

Diminished CD2 Expression in T cells Permits Tumor Immune Escape

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Abstract

CD2 is an adhesion molecule present on the cell surface of T and natural killer (NK) cells, and its interaction with CD58 on antigen-presenting cells plays an important role in their immune reaction. Downregulation of CD58 is a frequent mechanism for immune escape in hematological malignancies, whereas there are very few reports that decreased CD2 expression in immune cells is associated with tumor development. We report here a patient who developed Epstein-Barr virus-associated lymphoproliferative disorder (EBV-LPD) along with diminished CD2 expression in T and NK cells. The patient exhibited severely decreased numbers of peripheral T cells and with Th2 cell-biased cytokine production. Although EBV-LPD was refractory to chemotherapy, the patient was treated successfully with allogeneic stem cell transplantation from a donor with normal CD2 expression. It is suggested that CD2-CD58 interactions play a critical role in the anti-tumor immune response, and restoration of this signaling is considered to be an important strategy for anti-tumor therapy when this signaling is blocked.

Keywords: CD2; CD58; Epstein-Barr virus; Lymphoproliferative disorder; anti-tumor immunity

Introduction

CD2 is an adhesion molecule belonging to the immunoglobulin superfamily, known as one of the pan-T cell markers, and it is also expressed in the majority of natural killer (NK) cells. Its ligand, CD58, is expressed on the surface of various kinds of normal and malignant cells, and the CD2-CD58 interaction plays a crucial role in T and NK cell-mediated immune reactions. Disruption of CD58 is often involved in the pathogenesis of hematological malignancies, whereas there are very few reports that decreased CD2 expression in the immune cells is associated with tumor development. We report here a patient who developed a life-threatening Epstein-Barr virus (EBV)-associated lymphoproliferative disorder (EBV-LPD) with diminished CD2 expression in T and NK cells. The clinical course of the patient highlighted the importance of the CD2-CD58 axis in anti-tumor treatment.

Case Presentation and Results

A 40-year-old, previously healthy male stockbreeder was referred to our hospital with persistent dry cough and high fever, which was resistant to antibiotic therapy. According to the result of a computed tomography (CT) scan and polymerase chain reaction (PCR) examination of bronchoalveolar lavage fluid, he was diagnosed with *Pneumocystis jirovecii* pneumonia and treated with sulfamethoxazole-trimethoprim. During the diagnostic process, he was found to have multiple lymph node swelling, and a positron emission tomography (PET)-CT scan demonstrated systemic lymphadenopathy and liver tumors (Figure 1A). Additionally, a space-occupying lesion was suspected in the brain, and brain magnetic resonance imaging (MRI) disclosed a tumor in the right basal ganglia (Figure 1B).

Core needle biopsy of the liver and brain led to the diagnosis of diffuse large B-cell lymphoma (DLBCL), whereas histological examination of the inguinal lymph node indicated Hodgkin lymphoma (HL) (Figure 1C). In spite of discrepancy in the histological diagnoses, both tumors were positive for EBV-encoded small RNA (EBER) *in situ* hybridization.

His clinical presentation was strongly suggestive of cellular immunodeficiency, and flow cytometric analysis of peripheral blood cells revealed a significant decrease in CD3⁺ T-cell counts (111 cells/ μ l) with 32 cells/ μ l CD4⁺ T cells. The patient was suspected of having human immunodeficiency virus (HIV) infection, whereas his serum was negative for both anti-HIV antibody and HIV-RNA. The patient had no family history of immunodeficiency or consanguineous marriage.

Of note, CD2 expression in both T and NK cells was found to be extremely diminished (Figure 2). Only 17.2% of CD3⁺ T cells and 5.0% of CD56⁺ NK cells were positive for CD2. Additionally, nearly half of the T cells were CD4/CD8 double negative (45.9% of CD3⁺ T cells and 47.2% of TCR $\alpha\beta$ ⁺-gated CD3⁺ T cells). Anti-leukocyte antibody was not detected in serum samples, suggesting that these abnormalities in T cells were not caused by an autoimmune mechanism. Reverse transcription (RT)-PCR and Sanger sequencing detected a decreased, but intact, CD2 transcript in blood samples (data not shown). A cytokine production assay with peripheral T cells demonstrated a Th2 cell-dominant pattern, with suppressed interleukin (IL)-2 and increased IL-4 and IL-13 production (Figure 3). To examine the role of CD2 in anti-EBV immune response *in vitro*, an EBV-immortalized lymphoblastoid B-cell line (LCL) derived from a healthy subject was co-cultured with autologous T cells in the presence or absence of CD2-blocking antibody (Figure 4). The result showed that the CD2-blocking antibody inhibited interferon (IFN)- γ production in T cells, especially in CD4⁺ T cells, suggesting that CD2-CD58 binding is crucial for T-cell immune responses against EBV-infected cells.

