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Insufficient *ex vivo* expansion of Vα24$^+$ natural killer T cells in malignant lymphoma patients related to the suppressed expression of CD1d molecules on CD14$^+$ cells

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Background

Vα24$^+$ natural killer T (NKT) cell is a human counterpart of mice Vα14$^+$ NKT cell that has a regulatory role for innate and acquired potential antitumor activity. The efficient expansion of NKT cells is an obstacle to the clinical application of Vα24$^+$ NKT cells for immunotherapy.

Methods

We used mononuclear cells (MNC) obtained from the peripheral blood (PB) of normal healthy donor (HD) and malignant lymphoma (ML) patients before and after granulocyte colony-stimulating factor (G-CSF) treatment. MNC were cultured for 12 days with α-galactosylceramide (100 ng/mL) and interleukin-2 (IL-2; 100 U/mL).

Results

The fold expansion of Vα24$^+$ NKT cells was higher in HD than in ML patients (208 versus 0.00), despite comparable numbers of Vα24$^+$ NKT cells before culture. G-CSF administration enhanced the predominance of Vα24$^+$ NKT cell fold expansion in HD compared with ML patients (1935 versus 1.95). After treatment with G-CSF, the expression of CD1d molecules was up-regulated in CD14$^+$ cells from HD but not ML patients. The fold expansion of Vα24$^+$ NKT cells and CD1d expression on CD14$^+$ cells was strongly correlated in both HD and ML patients ($r^2 = 0.84$). However, replacement of a patient's CD14$^+$ cells with HD cells did not increase the efficacy of Vα24$^+$ NKT cell expansion.

Discussion

G-CSF-mobilized PB from ML patients has inhibitory characteristics for Vα24$^+$ NKT cell expansion as a result of both monocytes and Vα24$^+$ NKT cells. Multiple procedures would be needed for the expansion of patients' Vα24$^+$ NKT cells.

Keywords

CD1d, α-galactosylceramide, granulocyte colony-stimulating factor, malignant lymphoma, natural killer T cells.

Introduction

Vα24$^+$ natural killer T (NKT) cells are the human counterpart of murine Vα14$^+$ NKT cells. Human NKT cells express a CD1d-restricted, invariant T-cell receptor (TCR) chain, the Vα24-JαQ α-chain, which is preferentially coupled with Vβ11 chains; human NKT cells share some common immunobiologic characteristics with murine NKT cells [1]. NKT cells recognize and respond to glycolipid antigen (Ag) presented by the CD1d molecule and produce large amounts of cytokines, such as interferon (IFN)-γ and interleukin (IL)-4, which exert strong cytotoxicity for various cancer cells and regulate autoimmunity, respectively [2,3]. The production of these cytokines has a critical and crucial role for initial immune

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responses and tumor rejection directly or indirectly via dendritic cells (DC), activated T cells and natural killer (NK) cells [4].

NKT cells are reactive to the non-classical class I Ag-presenting molecule CD1d and they recognize glycolipid Ag [2,5]. The CD1d family of MHC-unlinked class Ib molecules is conserved through mammalian species [6,7]. The CD1d molecule is necessary for immune responses to several microbial infections in mice [8]. With regard to tumor immunology, two major subpopulations of NKT cells, CD1d-restricted and CD1d-unrestricted, have been identified; CD1d-restricted NKT cells are mainly involved in tumor immunity but little is known about the characteristics of CD1d-unrestricted NKT cells [9]. What is known is that no NKT cells develop in the absence of CD1d [10].

α-Galactosylceramide (α-GalCer) is a specific ligand for human $V\alpha24^+$ NKT cells and murine $V\alpha14^+$ NKT cells [2]. Both types of NKT cells are activated by α-GalCer presented by CD1d. Because CD1d is a class I molecule expressed mainly on Ag-presenting cells (APC), such as DC, macrophages and B cells, it is speculated that NKT cells interact primarily with APC [5,11]. After stimulation with α-GalCer, $V\alpha24^+$ NKT cells exhibit CD1d-dependent cytotoxicity against various types of tumor cells [11,12]. In contrast, some researchers report that $V\alpha24^+$ NKT cells are cytotoxic against CD1d-negative cells; this observation suggests that α-GalCer is not essential for CD1d-independent cytotoxicity. Thus it is possible that $V\alpha24^+$ NKT cells involved in tumor immunity are activated through the recognition of the α-GalCer–CD1d complex, although the CD1d molecule is not necessary in some killing stages.

We are attempting to use NKT cells for adaptive immunotherapy [11,13]. However, the extremely low frequency of $V\alpha24^+$ NKT cells in human peripheral blood (PB) [2,14,15], which is even lower in cancer patients than in healthy individuals [12,16,17], is an obstacle for their clinical application. To overcome this problem, the establishment of an effective *in vitro* expansion system for $V\alpha24^+$ NKT cells by stimulation with α-GalCer has been explored by several research groups, including ours. Previously, we observed that $V\alpha24^+$ NKT cells could be expanded effectively from human granulocyte colony-stimulating factor (G-CSF)-mobilized PB cells upon stimulation with α-GalCer and IL-2 [18], and we established an efficient non-fetal bovine serum (FBS) expansion system for $V\alpha24^+$ NKT cells to remove the potential risks related with FBS [19]. Consequently, we reported the essential effect of $CD14^+$ cells for *ex vivo* expansion of human NKT cells [20]. In the present report, we show that one mechanism of reduced expansion of $V\alpha24^+$ NKT cells in mononuclear cells (MNC) obtained from malignant lymphoma (ML) patients is the suppressed expression of the CD1d molecule on $CD14^+$ cells, and we discuss the important roles of the CD1d molecule on monocytes in the *ex vivo* expansion of human NKT cells.

Methods

Cells and plasma derived from healthy donors and ML patients

This study was approved by the National Cancer Center Institutional Review Board and written informed consent was obtained from the healthy donor (HD) volunteers and ML patients. PB and apheresis products were obtained from normal healthy individuals who donated PB stem cells for allogeneic transplants and from consecutive patients (from July 2004 to December 2004) with ML who would undergo autologous stem cell transplantation. In using apheresis products, leftover blood was used for healthy and autologous donors. Before and after G-CSF mobilization (pre- and post-G-CSF), the samples were manipulated immediately, and the cell fraction and plasma were separated by centrifugation at 3000 r.p.m. for 15 min. Plasma samples from both the PB and apheresis products were heat-inactivated immediately after separation and stored at $-80^{\circ}C$ before use. MNC were isolated from PB and apheresis products by Ficoll–Hypaque (Immuno-Biological Laboratories, Gunma, Japan) density-gradient centrifugation.

G-CSF procedure for apheresis donors

Apheresis was indicated for HD whose related patients needed PB stem cell transplantation or patients who would receive autologous stem cell transplantation after high-dose chemotherapy. This indication was determined by the clinical team of the stem cell transplantation unit in our hospital (National Cancer Center). G-CSF was administered subcutaneously at a dose of 300 $\mu g/m^2$ divided twice a day for 3 days just before the apheresis procedure. On the morning of the apheresis day, additional G-CSF was administered.

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