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【 CASE REPORT 】

Expansion of NKG2C-expressing Natural Killer Cells after Umbilical Cord Blood Transplantation in a Patient with Peripheral T-cell Lymphoma with Cytotoxic Molecules

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Abstract:
A 64-year-old woman presented with generalized lymphadenopathy and systemic manifestations. The examination of a biopsy specimen revealed peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) expressing cytotoxic molecules. Umbilical cord blood transplantation was successful during a partial remission state after the administration of salvage chemotherapy. The donor-derived large granular lymphocytes started to increase as a result of cytomegalovirus reactivation. The fraction of natural killer (NK) cells expressing the NKG2C molecule accounted for one-third of the total lymphocytes for almost two years. We implicitly indicate the association between the persistence of donor-derived NKG2C$^+$ NK cell-expansion and maintaining a complete remission in similar cases of aggressive PTCL-NOS.

Key words: umbilical cord blood transplantation, natural killer cell, peripheral T-cell lymphoma not otherwise specified

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Introduction

The NKG2C molecule is an activating natural killer (NK) cell receptor of the C-type lectin superfamily that binds to human leukocyte antigen (HLA)-E and which is a triggering receptor for anti-cytomegalovirus (CMV) immunity (1). The reactivation of CMV after allogeneic hematopoietic stem cell transplantation (HSCT) was associated with a lower risk of relapse in patients with acute myeloid leukemia (AML) (2). The two-year relapse rate of hematological malignancies was low patients who underwent allogeneic HSCT who showed CMV reactivation and in whom the number of peripheral blood CD56^{dim}/CD57$^+$/NKG2C$^+$ NK cells increased to >2.5×10^6/L at six months after allogeneic HSCT (3). An *in vitro* analysis revealed that NKG2C$^+$ NK cells were capable of producing tumor necrosis factor and interferon gamma against a leukemia cell line (3). Thus, a bold hypothesis has been proposed: that the CMV reactivation induces an increase in the number of NKG2C$^+$ NK cells, which have a direct graft-versus-leukemia effect (3). The literature lacks reports on the association between the expansion of NKG2C$^+$ NK cells and the outcome of peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) after allogeneic HSCT, which prompted us to conduct the present study.

Case Report

A 64-year-old woman presented with general fatigue, systemic edema, pleural effusion, hypoxemia, lymphadenopathy, and hepatosplenomegaly. A blood analysis revealed the following findings: hemoglobin, 6.6 g/dL; platelet count, 5.8 ×10^{10}/L; white blood cell (WBC) count, 5.87×10^{10}/L with 23% of the abnormal lymphoid cells (Fig. 1A), with a phenotype of CD2$^+$, CD3$^+$, CD4$^+$, CD5$^-$, CD7$^+$, CD56$^+$, CD57$^-$, T-cell receptor (TCR) αβ$^+$, and HLA-DR$^+$. A clonal TCR beta chain gene rearrangement was confirmed. The serum