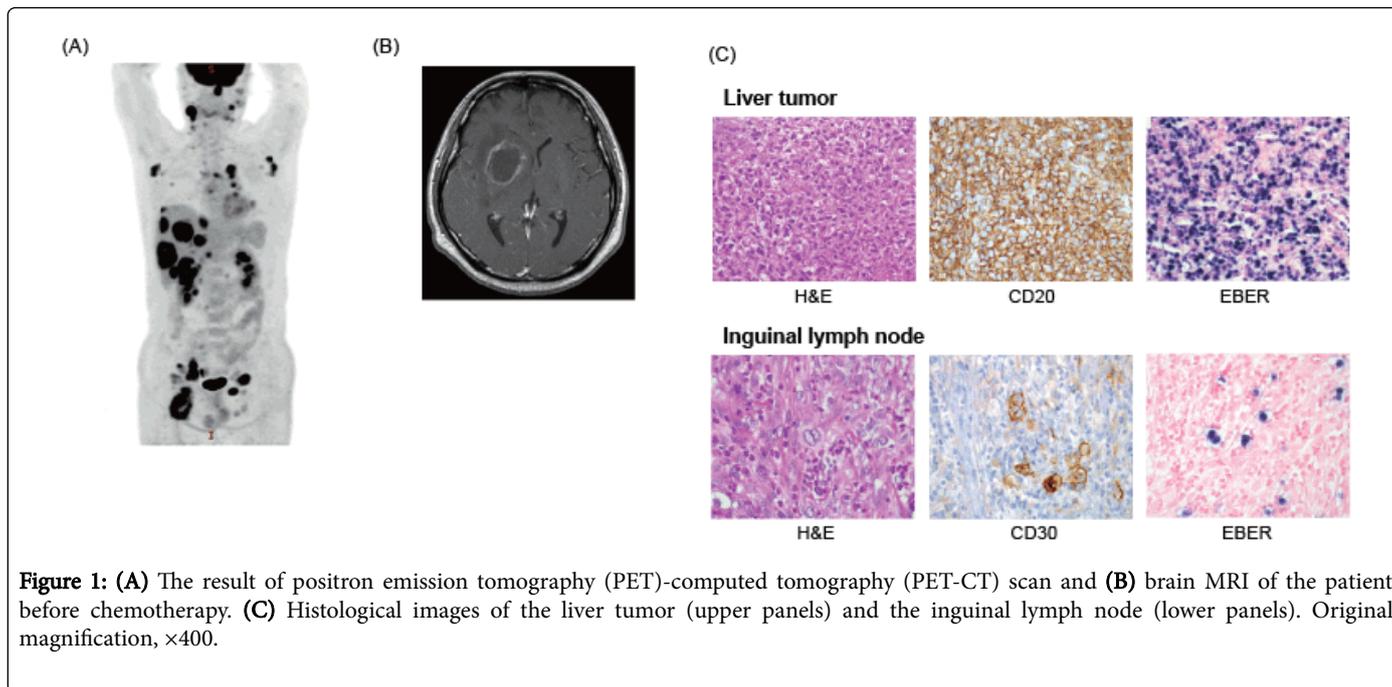


Figure 1: (A) The result of positron emission tomography (PET)-computed tomography (PET-CT) scan and (B) brain MRI of the patient before chemotherapy. (C) Histological images of the liver tumor (upper panels) and the inguinal lymph node (lower panels). Original magnification, $\times 400$.

