

Supplementary Materials

Interferon- γ Produced by EBV-Positive Neoplastic NK-Cells Induces Differentiation into Macrophages and Procoagulant Activity of Monocytes, Which Leads to HLH

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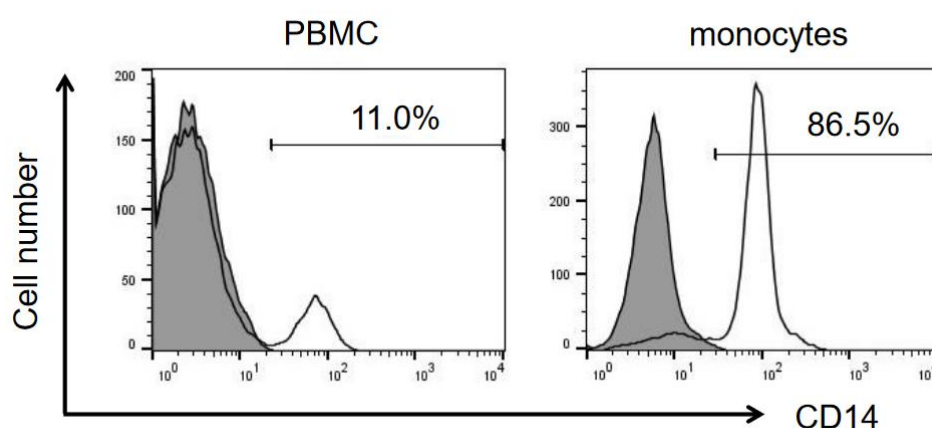


Figure S1. Enrichment of human monocytes. Human monocytes were isolated using an indirect magnetic labeling system from the PBMC. The CD14-positive monocytes which were 11.0% in the PBMC were enriched by 86.5% using the magnetic system.

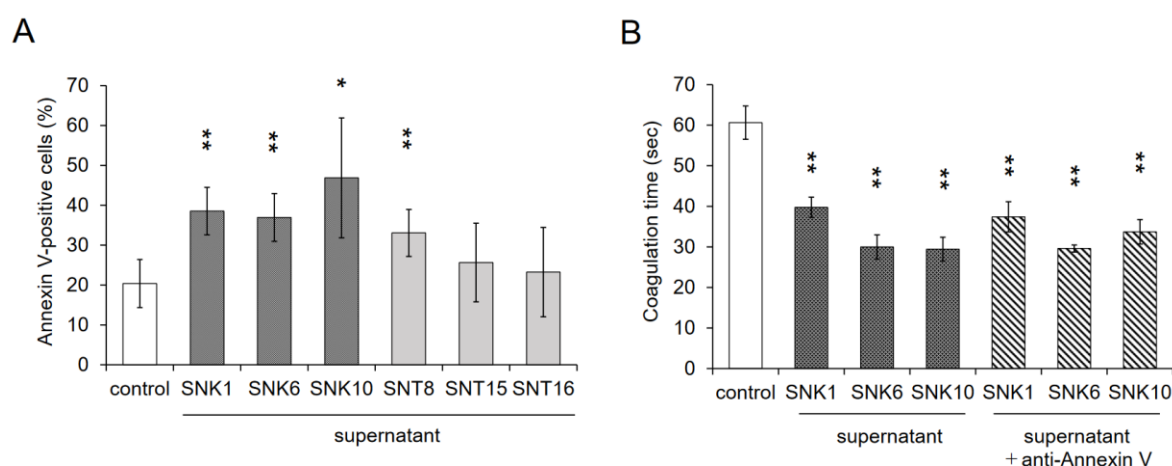


Figure S2. Induced phosphatidylserine was not related to the procoagulant activity. THP-1 cells were co-cultured with each supernatant of EBV-positive T- or NK- cell lines for 24 h. After the co-culture, the cells were subjected to each assay. (A) Cell surface phosphatidylserine (PS) antigen on THP-1 cells was analyzed by flow cytometry using an Annexin V antibody. The rate of Annexin V-positive cells was shown as mean \pm SD ($n = 4$). (B) To investigate the effects of cell surface PS on PCA, THP-1 cells were treated with Annexin V antibody. After the treatment of antibody, cell surface PCA was assessed. The data are shown mean \pm SD ($n = 4$). Significant differences are indicated as * $p < 0.05$ and ** $p < 0.01$ comparing to the control.