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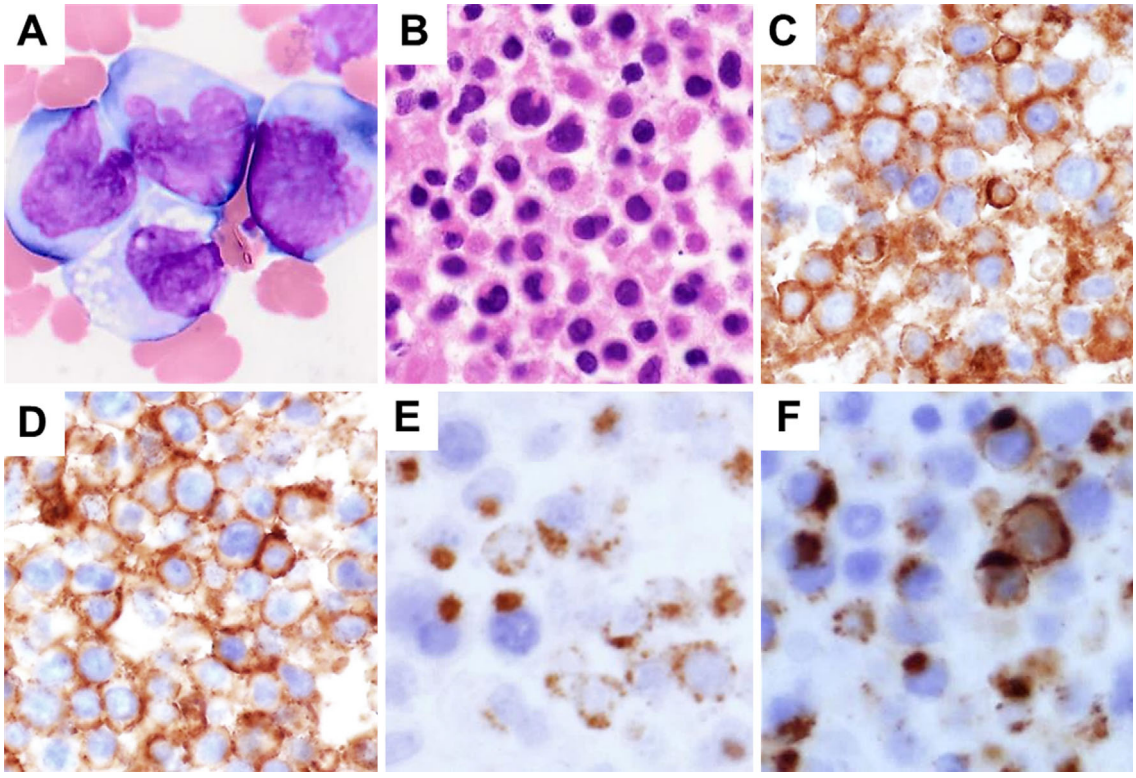

Figure 1. **(A) Peripheral blood smear with circulating lymphoma cells (Wright-Giemsa, ×1,000). (B) Hematoxylin and Eosin staining of an axillary lymph node sample (×400). The immunohistochemical findings of an axillary lymph node sample (×400): (C) CD4, (D) CD56, (E) TIA-1, and (F) granzyme B**

levels of lactate dehydrogenase, serum soluble interleukin (IL)-2 receptor and ferritin increased to 2,542 IU/L, 21,800 U/mL, and 4,468 ng/mL, respectively. The patient was seronegative for human T-cell lymphotropic virus type I. Bone marrow aspiration revealed 40.4% abnormal cells with a G-banding karyotype of 52,XX,+X,+del(2)(q?),+5,+7,+17,-18, add(19)(p13),+r1,+mar1 [7 cells] / 46,XX [13 cells]. An axillary lymph node biopsy specimen showed the diffuse proliferation of medium-to-large lymphoid cells with an abundant cytoplasm and large pleomorphic nuclei (Fig. 1B). Immunohistochemical staining of an axillary lymph node biopsy specimen was positive for CD3, CD4 (Fig. 1C), CD56 (Fig. 1D), T-cell intracellular antigen-1 (Fig. 1E), and granzyme B (Fig. 1F), and negative for CD8, CD10, CD30, B-cell lymphoma (BCL)-6, anaplastic lymphoma kinase, and programmed cell death 1. *In situ* hybridization for Epstein-Barr virus (EBV)-encoded small nuclear RNA was negative. A pathological diagnosis of PTCL-NOS with cytotoxic molecules was confirmed. Computed tomography (CT) revealed massive pleural thickening on the left side, and prominent hepatosplenomegaly (Fig. 2A and B). An endoscopic examination showed that the diffuse infiltration of lymphoma cells had caused a large gastric ulcer (Fig. 2C and D). The patient was classified as group 4 according to the prognostic index for PTCL-NOS (4).