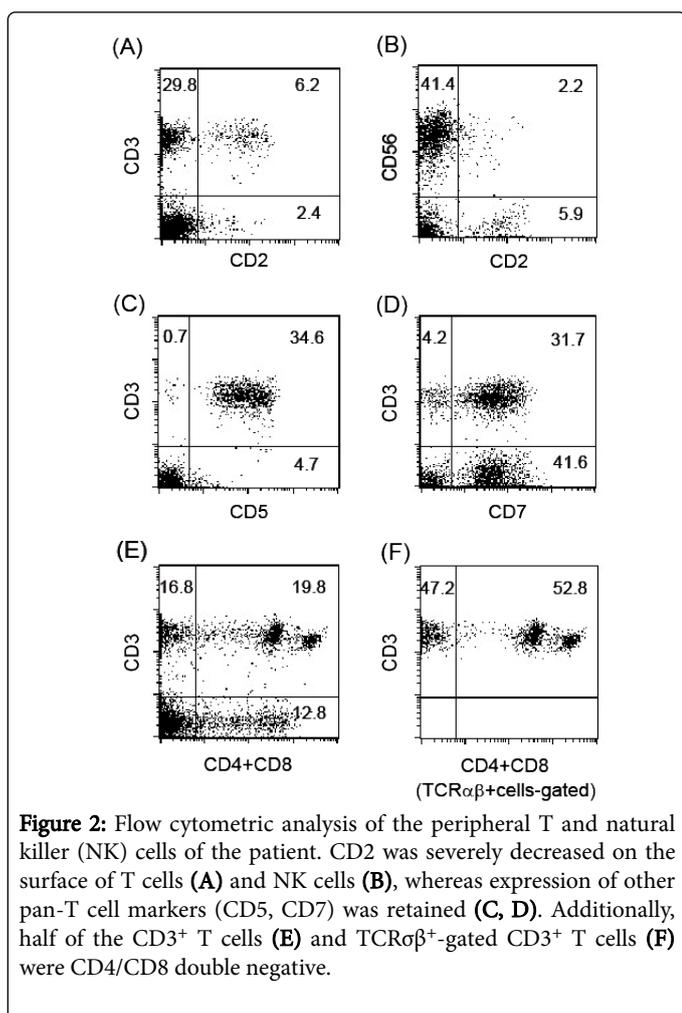


Figure 2: Flow cytometric analysis of the peripheral T and natural killer (NK) cells of the patient. CD2 was severely decreased on the surface of T cells (A) and NK cells (B), whereas expression of other pan-T cell markers (CD5, CD7) was retained (C, D). Additionally, half of the CD3⁺ T cells (E) and TCR $\alpha\beta$ ⁺-gated CD3⁺ T cells (F) were CD4/CD8 double negative.

According to the disturbed T-cell immunity, the patient's tumors were diagnosed as immunodeficiency-associated EBV-LPD. We initially treated with high-dose methotrexate (5 g/m²) and rituximab, but these treatments led to severe myelosuppression with limited therapeutic effect. Next, we performed one cycle of ProMACE-MOPP, and although lymph node and liver tumors decreased in volume, the brain tumor did not respond to treatment. During chemotherapy, there was no natural recovery of CD2 expression on T cells, and T cell numbers decreased further. It was considered difficult to treat his tumors without restoring T-cell function by allogeneic transplantation. After debulking whole-brain irradiation of 10 Gy, non-myeloablative bone marrow transplantation was performed from an unrelated, one-locus (HLA-C) mismatched donor. After transplantation, his tumors gradually decreased in size with successful engraftment of the donor cells with normal CD2 expression. The patient remains alive after 5 years without any residual disease or complications after transplantation (Figure 5).

Discussion

We report here the clinical course of a patient with CD2 deficiency accompanied with EBV-LPD, who was treated successfully with allogeneic stem cell transplantation. CD2 is known to play a major role in the direct cellular contact of T and NK cells with antigen-presenting cells [1]. CD2 not only has a role in cell adhesion, but it also enhances the immune reaction of T and NK cells. CD2 signaling activates T cells independently [2,3] or by positively regulating the T-cell receptor (TCR) signaling [4,5]. It is also shown to promote the cytotoxic activity of NK cells [6,7]. Disruption of CD58, the ligand for CD2, was reported to be a recurrent mechanism for immune escape by hematological malignancies such as DLBCL [8,9], adult T-cell leukemia [10], and peripheral T-cell lymphoma [11].