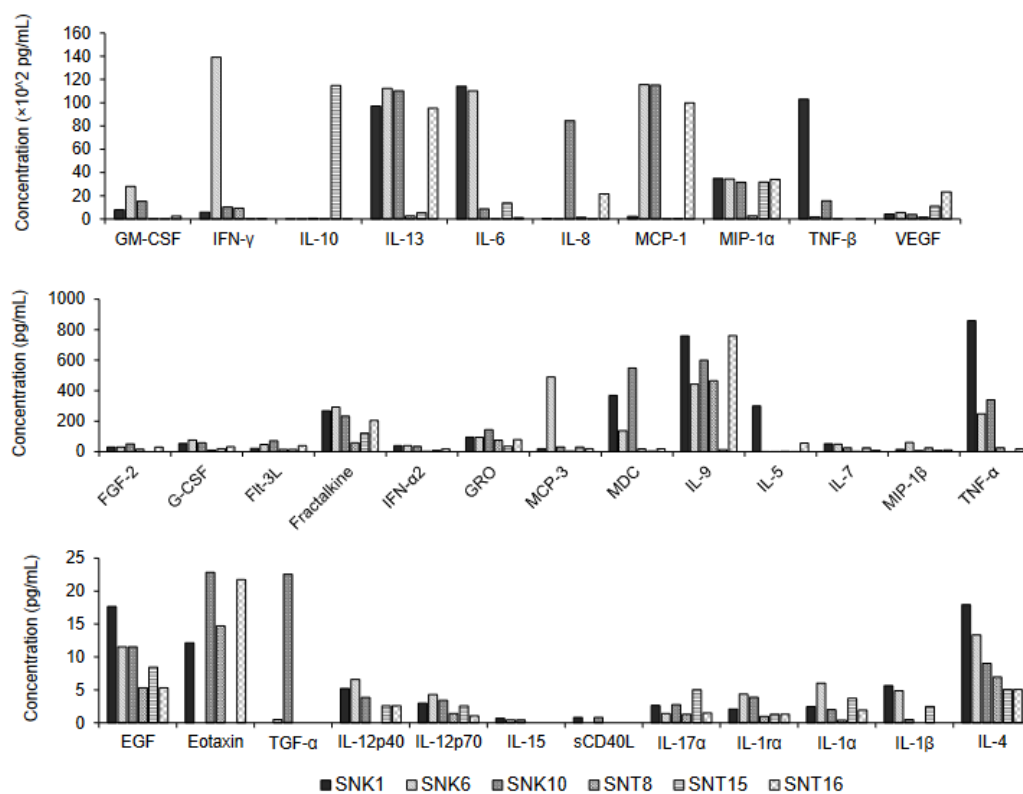


Figure S3. Identification of cytokines in the supernatants of EBV-positive T- or NK-cell lines. The cytokine concentrations in the supernatants of EBV-positive T- or NK-cell lines were examined using cytokine multiplex assay.

