Enhanced Suppression of Pulmonary Metastasis of Malignant Melanoma Cells by Combined Administration of α-Galactosylceramide and Interleukin-18

INTRODUCTION:
Administration of α-galactosylceramide (α-GalCer) into mice has been shown to activate natural killer (NK) T cells, which have the additional effect of stimulating NK cells and play a major role in the α-GalCer-induced antitumor effect in vivo. Interleukin (IL)-18 also exhibit antitumor effects through both NK and NKT cells. Thus, it is postulated that combined administration of IL-18 and α-GalCer may induce further increased antitumor effects. In this study, we analyzed the effect of combined administration of α-GalCer and IL-18 on the growth of B16 melanoma cells metastasized to the lung as compared to isolated administration.

METHODS:
Experimental animal: C57BL/6 mice 20 mice per group were used in the study.

Experimental pulmonary metastasis: B16 melanoma cell suspension (5x10^5 cells/0.2 ml) was injected into a tail vein of each mouse. The mouse was sacrificed 14 days later, and metastatic foci on the lung were counted under a dissection microscope.

Administration of α-GalCer, IL-18: α-GalCer (2 µg) was dissolved in 0.2 ml PBS and injected into a tail vein of a mouse. IL-18 (2 µg) was dissolved in 0.2 ml PBS containing 0.1% mouse serum and injected into a mouse intraperitoneally.

Cytotoxicity assay: Purified NK cells (DX-5^+NK1.1^+TCR-β^+) were obtained from the spleen of mice treated with vehicle, α-GalCer, or IL-18 alone or α-GalCer in combination with IL-18. B16 melanoma cells labeled with ^51Cr (target cells [T]) were mixed with purified NK cells (effector cells [E]) at various T:E ratios (1/100–1/12.5). The cells were incubated for 4 h. The released radioactivity was determined.

Effect of anti-asialo GM1 serum on populations of NK cells and NKT cells of lymphocytes obtained from the lung or spleen by a percentage of anti-asialo GM1 mAb for analysis of the NKT-cell population. Cells were analyzed by three-color flow cytometric analysis with APC-conjugated streptavidin–biotin-labeled anti-TCR-α mAb and PE-labeled anti-mouse IgG1 mAb and FITC-labeled anti-TCR-β mAb.

Numbers of NK cells and NKT cells in the lung and spleen: Splenic and lung lymphocytes were incubated with FITC-labeled anti-TCR-β mAb and PE-labeled anti-NK1.1 mAb for analysis of the NK cell population, or with FITC-labeled anti-TCR-β mAb and α-GalCer-loaded Dimer X conjugated with PE-labeled antimonimouse IgG1 mAb, and FITC-labeled anti-TCR-β mAb.

RESULTS:
Effect of administration of α-GalCer and IL-18 on pulmonary metastasis: α-GalCer alone or IL-18 alone decreased the number of pulmonary metastatic foci by approximately 50%, and in combination by approximately 80%. Treatment with anti-asialo GM1 serum markedly increased the number of pulmonary metastatic foci and the combination of IL-18 and α-GalCer did not reduce it significantly. Treatment of mice with anti-asialo GM1 serum completely erased the effect of the combined administration of α-GalCer and IL-18.

Effects of administration of α-GalCer and IL-18 on the cytotoxicity of NK cells and numbers of NK and NKT cells in the lung and spleen. α-GalCer or IL-18 alone increased the antitumor cytotoxicity of NK cells significantly, and the combination of α-GalCer and IL-18 further enhanced the antitumor cytotoxicity. IL-18 had no effect on the numbers of NK cells and NKT cells in the lung, and α-GalCer increased these cells slightly. In contrast, the combination of α-GalCer and IL-18 greatly increased the numbers of these cells. In the spleen, IL-18 alone did not significantly change the numbers of NK cells and NKT cells, but α-GalCer alone increased these cells. No cooperative effect of α-GalCer and IL-18 was observed in the spleen.

Effect of administration of α-GalCer and IL-18 on the NKT cell-dependent and NK cell-independent secretion of cytokines. NKT cell-dependent and NK cell-independent secretion of these cytokines was evaluated by a difference in the serum cytokine levels between normal and NKT cell-deficient mice treated with anti-asialo GM1 serum. α-GalCer induced NKT cell-dependent and NK cell-independent secretion of IL-2, IL-4, IFN-γ, IL-12, GM-CSF, TNF-α, and IL-10 after the α-GalCer injection. By contrast, IL-18 had little effect on NKT cell-dependent and NK cell-independent secretion of IL-2, IL-4, IFN-γ, and GM-CSF, although IL-18 slightly enhanced that of IL-12, TNF-α, and IL-10. IL-18 in combination with α-GalCer had an enhancing effect on the NKT cell-dependent and NK cell independent secretion of IL-2, IL-4, IFN-γ, and GM-CSF, but the effect was subtle.

DISCUSSION:
Results of this study suggest that α-GalCer-induced antitumor effect is achieved by activation of NK cells through the α-GalCer-induced activation of NKT cells. The enhancement of the antitumor effect by α-GalCer and IL-18 seems to be induced by their effects on the number and cytotoxic activity of NK cells in the lung. Based on these findings, it is thought that combined administration of IL-18 and α-GalCer exerts an antitumor effect on NK cell-sensitive tumors primarily by the direct stimulation of NK cells by IL-18 and the indirect stimulation of NK cells by α-GalCer through its activation of NKT cells (Figure 1).

REFERENCE:

Figure 1. Mechanism of effects on NK cells induced by the combination of α-GalCer and IL-18.