Human interleukin-15 transgenic NOG mice support the long-term maintenance of human mature NK cells from peripheral blood

Ikumi Katano, Takeshi Takahashi, Ryoji Ito, Asami Hanazawa, Mamoru Ito

Central Institute for Experimental Animals, Kawasaki, Japan

CIEA SINCE 1952 科研費 KAKENHI

Introduction

We generated a novel NOD-*scid*, Il2rg^{null} (NOG) mouse by introducing the human interleukin-15 (hIL-15) gene, which is essential for the homeostasis of natural killer (NK) cells. We investigated whether peripheral blood (PB)-derived human mature NK cells could be maintained in the NOG-hIL-15 transgenic (NOG-hIL-15 Tg) mice.

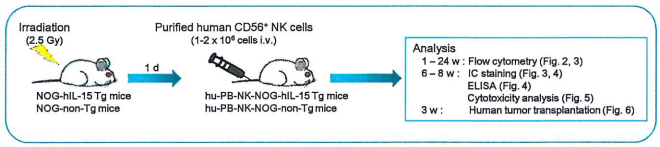
Materials and methods

Development of human interleukin-15 transgenic NOG mice

We introduced the human IL-15 gene into NOG mice. NOG-hIL-15 transgenic mice were generated by injection of a cytomegalovirus promoter – human IL-15 DNA segment into fertilized eggs of NOD/Shi-IL2rg^{null} mice. As a result, we obtained founder human IL-15 transgenic strain. The mouse was further backcross-mated with NOG mice to obtain NOG-hIL-15 Tg mice. We quantified the amount of human IL-15 protein in plasma of NOG-hIL-15 Tg mice using ELISA.

Human mature CD56$^+$ natural killer (NK) cell into immunodeficient mice

NOG-hIL-15 Tg and NOG-non-Tg mice were irradiated (2.5 Gy) and then intravenously transplanted with 1-2 x 10^6 peripheral blood (PB) -derived human CD56$^+$ NK cells (hu-PB-NK). Human PB was obtained from healthy volunteers after acquiring their informed consent. Human NK cells were negatively selected using human NK cell isolation kit (Miltenyi).



Analysis of hu-PB-NK-NOG-hIL-15 Tg mice

Human cells in peripheral blood and various tissues of these mice were analyzed using flow-cytometry and used for cytotoxicity assay.

- Flow-cytometric analysis: Human NK cells in mononuclear cells from PB and various tissues were prepared according to standard protocols. After staining with surface antigen-specific antibodies, they were analyzed by FACSCanto and FACSDiva software.
- In vitro human cytokine stimulation: Human NK cells were cultured in the presence or absence of 1 ng/mL rhIL-2, rhIL-15 or combination of rhIL-12 & rhIL-18. These stimulated-human NK cells were used for intracellular (IC) staining and cultured with target cells for cytotoxicity analysis.
- IC staining: Human NK cells were cultured in the presence of 3 μg/ml Brefeldin A for 20 hours. After staining with antibodies for surface antigens, they were fixed in paraformaldehyde and subsequently permeabilized using Intracellular Staining Permiabilization Wash Buffer (BioLegend). The permeabilized cells were stained with antibodies for intracellular antigens and analyzed by flow-cytometry.
- Measurement of CTL proteins: Plasma of healthy volunteers and mouse plasma 6 weeks post human NK cell transfer were collected for quantification of human perforin.
- Cytotoxicity analysis: Cytotoxic activity of human NK cells was examined using human myeloma cell line K562 as target. Human NK cells were cultured with K562 for 4 hours. The percentages of target cell lysis were measured by the release of LDH into the culture supernatants using a CytoTox96 Non-Radioactive Cytotoxic Assay kit (Promega).
- In vivo anti-tumor activity: 2.5x10^5 K562 were subcutaneously transplanted 3 weeks after transplantation of hu-PB-NK cells into NOG-hIL-15 Tg mice. Tumor size was weekly measured using a caliper.