Shortly after undergoing chemotherapy with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisolone), she developed nuchal lymphadenopathy and an extramedullary tumor in her left lower leg. The abnormal cells persisted in the peripheral blood. A partial remission (PR) was achieved with the DeVIC regimen (dexamethasone, etoposide, ifosfamide, and carboplatin) followed by high-dose cytarabine. She had no HLA-matched sibling and there was no time to identify a matched unrelated donor. After reduced-intensity conditioning with melphalan, fludarabine, and total body irradiation, umbilical cord blood (UCB) transplantation was performed with 3.02×10^7/kg body weight of total nucleated cells (Fig. 3). There were no mismatches in the graft-versus-host direction in two HLA alleles (A and DR loci). Tacrolimus was administered by continuous infusion, and minidose methotrexate (MTX) was administered intravenously on post-transplant days 1, 3, and 6, at a dose of 5 mg as prophylaxis against graft-versus-host disease (GVHD). She developed acute GVHD (involving cutaneous lesions) on day 35; this was alleviated with low-dose steroid therapy. An antigenemia assay for CMV pp65 was performed weekly, and was positive at six weeks and ten weeks after umbilical cord blood transplantation (UCBT). The monocyte count at 6 weeks after UCBT was 1.058×10^9/L. The average trough level of tacrolimus was 9.2 ng/mL from 5 to 10 weeks after UCBT. The number of large granular lymphocytes (LGL) with a donor-type karyotype started to increase at 8 weeks after UCBT and stabilized at 23-36% of the WBCs (Fig. 3). At 17 weeks after UCBT, ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET)/CT showed a nodule of 20 mm diameter in

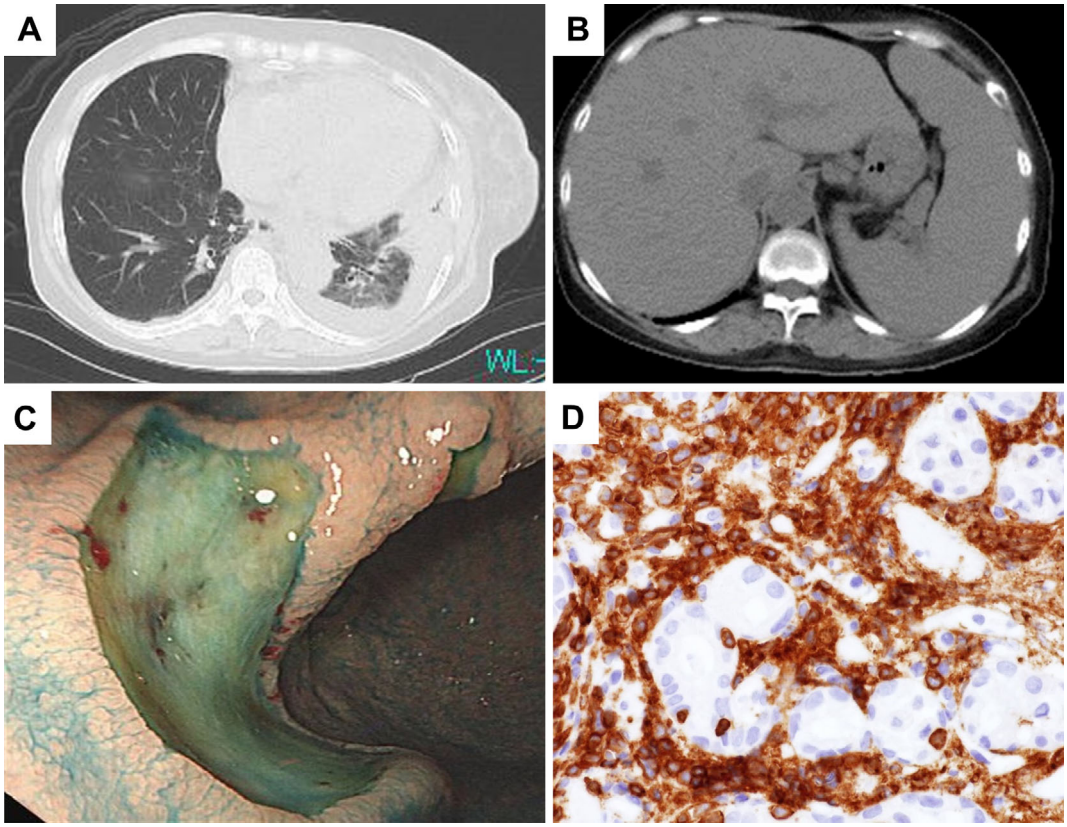

Figure 2. **Computed tomography revealed massive pleural thickening on the left side (A) and prominent hepatosplenomegaly (B). An endoscopic examination showed multiple gastric ulcers (C) caused by the diffuse infiltration of $CD3^+$ lymphoma cells (D).**

