자연살해세포 치료

한국생명공학연구원 줄기세포 연구단

최 인 표

Natural killer cell therapy

Inpyo Choi, M.D.

Stem Cell Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon, Korea

자연살해세포는 면역세포 중 3번째로 많은 수를 가지고 있으며 말초혈액의 면역세포 중 약 10%가 자연살해세포이다. 자연살해세포는 CD56과 CD16을 가지고 있으며 여러 암세포를 죽이는 기능을 가지고 있다. 자연살해세포의 활성은 여러 경로를 통해 나타낸다. 자연살해세포는 조혈줄기세포로부터 분화하는데 이에 관련하는 여러 가지 유전자가 동정되었다. 이러한 유전자를 활용한 자연살해세포의 분화와 활성을 유도하고 활성화된 자연살해세포를 이용한 자연살해세포 치료법이 개발되고 있다.

색인단어: 자연살해세포, 분화, 활성, 세포치료법

Natural killer (NK) cells are a unique and the third most common subset of lymphocytes residing in the marrow, spleen, and peripheral blood. Approximately 10% of lymphocytes in the peripheral blood are NK cells. Phenotypically, NK cells are positive for CD56 and CD16 but negative for CD3. Unlike T-cells, NK cells kill tumor cells and virally infected cells without previous stimulation and MHC-restriction, and do not express clonally rearranged receptors. NK cell cytotoxicity involves exocytosis of perforin- and granzyme-containing cytoplasmic granules, as well as FasL- and TRAIL-mediated pathways. NK cells produce various cytokines, notably IFN-gamma, TNF-alpha, GM-CSF, and IL-10. NK cells mediate important functions in innate immunity and are capable of eliminating major histocompatibility complex (MHC) class I-deficient virus-infected cells and tumor cells. In addition, NK cells are involved in many autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, experimental autoimmune encephalomyelitis, and allograft rejection.
NK cells are derived from hematopoietic stem cells (HSCs) in the bone marrow (BM), which is the main site of NK cell generation. The NK cell development from HSCs consists of multiple steps, which are not yet completely defined, and requires growth and differentiation signals from stromal cells. In the embryo, HSCs give rise to NK precursors (pNKs), which are found in the fetal thymus, blood, spleen, and liver. In the adult, pNKs are mainly in the BM, pNKs are lineage-negative and characterized by the presence of CD122 (IL-2 receptor β) and the absence of both NK1.1 and DX5 NK markers. The murine pNKs are noncytolytic and do not produce large amounts of IFN-γ. Following in vitro culture in the presence of IL-15 or IL-2, pNKs become NKR-P1 positive mature NK cells (mNK; NK1.1+ NK in mice and CD161+ NK in human).

NK cells express various cell surface receptors, which are involved in the activation or inhibition of NK cells, as well as cell adhesion. NK cells recognize MHC class I molecules via killer immunoglobulin-like receptors (KIRs), many of which are inhibitory. Lack of engagement results in target cell lysis. Efforts to utilize the biologic characteristics of NK cell in cancer therapeutics are underway. In murine HCT experiences, Ruggeri et al showed that NK cells given after haploidentical HCT decreased relapse of leukemia and enhanced donor cell engraftment, but did not cause GVHD. In a study of Passweg et al, NK cells, negatively selected on the basis of lack of CD3 expression by CliniMacs system, were given to 5 patients who had undergone T cell depleted haploidentical BMT and subsequently experienced mixed chimerism (n=3), graft failure (n=1), or relapse (n=1). Median NK cell dose was 1.61×10^7/kg (range, 0.21~2.2). Infusions were well tolerated and none developed GVHD. Increase in donor chimerism was observed in a relatively small number of patients of 2 of 5 patients. No effect was observed for the relapse of acute myelogenous leukemia. In addition, NK cell can be generated in vitro from CD34+ hematopoietic stem cells.

To analyze the genome scale expression pattern for NK differentiation, we performed SAGA for 4 different stages of NK development—HSC, pNK, mNK (−OP9), and mNK (+OP9). We set up the database of 35,000 unigenes related to NK differentiation (Fig. 1). Among them, in vivo functions of VDUP1 expressed in pNK and mNK were analyzed. We found VDUP1 is critical for NK development in vivo and in vitro (Fig. 2).

Based on these observations, we have developed the efficient methods to expand and differentiate mature NK cell in vitro. Using these mature NK cells differentiated from HSC, anti-tumor activity for in vivo tumor models was tested. In vivo tests showed that mature NK cells eradicated tumors efficiently. Furthermore, the optimized protocols for anti-NK cell therapy are now under investigation to kill tumors with diverse NK repertoire.
Figure 1. A comparison of surface molecule expression during NK cell differentiation. (A) Cells in the different stages during NK differentiation were prepared as described in Methods. Murine hematopoietic stem cells (HSCs) derived from BM cells were differentiated into NK precursors (pNK) in the presence of mouse SCF, IL-7, and Flt3L for 6 days. After the initiation of cultures of HSCs for 6 days, to generate mature NK cells, the cells were further cultured without (−OP9) or with OP9 (+OP9) stromal cells in the presence of mouse IL-15 for an additional 6 days. Purity from the indicated cells was determined by two-color flow cytometric analysis. The numbers in each quadrant represent the percentages of cells. (B) Differentiation of NK precursors into mature NK cells by coculture with OP9 stromal cells were analyzed for the expression of NK cell–associated surface markers. Open histograms represent staining with the antibodies specified, and close histograms represent control staining. (C) Total cytoplasmic RNA was isolated from each cell type, and RT–PCR was performed for the expression of representative NK cell–associated genes from cells in the different stages during NK cell differentiation.

Figure 2. NK defection in VDUP1–deficient mice. (A) Single cell suspensions from the spleen, bone marrow (BM) and lung of WT and VDUP1−/− mice were stained with FITC–conjugated anti–CD3 and PE–conjugated anti–NK1.1 as described in Experimental Procedures. The percentage of the NK cells was evaluated from the gated lymphocyte population. (B) To detect another surface marker expression for the NK cells, DX5 expression was analyzed from the gated lymphocyte population of BM and lymph nodes (LN) using FITC–conjugated anti–DX5 and PE–conjugated anti–CD3. (C) The different Ly49 and NKG2 receptor expression was analyzed from the gated lymphocyte population of splenocytes and BM cells. The results are a representative of at least another eight independent experiments with similar results.
References