once sufficient numbers of mice in other colonies are observed to 1 year and are subjected to similar analyses. If disease progression is unique to mice in our facility, it might suggest that environmental or infectious agents can play a role in the progression to the more severe phenotype in a subset of mice in the context of 12/15-lipoxygenase deficiency. It is important to stress, however, that the defects we reported in the majority of Alox15 mice that are asymptomatic do not in our opinion appear to be unique to the Wistar colony, and these mice remain a valuable tool for defining the role of 12/15-lipoxygenase in hematopoiesis.

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To the editor:

Dasatinib enhances the expansion of CD56$^+$CD3$^-$ NK cells from cord blood

Dasatinib can inhibit T-cell activation through inhibition of the Scr family of tyrosine kinases such as p56 (Lck).[1] It has been reported that some chronic myeloid leukemia (CML) patients who were treated with dasatinib developed chronic large-granular lymphocytosis (LGL) with natural killer (NK) or NK T-cell lineage, and that these patients achieved optimal molecular response.[2] In addition, Mustjoki et al reported clonal expansion of NK T cells during dasatinib therapy.[3] Kreutzman et al reported that mono/oligoclonal T and NK cells were present in CML patients at diagnosis and expanded during dasatinib therapy and that LGL expansion is linked to cytomegalovirus infection.[4,5] Therefore, dasatinib may have a favorable effect on NK-cell proliferation. In this study, we analyzed the effects of dasatinib on the expansion of NK cells from cord blood and transcriptional factors during expansion.

Umbilical cord blood cells (1×10^6/mL; Hokkaido Cord Blood Bank) were cultured with IL-15 (10 ng/mL; PeproTech), IL-2 (5 ng/mL; R&D Systems), and anti-CD3 mAb (OKT3, 10 ng/mL; Janssen Pharmaceutical); with or without dasatinib (10nM; a kind gift from Bristol-Myers Squibb) in culture medium stem cell growth medium (CeeGenix) with 5% human AB serum in 24-well plates, as we reported previously.[6] After a 7-day culture of umbilical cord blood cells (1×10^6/mL), the absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with the culture with cytokines only (before culture $5.3 \pm 1.4 \times 10^4$ in 10^6 cord blood cells, after culture with IL-2 + IL-15 $26.0 \pm 17.8 \times 10^4$, and after culture with IL-2 + IL-15 and dasatinib $66.6 \pm 29.1 \times 10^4$; $P < .05$, means ± SDs, n = 6; Figure 1A). In addition, the proportion of CD56$^+$CD3$^-$ cells, CD56$^+$NKG2D$^+$ cells, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib (Figure 1B).

We analyzed the transcriptional factors Eomesodermin (Eomes) and T-bet using an Applied Biosystems 7300 Real-Time PCR System and GAPDH as an endogenous control. Before stimulation, cord blood of CD56$^+$ cells showed increased expression of Eomes and T-bet compared with the expression in unfractionated whole cord blood cells and CD3$^+$ cells. After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with cells cultured with cytokines only (5.96 ± 3.95 vs 0.81 ± 0.62, $P < .05$; Figure 1C).

At present, there are only a few transcription factors that are known to play an essential role in NK-cell development, especially in humans. T-box proteins, T-bet, and Eomes are involved in NK-cell development.[7-9] T-bet and Eomes are both later required for the differentiation in DX5$^+$(CD49b) CD11b$^+$ NK cells. In addition, Eomes is highly expressed in fully differentiated NK cells. In this study, we showed NK-cell expansion after culture with dasatinb and increased expression of Eomes after 24 hours. Therefore, dasatinib has some role in NK-cell expansion from cord blood under the condition of IL-2 and IL-15 stimulation through increased expression of transcription factors such as Eomes. This observation may have potentially important implication for the treatment of other diseases with dasatinib.[10]

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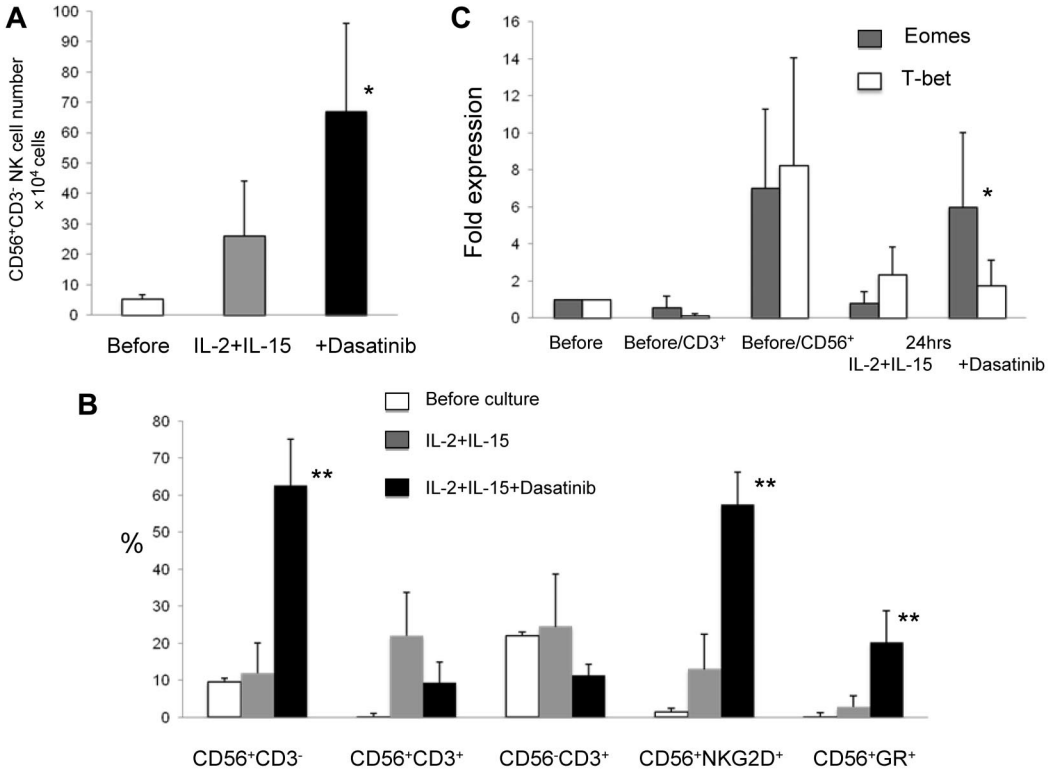

Figure 1. Expansion of CD56$^+$CD3$^-$ NK cells with dasatinib from cord blood cells. (A) The absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with those in the culture with cytokines only. (B) The proportion of CD56$^+$CD3$^-$, CD56$^+$NKG2D$^+$, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib compared with culture without dasatinib. (C) After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with that in cells cultured with cytokines only. T-bet expression was increased after 24 hours culture compared with that before culture, but there was no significant difference between expression level in culture with dasatinib and that without dasatinib (bars indicate means ± SDs, n = 6; *$P < .05$ and **$P < .01$).

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