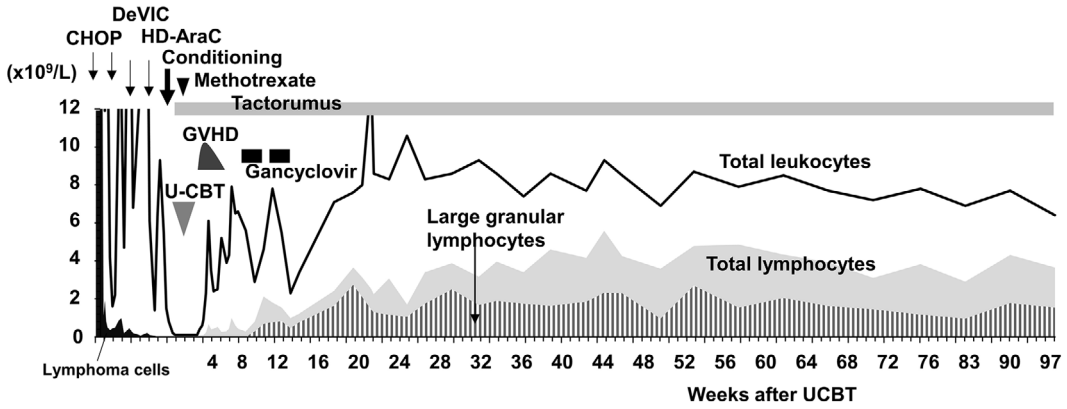

Figure 3. **Time course of the measurement of the leukocyte subsets in the peripheral blood before and after umbilical cord blood transplantation. Arrows indicate two cycles of CHOP, DeVIC, high-dose cytarabine (HD-AraC), and a conditioning regimen (in that order). The gray area indicates the skin manifestations of graft-versus-host disease.**

the left upper lobe and accumulation with a standardized FDG uptake value (SUV) of 1.81. There was no pathological uptake of FDG in the other parts of the body. At 20 weeks after UCBT, the patient underwent video-assisted thoracic surgery and left upper wedge resection. The pathological diagnosis was well-differentiated adenocarcinoma. The regional lymph nodes showed anthracosis; however, we did not detect metastasis or the invasion of lymphoma cells. Thereafter, the number of LGLs persistently increased. Microscopic examinations revealed 2.494×10^9/L LGLs at 29 weeks after UCBT and 1.536×10^9/L LGLs at 97 weeks after UCBT (Fig. 3). Flow cytometry revealed that the total number of $CD56^{dim}/CD57^+/NKG2C^+$ NK cells was 8.1×10^8/L at 29 weeks and 8.7×10^8/L at 97 weeks (Fig. 4A). The frequency of $CD4^+$ cells, $CD8^+$ cells, and $CD56^{dim}/CD57^+/NKG2C^+$ NK cells had among the total lymphocyte populations increased from 6% to 8%, 9% to 14%, 21% to 24%, respectively, between 29 weeks and 97 weeks after UCBT

