Mini Review

Cancellation of NKT cell immunosuppression targeting myeloid suppressor cells

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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 $CD11b^+$ $Gr\text{-}1^+$ 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 $CD11b^+$ $Gr\text{-}1^+$ cells suppressed NKT cell function in a nitric oxide-mediated fashion. To reduce the population of $CD11b^+$ $Gr\text{-}1^+$ cells, we administered a retinoic acid to cancer-bearing mice. This treatment significantly reduced the population of $CD11b^+$ $Gr\text{-}1^+$ 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 $CD11b^+$ $Gr\text{-}1^+$ cell functions.

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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 humans[1)]. NKT cells recognize α-galactosylceramide (α-GalCer) and its analogues; glycolipids that can

be presented by CD1d[2]. It has been shown that α-GalCer selectively stimulates NKT cells to produce large amount of both T helper 1 (Th1) and Th2 cytokines, and that α-GalCer-activated NKT cells exhibit cytolytic activity and exert anti-tumor effects[2]. Therefore, manipulation of immune system with α-GalCer has a potential to become an effective tool in cancer immunotherapy. In fact, several clinical trials against cancer using α-GalCer have already been reported[3]. Considering its clinical applications, it seems important to examine the α-GalCer-induced immune responses in cancer-bearing hosts. Using clinical samples obtained from cancer patients, we have reported that responses of Vα24 NKT cells against α-GalCer to proliferate or produce cytokines were impaired[4,5]. These observations prompted us to investigate α-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 α-GalCer-induced cell proliferation and cytokine production[6]. Mouse splenocytes from either naïve or cancer-bearing mice were stimulated with α-GalCer. In the culture of splenocytes from naïve mice, NK1.1$^+$ TCR β$^+$ population expanded well by day 7. In contrast, this expansion of NK1.1$^+$ TCR β$^+$ cells in B16- or 3LL Lewis lung cancer-bearing mice was significantly lowered. Thus, the α-GalCer-induced cell expansion of NK1.1$^+$ TCR β$^+$ population is impaired in cancer-bearing mice. Upon α-GalCer injection, the levels of both IFN-γ and IL-4 in the sera of B16-bearing mice were significantly lower than those in naïve mice. When spleoncytes from cancer-bearing mice were stimulated with α-GalCer *in vitro*, reduced level of both IFN-γ and IL-4 production in the supernatants was observed compared with those from naïve mice.

We also examined whether α-GalCer-induced cytotoxic activity in the spleens differs between naïve and cancer-bearing mice[6]. Spleen cells obtained from naïve mice which had been injected with α-GalCer showed significant cytotoxicity against both YAC-1 and B16 cells. However, when B16-bearing mice were injected with α-GalCer, the cytotoxicity induced in the spleens was significantly reduced to both targets. These results indicate that α-GalCer-induced cytotoxicity in the spleen is impaired in the cancer-bearing state. We further evaluated the anti-metastatic effect of α-GalCer in cancer-bearing status. In naïve mice which had been i.v. injected with 3LL cells, treatment with α-GalCer effectively inhibited the formation of lung metastasis. In contrast, in cancer (3LL)-bearing mice, α-GalCer did not efficiently prevent the lung metastasis, indicating that anti-metastatic effect of α-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 α-GalCer in cancer-bearing mice, because the proportion and absolute number of CD11b$^+$ Gr-1$^+$ cells were increased in cancer-bearing mice[6]. CD11b$^+$ cells and Gr-1$^+$ cells were separately isolated from naïve and cancer-bearing mice, then added to freshly isolated naïve splenocytes cultured with α-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 α-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 α-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[6]. 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 α-GalCer-induced cytokine production from cancer-bearing mice, indicating that the ATRA treatment could reverse defective NKT cell response to α-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 Vα24 NKT cells of cancer patients[4]. 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-

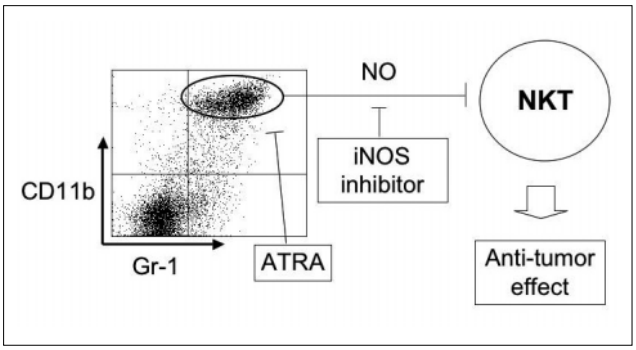

Fig.1 CD11b$^+$ Gr-1$^+$ cell-derived NO suppresses NKT cell function in cancer-bearing state
This could be canceled by the reduction of CD11b$^+$ Gr-1$^+$ population or blocking of NO.

served in cancer patients. It is possible that the myeloid suppressor cells can suppress NKT cells bearing non-Vα14Jα281T cell receptor.

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[9]. However, in our model, blocking of TGF-β did not restore the cytokine production by NKT cells[6], 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.

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