GalCer reactivity, we confidently expect that it will be possible to expand functional human NKT cells from human-iPS cells in vitro and to use the iPS-derived functional human NKT cells in the clinical setting.

10. Future directions for NKT cell-targeted therapy using iPS-derived NKT cells

Two immunological issues need to be addressed before clinical application of iPS-derived NKT cells: one is whether NKT cells induce GvHD, because various cell types, including conventional CD4T cells, are reported to induce GvHD, and the other is whether semi-allogeneic NKT cells will be functional in vivo, because semi-allogeneic iPS-derived NKT cells will be rejected by the patients soon after the cell transfer.

Related to the first question, allogeneic B6 NKT cells as well as syngeneic BALB/c CD4T cells as a control did not induce GvHD, whereas allogeneic B6 CD4T cells induced a fulminant GvHD characterized by weight loss, diarrhea, skin disease, or death when injected into BALB/c RAG-KO mice (Fig. 6A).

Concerning the second issue on the in vivo effects of semi-allogeneic NKT cells, we transferred (129 × B6) F1 NKT cells derived from cloned NKT-ES cells into B6 NKT-KO mice followed by immunization with α-GalCer in the OVA artificial tumor antigen model as described above (see Fig. 3B). A significant increase in the number of IFN-γ-producing OVA-specific CD8T cells (Fig. 6B) and the significant suppression of OVA-bearing tumor (EG7) growth (Fig. 6C) was detected. Since
the transferred semi-allogeneic F1 NKT cells will be eliminated in a few days, this strategy reduces the intrinsic anxiety on tumorigenicity of iPS-derived cells in vivo. The use of in vitro-generated NKT cells derived from iPS cells, therefore, offers a powerful option for the establishment of optimal NKT cell-targeted therapy. In 2013, the projects on the clinical application of human iPS-derived NKT cells was accepted as the Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan.

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Profile

Masaru Taniguchi received his M.D. and Ph.D. degrees at Chiba University School of Medicine (1974). He was appointed a Professor of Immunology there (1980–2004), and also served as the Dean of Chiba University School of Medicine (1996–2000) and President of the Japanese Society for Immunology (1997–1998). He was then appointed the founding Director of RIKEN Research Center for Allergy and Immunology in 2001, and is now Senior Advisor and Group Director at RIKEN Center for Integrative Medical Sciences.

The NKT cell that he identified in 1986 is known as an important lymphocyte that bridges the innate and acquired immune systems and mediates adjuvant activity for both innate and the acquired immune cells. He also identified an NKT cell ligand, \( \alpha \)-galactosylceramide (Science 278: 1626, 1997a) and developed NKT cell-deficient mice (Science 278: 1623, 1997b), which are essential for NKT cell studies.

He has published more than 400 papers. Among his papers, the highest number of citations is from the two aforementioned 1997 Science papers, which have been cited 2,357 times, highest among all NKT cell papers so far published. One of his papers on the first identification of NKT cells in vivo, published in PNAS in 1990, was selected by the American Association of Immunologists in 2014 as one of the “Pillars of Immunology” for being pivotal to the advance of the immunology field. His scientific activity has been distinguished with several prizes and he was awarded the Hideyo Noguchi Memorial Medical Prize in 1993, the Uehara Prize in 2004, and the Medal with Purple Ribbon in 2004.