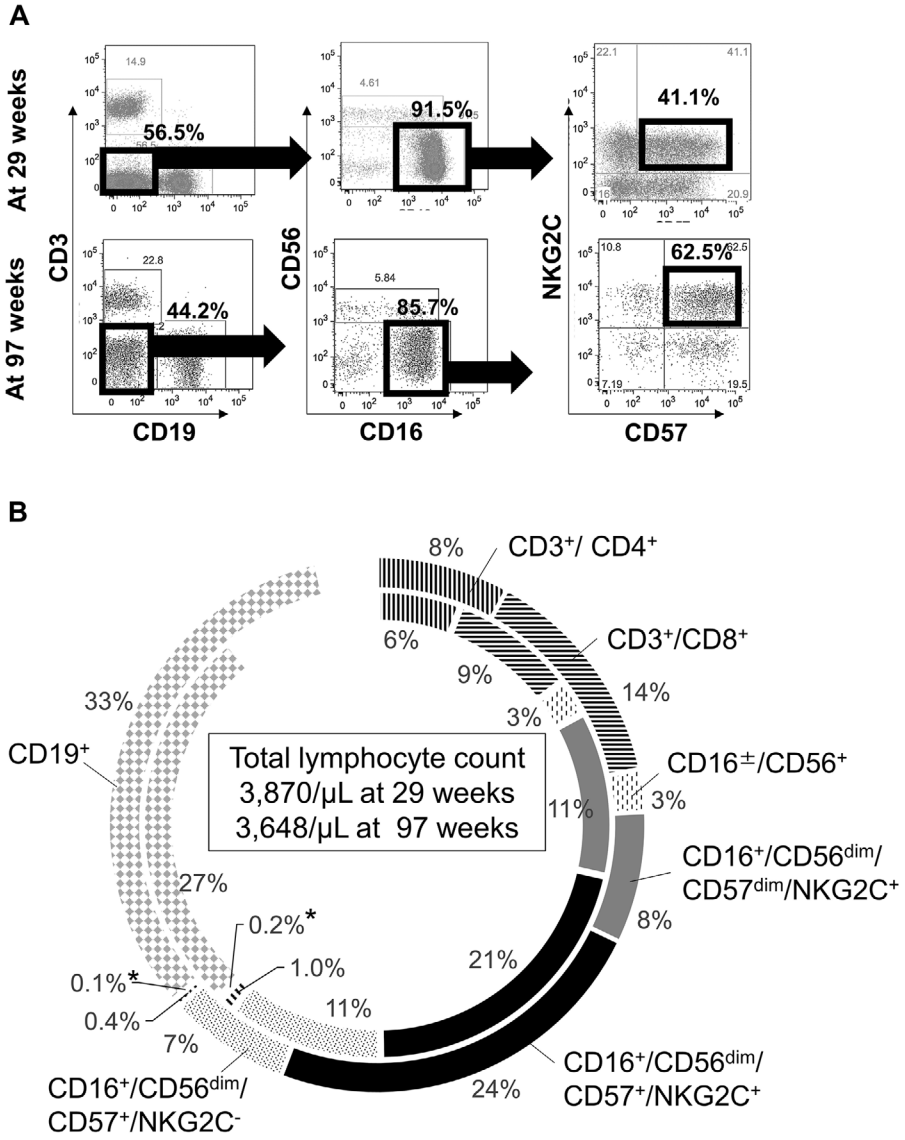

Figure 4. **Flow cytometric analysis of peripheral blood obtained at 29 weeks and at 97 weeks after umbilical cord blood transplantation (UCBT). A: The $CD3^-/ACD19^-$ lymphocyte-gated cells were displayed on a plot of CD16 versus CD56 expression. The fraction of mature NK cells with $CD56^{dim}/CD16^+$ was gated, and the fraction of the terminal differentiated NK cells with $CD56^{dim}/CD57^+/NKG2C^+$ was elucidated. B: The pie charts show the fraction of each peripheral lymphocyte population at 29 weeks (inside charts) and at 97 weeks (outside charts) after UCBT. Each type and its content among total lymphocyte count was indicated. The remaining small fractions contained $CD16^+/CD56^{dim}/NKG2A^+$ cells (oblique lines) and $CD16^+/CD56^{dim}/CCR7^+$ cells (asterisks). The remaining uncharacterized populations are blanked.**

(Fig. 4B). No reactivation of CMV was observed after three months post-UCBT and a complete remission (CR) from PTCL-NOS has been maintained for 27 months, with a Karnofsky performance status of 90-100%.

