Human natural killer (NK) cells are one of the attractive candidates for cell-based therapy against any cancers due to their strong cytotoxicity. However, there are few convenient and efficient methods to obtain a large amount and high purity of functional NK cells from peripheral blood mononuclear cells (PBMCs) derived from a small amount of blood. Thus, we have developed a robust NK-cell expansion method using OK-432 and RetroNectin™, and treated to suppress the growth potential (modified RN-T cells). NK cells could be expanded from PBMCs stimulated with modified RN-T cells, OK-432 and IL-2, then cultured for more than 16 days. In our large-scale culture system using gas-permeable culture bag (Cultlife™215 and Cultlife™Evax), we could obtain 10⁹–10ⁱ⁰ cells containing a high proportion (>90%) of CD3⁺CD56⁻ NK cells from 50ml of peripheral blood. Furthermore, almost all cells displayed functional cell surface molecules such as NKGD2 and CD16 implicated in cytotoxicity and antigen dependent cell cytotoxicity (ADCC). Thus, we investigated the antitumor effect of the expanded NK cells combined with Trastuzumab against HER2-positive human gastric cancer cell line NCI-N87 in hIL-2 Tg NOG mice (hIL-2 NOG mice; Central Institute for Experimental Animals). In this experiment, we used purified NK cells to reduce GVHD risk caused by human CD3⁺ cells including in the expanded cells. As a result, the combination of the NK cells and Trastuzumab dramatically enhanced the antitumor activity compared with each treatment alone. The chimerism of human NK cells in mouse peripheral blood was observed during the observation period without any GVHD symptoms and functional NK-cell surface markers such as CD16 and NKGD2 also expressed in human NK cells. Furthermore, human NK cells were observed in tumor tissue even in 3 months after administration. Overall, we have established a robust NK-cell expansion system and the expanded cells showed strong antitumor activity in a xenogenic mouse model. It is considered that our expansion system could be used for chimeric antigen receptor (CAR)-NK cell processing or pluripotent stem cell derived NK-cell manufacture for future application.

The issues of NK-cell expansion for therapeutic application

- Low purity of NK cells after expansion
- Low proliferation of NK cells

The important factors for NK-cell expansion are... We developed a large-scale NK-cell expansion method using RetroNectin™-induced T-cells.

procedure for NK-cell expansion

1) Culture of RN-T (RetroNectin™ induced T-cells)
   10ml peripheral blood
   PBMC
   Day-7
   DO
   D7
   Takara
   Anti-CD3Ab (GMP)
   GMP grade
   RetroNectin™
   IL-2
   Modified RN-T

2) Culture of NK cells
   20-40ml peripheral blood
   PBMC
   Day 0
   IL-2
   Culture bag Cultlife™215
   Culture bag Cultlife™Evax
   %CD56
   8.6%
   Day17-21
   46.6%

NK-cell expansion using modified RN-T

A hIL-2 Tg NOG mice model and study design for evaluating in vivo ADCC activity

No significant differences in weight loss were observed among the groups.

Chimerism of human CD45⁺ cells in PBL

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The moderate effects were observed in monotherapies. NK-cell treatments with Trastuzumab significantly reduced tumor volume and led to complete tumor regression in 2 out of 4 animals.

Additional experiment

Even in only single cycle of treatment, NK-cell with Trastuzumab significantly reduced tumor volume. Cryopreserved NK cells also showed anti-tumor effect in combination with Trastuzumab. Ex vivo expanded human NK cells infiltrated in tumor tissue and they were observed even at 3 months after injection.

Summary

- High purity of NK cells can be obtained from small amount of peripheral blood by using our expansion system.
- The expanded NK cells showed in vivo as well as in vitro anti-tumor activity.
- Our NK-cell expansion system is highly potent tool to produce a large amount of functional NK cells.