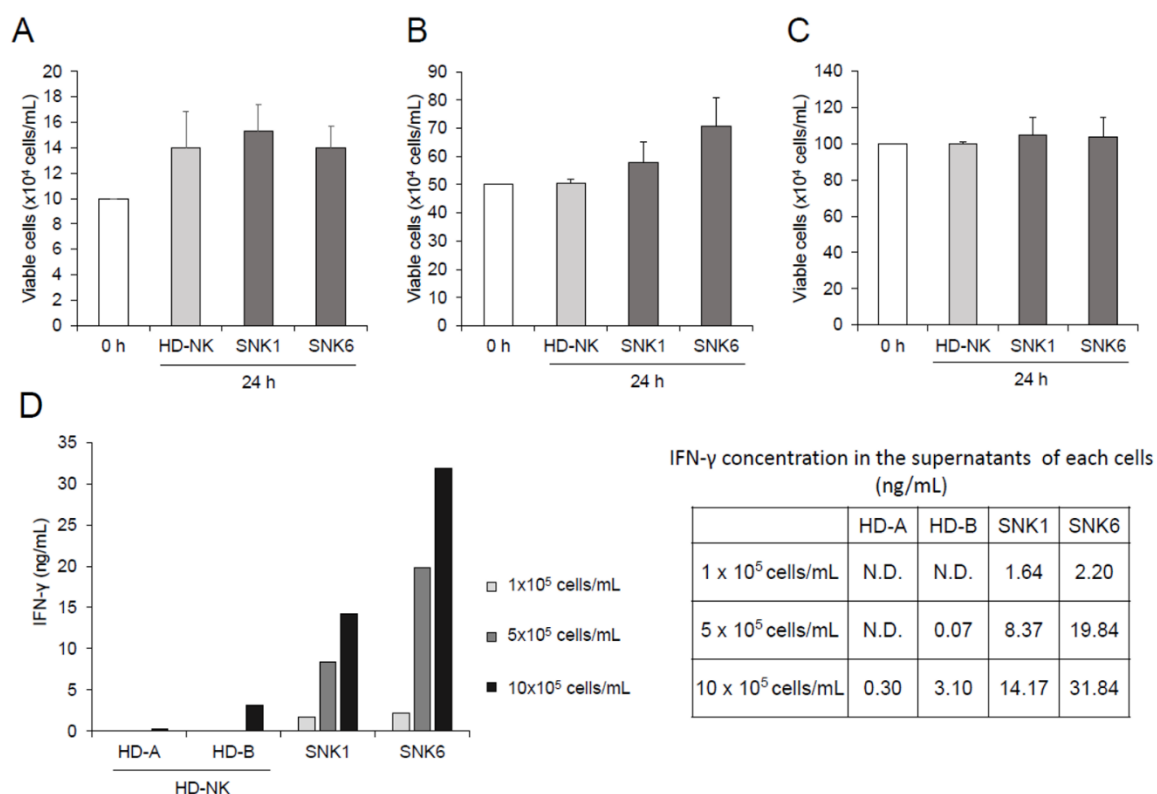


Figure S4. The concentration of IFN- γ in the supernatant of healthy donors' NK cells and EBV-positive NK-cell lines. NK cells (HD-NK: HD-A and HD-B) of two healthy donors and EBV-positive NK-cell lines, SNK1 and SNK6, were cultured for 24 h and the supernatants were collected. We examined the proliferation of the cells in three different concentrations of 1×10^5 cells/mL (**A**), 5×10^5 cells/mL (**B**), and 10×10^5 cells/mL (**C**). The concentration of IFN- γ in each supernatant was measured (**D**). N.D.; not detected.

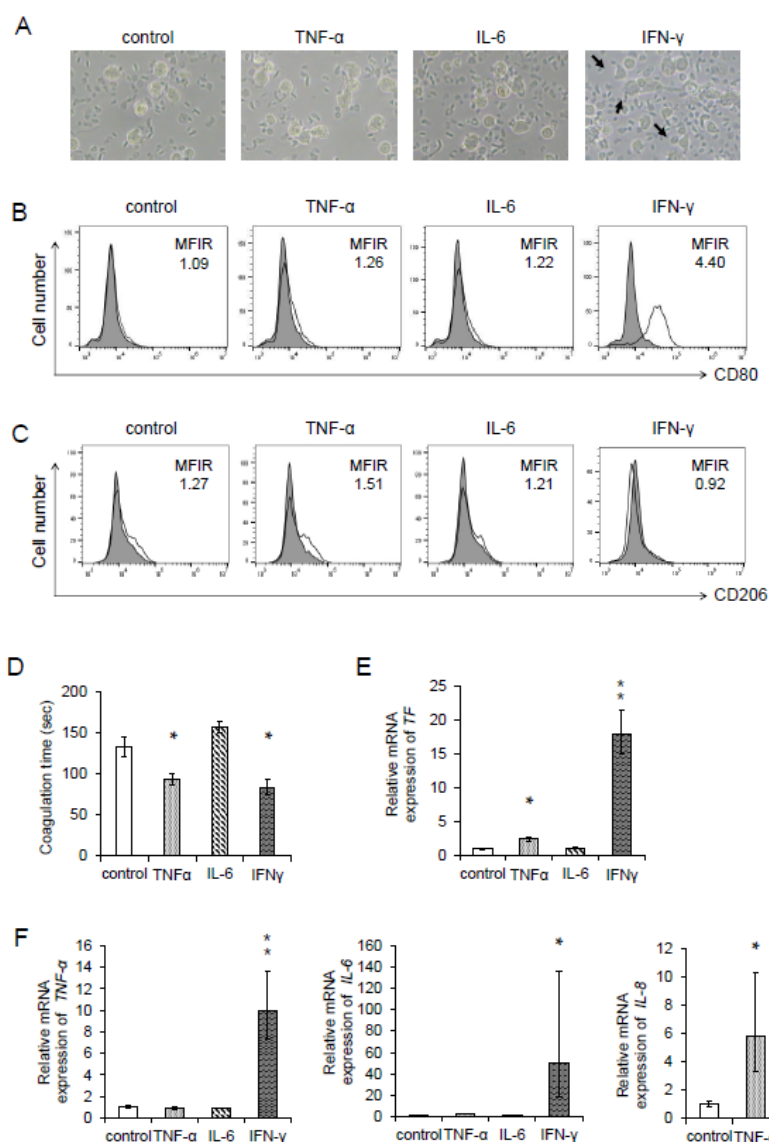


Figure S5. Effects of TNF- α , IL-6 and IFN- γ on the differentiation and procoagulant activity of human monocytes. Human monocytes were treated with 1.0 ng/mL of TNF- α , 20 ng/mL of IL-6, and 10 ng/mL of IFN- γ for 24 h. After the treatment, the target cells were subjected to each assay. **(A)** Representative morphology was pictured by an optical microscope. **(B,C)** The CD80 (B) and CD206 (C) expression on human monocytes was determined by flow cytometry. The representative figure is shown on the left panel indicating CD80 (open histogram) or isotype-matched control (gray, shaded histogram). The mean fluorescent intensity of CD80 was normalized by that of isotype-matched control and expressed as MFIR. **(D)** PCA was measured by normal plasma-based recalcification time. Shortening of the coagulation time indicates increased PCA. **(E,F)** The mRNA expression of TF, TNF- α , IL-6 and IL-8 was analyzed by qRT-PCR assay. The expression was normalized to *GAPDH* mRNA. The data are shown as mean \pm SD of three independent healthy donors ($n = 3$). Significant differences are indicated as * $p < 0.05$ and ** $p < 0.01$ comparing to the control.