Discussion

The prevalence of PTCL-NOS expressing cytotoxic molecules is low, and the prognosis is quite poor (Table). The majority of patients with this subtype of PTCL-NOS have shown a poor performance status, B-symptoms, and extranodal involvement (5, 6). Only 11 of 144 patients with PTCL-NOS were found to express the cytotoxic T-cell signature (7) and the patients with this signature showed a five-year overall survival (OS) rate of <20% (8). The five-year OS rate in a study that investigated 39 patients who were EBV-negative with nodal PTCL expressing cytotoxic molecules, was 17% (9). The present case was consistent with these conditions, and a poor outcome had been predicted. In patients with relapsed PTCL, salvage autologous HSCT is associated with a 5-year OS rate of 33% and a 5-year progression-free survival rate of 24% (10); a CR before salvage autologous HSCT is a significant factor for a favorable outcome (10). In the present case, we were concerned about

Table. Comprehensive Review of Case Series with PTCL-NOS Expressing Cytotoxic Molecules.

N	Characteristics	Outcome	References
3	Lymphadenopathy, leukemic presentation, splenomegaly, $CD3^+$, $CD56^+$, and EBV negative	Allo-HSCT in one, planning to allo-HSCT in another, and death at 6 months in the other patient.	[5]
41	Onset age median 55 yr, B-symptoms 68%, bone marrow involvement 33%, extranodal involvement>1 37%, stage IV 68%, IPI high 46%, and PIT group 4 46%	Chemotherapy with anthracycline 71%, auto HSCT in 3 patients, CR 30%, no response 41%, 5-year OS 17%, and median OS 4.2 months.	[6]
11	$CD4^+$: 6/11 patients. IFN responsive genes, granule secretion, T-bet (TBX21), eomesodermin, CXCR3, IL-2RB, CCL3, and mTOR pathway were highly expressed.	Median PFS less than 1 year in 11 patients	[7]
ND	TBX21, cytotoxic molecules, CXCR3, CXCL12, and CCL-2,-3,-6,-11 were highly expressed.	Poor prognosis among PTCL-NOS	[8]
39	Nodal & EBV negative PTCL: $CD4^+$65%, $CD56^+$23%, onset age median 62 yr, PS 3/4 25%, stage III/IV 72%, IPI high-intermediate/high 57%, PIT group 3/4 70%	CR 37%, PR 17%, autologous HSCT 15%, median OS 4.7 months	[9]

N: number of patients, Allo-HSCT: allogeneic hematopoietic stem cell transplantation, IPI: the international prognostic index, PIT: prognostic index for PTCL-NOS, OS: overall survival, CR: complete remission, GEP: gene expression profiling, IFN: interferon, PFS: progression free survival, ND: not described

persisting monoclonal T-cells in the peripheral blood and the possibility that they would contaminate the auto-grafting of the transplanted cells. Thus, allogeneic HSCT was planned as an alternative strategy. A small study demonstrated promising results with a 3-year OS rate of 87% among patients with PTCL who had achieved a CR/PR before allogeneic HSCT (11). The present patient had achieved a PR state before UCBT. According to a comparative study of matched unrelated donor (MUD) transplantation and UCBT for mature lymphoid malignancies, the incidence of chronic GHVD at 3 years in MUD recipients was higher than that in UCB recipients (52% vs. 26%, <0.0001), but the relapse rates at three years were similar in patients with lymphoid malignancies (35% vs. 28%, not significant) (12). The present report is also consistent with the fact that UCB might represent an alternative donor source for PTCL-NOS.

