Mini Review

Cancellation of NKT cell immunosuppression targeting myeloid suppressor cells

Haruka Wada¹), Kazuhiko Yanagisawa²), and Ken-ichiro Seino¹, *)

¹)Division of Bioregulation Research, Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa, Japan
²)Department of Surgery, School of Medicine, University of Tsukuba, Ibaraki, Japan

CD1d-restricted natural killer T (NKT) cells are one of immunoregulatory cells. NKT cells can be specifically activated by a synthetic glycolipid, α-galactosylceramide (α-GalCer). Using some glycolipids such as α-GalCer, it is expected to develop a new NKT cell-mediated therapeutic strategy against cancer. However, it is known that, in human cancer patients, NKT cells express a degree of hyporesponsiveness to α-GalCer. For example, we have reported that, in gastrointestinal cancer patients, NKT cell proliferation and cytokine production were impaired. We have further examined the mechanism by which hyporesponsiveness to α-GalCer can be induced using cancer-bearing mice. In the animal study, α-GalCer-induced NKT cell expansion, cytokine production, cytotoxicity, and anti-metastatic effect in vivo were all significantly impaired. In fact, α-GalCer could eliminate metastatic disease in naïve animals, but failed to protect cancer-bearing mice. We found that CD11b⁺ Gr-1⁺ cells were particularly increased in cancer-bearing mice and were necessary and sufficient for the suppression of NKT cells to α-GalCer. We also found that the increased CD11b⁺ Gr-1⁺ cells suppressed NKT cell function in a nitric oxide-mediated fashion. To reduce the population of CD11b⁺ Gr-1⁺ cells, we administered a retinoic acid to cancer-bearing mice. This treatment significantly reduced the population of CD11b⁺ Gr-1⁺ cells and effectively restored α-GalCer-induced NKT cell responses. These results demonstrate a novel feature of NKT cell function in cancer, and suggest a new strategy to enhance NKT cell-mediated anti-cancer immune responses by suppressing CD11b⁺ Gr-1⁺ cell functions.


*Correspondence should be addressed to:
Ken-ichiro Seino, M.D., Ph.D., Division of Bioregulation Research, Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan. Phone: 81-44-977-8165, Fax: 81-44-977-8165, email: seino@marianna-u.ac.jp.

Conflict of interest statement: No conflict declared.

Key words NKT, α-galactosylceramide, nitric oxide, myeloid suppressor cell, ATRA

NKT cell and α-galactosylceramide

CD1d-restricted natural killer T (NKT) cells are a lymphoid lineage characterized by expression of unique invariant T cell receptor encoded by Vα14-Jα281 gene segments in mice and Vα24-Jα18 in humans¹. NKT cells recognize α-galactosylceramide (α-GalCer) and its analogues; glycolipids that can
be presented by CD1d\(^5\). It has been shown that \(\alpha\)-GalCer selectively stimulates NKT cells to produce large amount of both T helper 1 (Th1) and Th2 cytokines, and that \(\alpha\)-GalCer-activated NKT cells exhibit cytotoxic activity and exert anti-tumor effects\(^6\). Therefore, manipulation of immune system with \(\alpha\)-GalCer has a potential to become an effective tool in cancer immunotherapy. In fact, several clinical trials against cancer using \(\alpha\)-GalCer have already been reported\(^9\). Considering its clinical applications, it seems important to examine the \(\alpha\)-GalCer-induced immune responses in cancer-bearing hosts. Using clinical samples obtained from cancer patients, we have reported that responses of \(\gamma\)\(\delta\) NKT cells against \(\alpha\)-GalCer to proliferate or produce cytokines were impaired\(^4\). These observations prompted us to investigate \(\alpha\)-GalCer-induced immune responses in cancer-bearing mice and examine corresponding mechanisms.

**Hyporesponsiveness of NKT cell in cancer**

Using mouse cancer model, we first examined \(\alpha\)-GalCer-induced cell proliferation and cytokine production\(^8\). Mouse splenocytes from either naïve or cancer-bearing mice were stimulated with \(\alpha\)-GalCer. In the culture of splenocytes from naïve mice, NK1.1\(^+\) TCR \(\beta\)^+ population expanded well by day 7. In contrast, this expansion of NK1.1\(^+\) TCR \(\beta\)^+ cells in B16- or 3LL Lewis lung cancer-bearing mice was significantly lowered. Thus, the \(\alpha\)-GalCer-induced cell expansion of NK1.1\(^+\) TCR \(\beta\)^+ population is impaired in cancer-bearing mice. Upon \(\alpha\)-GalCer injection, the levels of both IFN-\(\gamma\) and IL-4 in the sera of B16-bearing mice were significantly lower than those in naïve mice. When splenocytes from cancer-bearing mice were stimulated with \(\alpha\)-GalCer in vitro, reduced level of both IFN-\(\gamma\) and IL-4 production in the supernatants was observed compared with those from naïve mice.