Results

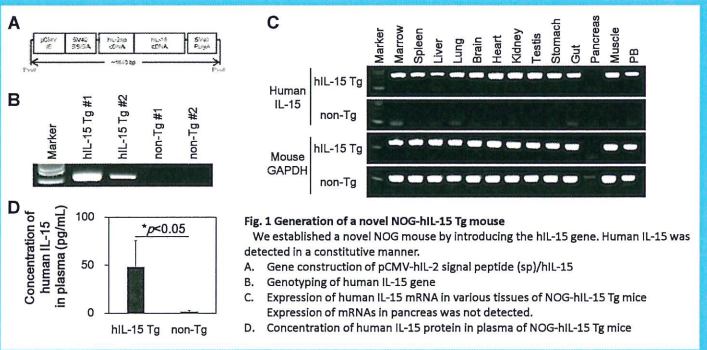


Fig. 1 Generation of a novel NOG-hIL-15 Tg mouse

We established a novel NOG mouse by introducing the hIL-15 gene. Human IL-15 was detected in a constitutive manner.

A. Gene construction of pCMV-hIL-2 signal peptide (sp)/hIL-15
B. Genotyping of human IL-15 gene
C. Expression of human IL-15 mRNA in various tissues of NOG-hIL-15 Tg mice
Expression of mRNAs in pancreas was not detected.
D. Concentration of human IL-15 protein in plasma of NOG-hIL-15 Tg mice

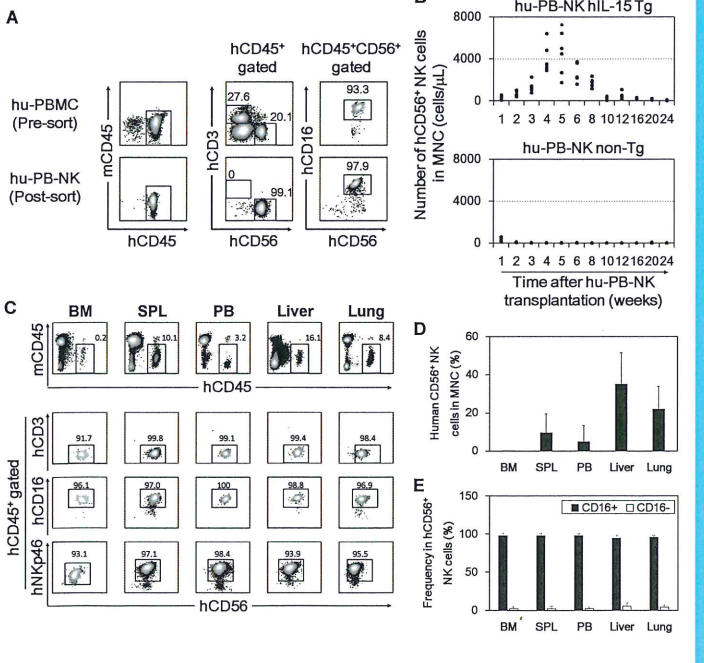


Fig. 2 Engraftment rates of human cells in NOG-hIL-15 Tg mice after human peripheral blood-derived-NK cell transfer

Human CD56$^+$ NK cells (1-2x10^6) were intravenously transferred into mice. Human CD45$^+$ (hCD45$^+$) cells in mouse mononuclear cells (MNC) were analyzed by FACSCanto over 24 weeks.

A. FACS pattern of human NK cells in human PBMC. Purified human CD56$^+$ NK cells (hu-PB-NK) were transplanted into NOG-hIL-15 Tg mice.
B. Engraftment of hCD56$^+$ NK cells in PB of hu-PB-NK-NOG-hIL-15 Tg mice: Human NK cells in NOG-hIL-15 Tg mice increased in number for initial 4 weeks and gradually decreased thereafter. In contrast, human NK cells in hu-PB-NK-NOG-non-Tg mice disappeared within 2 weeks.
C. FACS pattern of human NK cells in various tissues of hu-PB-NK-NOG-hIL-15 Tg mice
D. Frequencies of human NK cells in MNC in various tissues of hu-PB-NK-NOG-hIL-15 Tg mice
E. Frequencies of hCD16$^+$ or hCD16$^-$ subpopulations in human NK cells in each tissue of hu-PB-NK-NOG-hIL-15 Tg mice
F. Engraftment of human NK cells in spleen of hu-PB-NK-NOG-hIL-15 Tg mice at 24 weeks

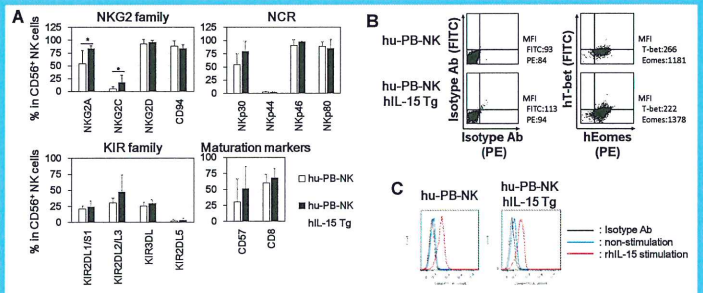


Fig. 3 Expression of NK-specific molecules in human NK cells in hu-PB-NK-NOG-hIL-15 Tg mice

FACS staining of activating and inhibitory NK receptors. Splenocytes of hu-PB-NK-NOG-hIL-15 Tg mice at 6-8 weeks after hu-PB-NK transplantation and normal human CD56$^+$ PB-NK were stained with the indicated antibodies and analyzed.