The rapid expansion of $NKG2C^+$ NK cells following CMV reactivation was first reported in 10 UCBT recipients; in 9 of these cases a CR from myeloid malignancy was achieved (13). Among 674 allogeneic HSCT-recipients, including 471 UCBT recipients, the association between the expansion of $NKG2C^+$ NK cells and the lower relapse rate was demonstrated as described (3). The relapse rates were compared between myeloid and lymphoid malignancies in cases with the expansion of $NKG2C^+$ NK cells; however no definitive conclusion was reached (3). We confirmed that the fraction of $NKG2C^+$ NK cells accounted for one third of the total lymphocytes at up to 2 years after UCBT. Although we could not demonstrate the direct graft-versus-lymphoma effect of $NKG2C^+$ NK cells, we insist that a significant interaction between the persistently increased the number of $NKG2C^+$ NK cells and played a role in the durable remission in this case of aggressive PTCL-NOS.

The previous study showed that the expansion of $NKG2C^+$ NK cells was maintained at up to 12 months after HSCT (3). We confirmed the expansion of $NKG2C^+$ NK cells at up to 22 months after UCBT. In the fraction of terminal differentiated $CD56^{dim}/CD57^+$ NK cells, the average frequency of $NKG2C^+$ was <5% in CMV-positive healthy individuals (14); however, in the $CD56^{dim}/CD16^+$ fraction in the present case it increased to approximately 41.1% at 29 weeks and 62.5% at 97 weeks (Fig. 4A). An *in vitro* assay revealed that cyclosporine and tacrolimus inhibited the proliferation of NK cells, and that mycophenolate mofetil (MMF) inhibited the proliferation more strongly while MTX did not (15). The patient in the present case was treated with tacrolimus and minidose MTX, which did not inhibit the expansion of $NKG2C^+$ NK cells. The present report focuses on a single case, and therefore, a large-scale comparison between MMF and MTX is required to evaluate the kinetics of the number of NK cells in order to generalize the results. The expansion of $NKG2C^+$ NK cells was also confirmed in the patients with no (or mild) GVHD (16). The present case was complicated by mild acute GVHD, which is consistent with their observation (16). The absolute monocyte count at the initial diagnosis of CMV antigenemia is positively correlated with the increase in the number of $NKG2C^+$ NK cells (3), and the present case also showed an increase in the absolute monocyte count to 1.058×10^9/L at that time. IL-12, which is produced by inflammatory monocytes, is considered to be responsible for the expansion of $NKG2C^+$ NK cells after CMV reactivation (17). The biological effects of IL-12 include lymphoid development, the proliferation of T and NK cells, and the secretion of interferon gamma (18). Patients with high serum levels of IL-12 (median 181.0 pg/mL) on days 4 and 7 after HSCT showed a lower relapse rate in comparison to patients with medium (median 20.5 pg/mL) or low (median 2.0 pg/mL) levels (18). An analysis at 116 weeks after UCBT in the present case revealed that the serum levels of IL-12 and interferon gamma were 2.1 pg/mL and 0.1> IU/mL, respectively (SRL, Tokyo, Japan). Further studies are required to clarify whether the serum levels of IL-12 in the early period after HSCT can induce the expansion of $NKG2C^+$ NK cells and their graft-versus-leukemia (GVL) activity.

The expression or loss of HLA-E on malignant cells is

crucial for discussing the functional association between NKG2C+ NK cells and the GVL effect. Physiologically, HLA-E is highly expressed on lymphocytes as well as leukemia-derived cell lines (19); thus, it is reasonable to consider that the lymphoma cells of the present case expressed HLA-E. However, there some tumor cell lines show a loss of the cell surface expression of HLA-E as well as a total loss of HLA class Ia expression (19). A previous study investigated the expression of HLA-E in tumor cell lines and verified the anti-tumor cytotoxicity of NKG2C+ NK cells (20). We confirmed the expression of HLA-DR on lymphoma cells by flow cytometry, but did not investigate the expression of HLA-E. In the future the HLA-E expression should be confirmed in patients with hematological malignancies and the functional association with NKG2C+ NK cells should be investigated.

Based on the fact that a CR was maintained in an aggressive case of PTCL-NOS without apparent chronic GVHD after UCBT, we hypothesize that there was a substantial association between the expansion of NKG2C+ NK cells after CMV reactivation and the graft-versus-lymphoma effect.

The authors state that they have no Conflict of Interest (COI).

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