We also examined whether \(\alpha\)-GalCer-induced cytotoxic activity in the spleens differs between naïve and cancer-bearing mice\(^9\). Spleen cells obtained from naïve mice which had been injected with \(\alpha\)-GalCer showed significant cytotoxicity against both YAC-1 and B16 cells. However, when B16-bearing mice were injected with \(\alpha\)-GalCer, the cytotoxicity induced in the spleens was significantly reduced to both targets. These results indicate that \(\alpha\)-GalCer-induced cytotoxicity in the spleen is impaired in the cancer-bearing state. We further evaluated the anti-metastatic effect of \(\alpha\)-GalCer in cancer-bearing status. In naïve mice which had been i.v. injected with 3LL cells, treatment with \(\alpha\)-GalCer effectively inhibited the formation of lung metastasis. In contrast, in cancer (3LL)-bearing mice, \(\alpha\)-GalCer did not efficiently prevent the lung metastasis, indicating that anti-metastatic effect of \(\alpha\)-GalCer is impaired in cancer-bearing status.

**Mechanism of the hyporesponsiveness**

What is the mechanism for the suppression of NKT cells in cancer-bearing state? We focused on the role of CD11b\(^+\) Gr-1\(^+\) cells in the hyporesponsiveness to \(\alpha\)-GalCer in cancer-bearing mice, because the proportion and absolute number of CD11b\(^+\) Gr-1\(^+\) cells were increased in cancer-bearing mice\(^8\). CD11b\(^+\) cells and Gr-1\(^+\) cells were separately isolated from naïve and cancer-bearing mice, then added to freshly isolated naïve splenocytes cultured with \(\alpha\)-GalCer. We found in this coculture experiments that cytokine production by NKT cells was significantly impaired by the addition of CD11b\(^+\) Gr-1\(^+\) cells derived from cancer-bearing mice. We further tested a possible role of nitric oxide (NO) in the \(\alpha\)-GalCer hyporesponsiveness. We pretreated the CD11b\(^+\) Gr-1\(^+\) cells with iNOS inhibitor (l-NMMA) and added them to the coculture. This pretreatment canceled the suppression, thus we concluded that CD11b\(^+\) Gr-1\(^+\) cells from cancer-bearing mice induce the hyporesponsiveness to \(\alpha\)-GalCer in a NO-mediated fashion.

We finally injected all-trans retinoic acid (ATRA) to the cancer-bearing to induce differentiation of the CD11b\(^+\) Gr-1\(^+\) cells\(^8\). In fact, the number of CD11b\(^+\) Gr-1\(^+\) cells in spleens of cancer-bearing mice was significantly reduced by the ATRA treatment. Accordingly, this treatment restored the \(\alpha\)-GalCer-induced cytokine production from cancer-bearing mice, indicating that the ATRA treatment could reverse defective NKT cell response to \(\alpha\)-GalCer in cancer-bearing mice.

**Discussion**

In our previous human study, T cell-depleted fraction in peripheral blood mononuclear cells (containing myeloid cell fraction) was responsible for the hyporesponsiveness of \(\gamma\)\(\delta\) NKT cells of cancer patients\(^8\). This is consistent with the fact in the animal study which indicated that CD11b\(^+\) Gr-1\(^+\) myeloid cells were responsible for the NKT cell suppression in cancer. Since CD11b\(^+\) Gr-1\(^+\) cells are a heterogeneous population of myeloid cells that comprises immature macrophages, granulocytes and dendritic cells (DCs), these cells have been called “immature myeloid suppressor cells”\(^7\). The myeloid suppressor cells are known, in fact, to be able to suppress diverse kind of immune cells, including T cells\(^8\). It has been also known that the myeloid suppressor cells can produce NO which induces cell-type-independent suppression. Therefore, the NO-mediated NKT cell suppression may be one of immunosuppressive events ob-
CD11b+ Gr-1+ cell-derived NO suppresses NKT cell function in cancer-bearing state. This could be canceled by the reduction of CD11b+ Gr-1+ population or blocking of NO.

CD11b+ Gr-1+ cells were also examined in another model of cancer-mediated immune dysfunction. Terabe et al. have reported that CD11b+ Gr-1+ cells, which are stimulated by IL-13 produced by non-Vα14Jα281 CD1d-reactive T cells, induce suppression of tumor immunosurveillance of 15-12RM tumor through their TGF-β production. However, in our model, blocking of TGF-β did not restore the cytokine production by NKT cells, suggesting a little contribution of TGF-β in this hyporesponsiveness. Instead, we have identified the NO-mediated suppression mechanism, which was restored by the differentiation of CD11b+ Gr-1+ cells with ATRA (Fig. 1).

When considering a cancer immunotherapy using α-GalCer, we should be careful in the suppression of NKT cell function. To overcome this, it could be beneficial to combine some therapies, including a differentiation-inducer which could reduce the size of the immature myeloid suppressor cell populations.

References