A. Expression of NK receptors: NKG2 family, killer immunogroblin-like receptor (KIR) molecules and other natural cytotoxicity receptors (NCRs). NKG2A$^+$ and NKG2C$^+$ NK cell subsets were increased in hu-PB-NK-NOG-hIL-15 Tg mice compared to normal human PB-NK cells. (*p<0.05)
B. Expression of T-bet and Eomes: Human NK cells in NOG-hIL-15 Tg mice maintained the expression of both T-bet and Eomes.
C. Phosphorylation of STAT5 in human NK cells in response to rhIL-15

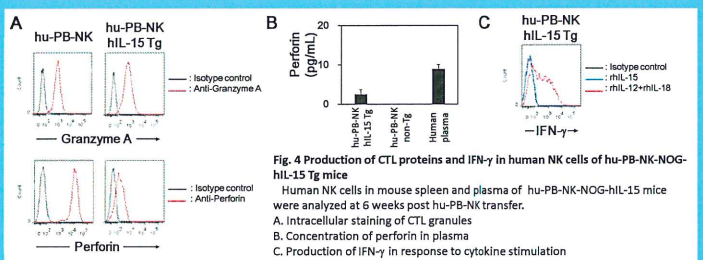


Fig. 4 Production of CTL proteins and IFN-γ in human NK cells of hu-PB-NK-NOG-hIL-15 Tg mice

Human NK cells in mouse spleen and plasma of hu-PB-NK-NOG-hIL-15 mice were analyzed at 6 weeks post hu-PB-NK transfer.

A. Intracellular staining of CTL granules
B. Concentration of perforin in plasma
C. Production of IFN-γ in response to cytokine stimulation

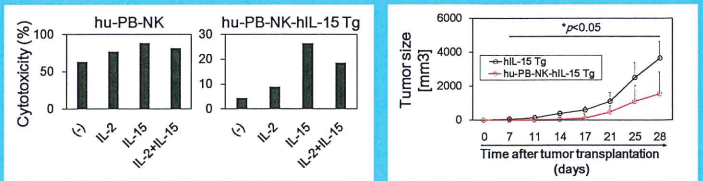


Fig. 5 CTL activity of human NK cells in hu-PB-NK-NOG-hIL-15 Tg mice

Cytotoxic activity of human NK cells from human PB and spleen of hu-PB-NK-NOG-hIL-15 Tg mice was measured after rhIL-2 and rhIL-15-stimulation for 2 days. Human cytokine-stimulated human NK cells were cultured with K562. The release of LDH in the culture supernatants was measured. The ratio of effector to target cells was 10:1.

Fig. 6 In vivo anti-tumor activity of human NK cells in hu-PB-NK-NOG-hIL-15 Tg mice

K562 tumor size was monitored for 4 weeks after tumor transplantation. As a control, non-transplanted NOG-hIL-15 Tg mice were used. Tumor growth was inhibited in hu-PB-NK-NOG-hIL-15 Tg mice (n=4) compared to in non-transplanted NOG-hIL-15 Tg mice (n=4).

Conclusions

① After transplantation of human NK cell, they proliferated for initial 4 weeks and were maintained up to 24 weeks in NOG-hIL-15 Tg mice.
② The human NK cells from hu-PB-NK-NOG-hIL-15 Tg mice maintained the expression of various NK cell-specific surface markers.
③ They produced Granzyme A, Perforin and IFN-γ.
④ They induced phosphorylation of STAT5 in response to rhIL-15 stimulation.
⑤ In vitro cytotoxicity of human NK cells from hu-PB-NK-NOG-hIL-15 Tg mice was lower than that of hu-PB-NK, but rhIL-15 stimulation augmented the activity.
⑥ The NK cells could delay tumor growth in vivo.

Collectively NOG-hIL-15 Tg mice will become a suitable animal model for analyzing human mature NK cells in vivo.