On the contrary, decreased expression of CD2 on immune cells is a very rare condition. There are only two case reports of patients with decreased CD2 expression [12,13]. Both patients were reported to

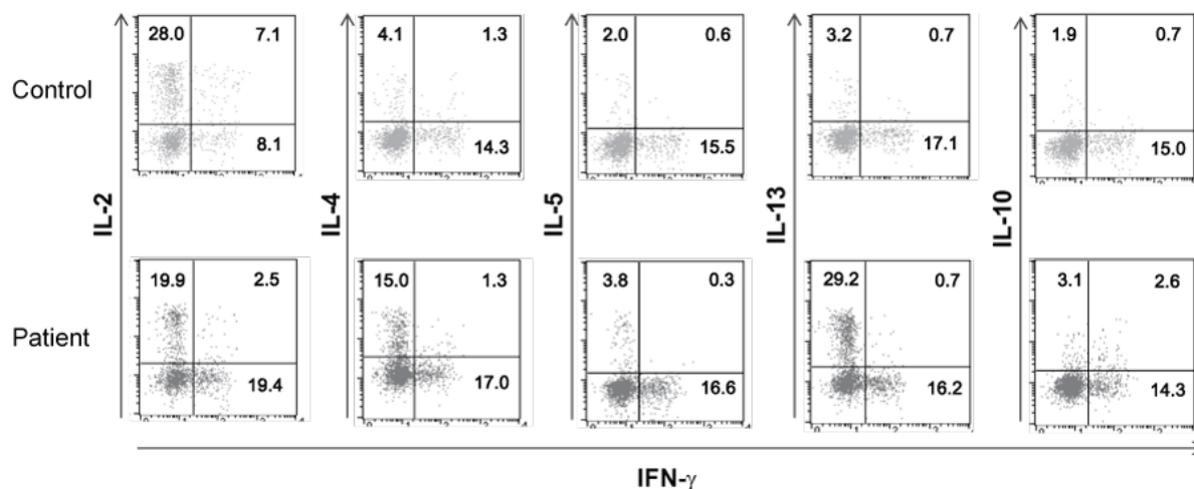


Figure 3: Cytokine production analysis. T cells from the patient had decreased interleukin (IL)-2 but increased IL-4, IL-5, IL-13, and IL-10 producing capacity, in comparison with a healthy control subject.

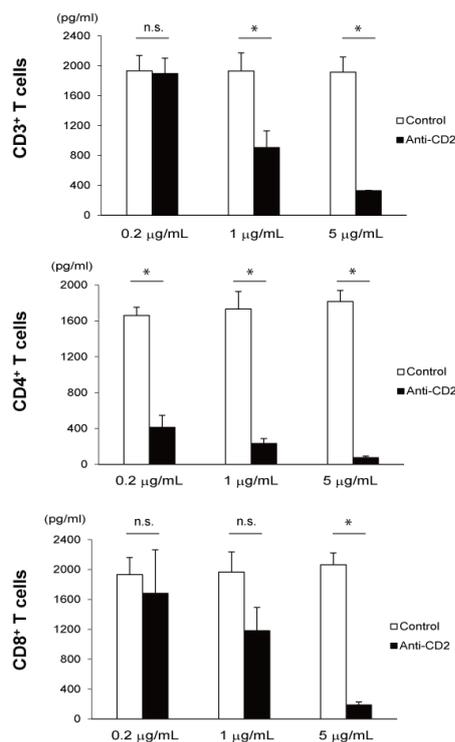


Figure 4: CD2 blocking analysis. A lymphoblastoid B-cell line (LCL) established from a healthy subject was co-cultured for 48 hours with autologous CD3⁺, CD4⁺, and CD8⁺ T cells in the presence of CD2-blocking antibody or isotype control, and interferon (IFN)- γ production from T cells was examined using an enzyme-linked immunosorbent assay (ELISA). Results are shown as mean \pm standard deviation of four independent experiments. *P<0.05; n.s., not significant by Mann-Whitney U test.

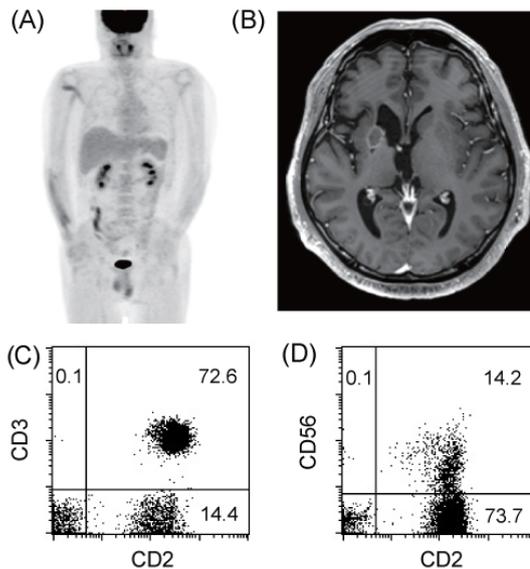


Figure 5: Clinical outcome of the patient after transplantation. (A) The result of PET-CT scan and (B) brain MRI of the patient after transplantation, showing the resolution of the tumors. (C) Flow cytometric analysis demonstrating the restoration of CD2 expression on T cells and (D) NK cells after transplantation.

