

Letter to the Editor

## **CD2 loss and CD8 expression by Epstein-Barr virus-associated NK leukemia cells in culture**

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We previously reported a case with Epstein-Barr virus (EBV) associated NK cell leukemia which expressed CD3 in a suspension culture [1]. Another case of EBV-related NK cell leukemia which lost CD2 and expressed CD8 in a culture is reported in this article. The patient (a 20-year old female) suffered from a high grade fever, and visited our hospital in November 1992. Hepatosplenomegaly without lymphadenopathy was present, and the laboratory examination showed pancytopenia with granular lymphocytosis (Hb 7.0 g/dl, WBC 2,300/ $\mu$ l, Plt 113  $\times 10^3$ / $\mu$ l, GL 96 %) and severe liver dysfunction (GOT 210 IU/l, GPT 360 IU/l, Alp 734 IU/l, LDH 841 IU/l,  $\gamma$ -GTP 101 IU/l, TB 4.6 mg/dl (dB 3.4 mg/dl)). The phenotype of granular lymphocytes was CD2/CD16/CD56/CD122-positive and CD3/CD4/CD8/CD25/CD57-negative. Bone marrow specimen showed typical hemophagocytosis. Monoclonal integration of EBV was present in blood granular lymphocytes by means of Southern blot analysis using long terminal repeat probe of EBV genome. Granular lymphocytes obtained from peripheral blood of the patient were cultured to examine the sensitivity to interleukin-2. The patient was administered with etoposide and vincristine, and the effect was minimal. After ten months of treatment in the hospital, she died of massive gastrointestinal bleeding.

Heparinized peripheral blood was obtained from the patient, light density mononuclear cells were suspended in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum, then cultured in the presence or absence of 500 JRU/ml of human recombinant interleukin-2 (Shionogi Pharmaceuticals, Osaka, Japan) for 4 days at 37°C in 5% CO<sub>2</sub> in humidified air. As shown in the

Figure, the cells before culture were CD2/CD16/CD56/CD122-positive and CD3/CD4/CD8/CD25/CD57-negative. After 4 days culture without IL-2, a minor population (CD2dim+/CD56dim+, 6.2%) appeared, and the majority of the cells expressed CD8 (68.0 and 71.4 %). On the other hand, about a half of the cells cultured in the presence of IL-2 for 4 days were CD56dim+/CD2-/CD8- (34.0 and 34.7 %), and the rest of the cells were CD56+/CD2+/CD8+ (38.2 and 38.7 %). CD3 was absent in the every culture days. Therefore, we concluded that the leukemic granular lymphocytes from the patient had the nature to express CD8, and the IL-2 sensitive cells developed another population of CD2-/CD8-/CD56dim+ in the culture.

Although the meaning of losing CD2 in the culture is unclear, these cells had the nature to express CD8 on the surface. The molecule of CD2 attaches the ligands, CD58, CD48 and CD59, and is supposed to have a important role for T-cells and NK-cells to recognize antigen presenting cells and tumor cells in the loci of immune reactions, respectively. However, CD2-/CD16+ NK cells were observed to have strong killer activity in a culture condition of killer cell induction using lymphokine activation [2]. Therefore, the CD2-/CD56+ cells in our report may have a normal counter part as a subpopulation of strong NK-cells induced in the presence of IL-2. As discussed in several report [3], EBV-related GL leukemia has a wide spectrum of subpopulation characterized using phenotype. Actually, the GL leukemia reported in our previous paper was CD3-/CD16+/CD56+/CD57- which expressed CD3 in a culture, therefore the cells were supposed to be intermediate between NK and

T-cell [1]. Moreover, CD56 positive T-cells exist in human blood as a minor population (3 to 5 % of circulating lymphocytes in human), presenting a GL appearance and perforin granules in the cytoplasm [4]. Therefore, the CD3+/CD56+ GL leukemia has normal counterpart in blood, as well. On the other hand, the cells presented in this paper showed CD2+/CD16+/CD56+/CD57- which lost CD2 in a culture in the presence of IL-2, hence the cells supposed to be capable to grow up to strong killer. This type of GL leukemia also have normal counterpart as discussed above. Therefore, we conclude that the cultivation of the wide range of CD122 positive NK cells with IL-2 may clarify the nature and function of these cells in the loci of tumor killing reaction in vivo through the alteration of phenotype in the culture.

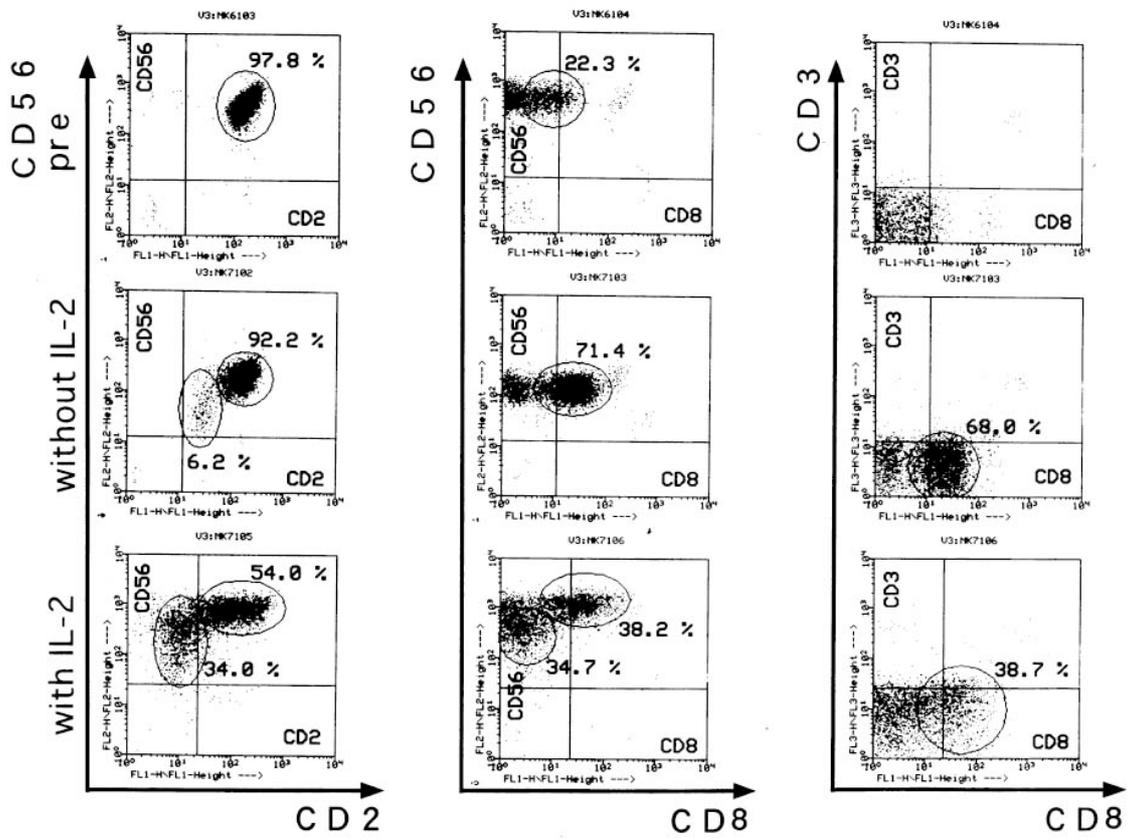
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Table 1 Phenotypic alteration of NK leukemia in culture.

Pre culture	CD2+/CD56+/CD3-/CD8-	97.8%
Without IL-2	CD2dim/CD56dim/CD3-/CD8-	6.2%
	CD2+/CD56*/CD3-/CD8+	68.0 and 71.4%
With IL-2	CD2-/CD56dim/CD3-/CD8-	34.0 and 34.7%
	CD2+/CD56*/CD3-/CD8+	38.2 and 38.7%

Preculture: before culture; without IL-2: cultured in the absence of IL-2; with IL-2: cultured in the presence of IL-2.



**Fig 1.** Phenotype alteration of leukemic NK cells in culture. NK leukemia cells were obtained from blood of the patient, and cultured in the absence and presence of interleukin-2. Pre, before culture; without IL-2, cultured for 4 days in the absence of IL-2; and with IL-2, cultured for 4 days in the presence of 500 JRU/ml of IL-2.