have developed fatal opportunistic complications with prominent decrease in T-cell numbers in their fifth decades. As with our patient, they were described as having no previous medical history of immunodeficiency, and the loss of CD2 expression was considered to be an acquired immune condition caused by some unknown mechanism. Diminished CD2 expression was associated with decrease in T-cell numbers in all patients, suggesting that CD2 signaling is required for the survival and/or maintenance of T cells. Cytokine production assays of T cells in this case demonstrated a Th2 cell-

dominant pattern, with suppressed IL-2 and increased IL-4 and IL-13 production. Previous reports examining the effect of CD2 signaling on cytokine production are conflicting, possibly due to various experimental designs. However, CD2 signaling was shown to enhance responsiveness of T cells to IL-12 [14], a key cytokine that promotes Th1 immune response. On the other hand, it was suggested to have less association with IL-13 production [15]. Thus it may be speculated that CD2 signaling is more functionally associated with Th1 response than Th2.

In addition, blockade of CD2 profoundly decreased IFN- γ production by normal T cells in response to LCL, suggesting that the CD2-CD58 interaction plays a critical role in T-cell immunity against EBV. Our results accord well with an observation of the increased occurrence of EBV-LPD in patients treated with an anti-CD2 antibody sipilizumab for T-cell malignancies [16]. IFN- γ production was not obviously decreased in the T cells of the patient, but we speculated that this was because they had already been extremely activated *in vivo*, and the cell condition might be different from those of the healthy control at baseline.

Although the patient's condition was not well controlled before bone marrow transplantation, it seemed that the donor cells began to exert anti-tumor immunity shortly after transplantation. The clinical course of the patient suggested that chemotherapeutic agents have limited effect in immunocompromised conditions, indicating that cellular immunity works cooperatively to kill tumor cells with chemotherapy. Immune-checkpoint inhibitors have recently been shown as highly promising in the treatment of several kinds of malignancies, whereas these drugs are considered to have efficacy on the premise of intact positive immune signals. Restoration of CD2-CD58 signaling would, thus, be an essential treatment strategy for the control of tumor cells, in cases when this signaling is inactivated by some mechanism.

Materials and Methods

Cytokine production assay

Peripheral mononuclear cells were separated using a Ficoll-Paque density gradient (Amersham Biosciences), and stimulated with phorbol 12-myristate 13-acetate (PMA; 50 ng/ml, Sigma) and A23187 (500 ng/ml, Sigma) for 6 hours. Brefeldin A (10 μ g/ml, Sigma) was added for the last 3 hours. After stained with CD3 antibody, cells were fixed, permeabilized, stained with fluorescein isothiocyanate-labeled IFN- γ and phycoerythrin-labeled IL-4, IL-5, IL-13, or IL-10 antibodies (BD Pharmingen) and analyzed using a FACSCalibur flow cytometer (BD Biosciences).

CD2 blocking analysis

We had established an LCL from a healthy subject. Peripheral mononuclear cells derived from the same person were separated using a Ficoll-Paque density gradient, and then CD3⁺, CD4⁺, and CD8⁺ T cells were positively collected using MACS Cell Separation Technology (Miltenyi Biotec). LCL (1 \times 10⁵ cells/well) and MACS-collected T cells (2 \times 10⁵ cells/well) were co-cultured in a 96-well round-bottom plate for at 37°C in 200 μ l of RPMI 1640 medium supplemented with 10% fetal calf serum, in the presence of CD2-blocking antibody (Antibody Systems Inc.) or isotype control (mouse IgG κ antibody, eBioscience). After culturing for 48 hours, supernatants were collected and examined for IFN- γ production using an enzyme-linked

immunosorbent assay (ELISA) (Biolegend). Statistical difference was tested by Mann-Whitney U test.

Disclosure of Conflicts of Interest

The authors have no financial conflicts of interest to disclose with regard to this report.

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