Cytotherapy, 2013; 15: 231–241

International Society for Cellular Therapy
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Ex vivo activation and expansion of natural killer cells from patients with advanced cancer with feeder cells from healthy volunteers

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Abstract

Background aims. Culturing natural killer (NK) cells from patients with advanced cancer is difficult and has restricted the generation of sufficient cell numbers for autologous adoptive NK-cell therapy. The aim of this study was to establish a novel method for *ex vivo* NK-cell expansion from patients with cancer. *Methods.* NK cells ($CD3^-CD56^+$) were isolated from peripheral blood mononuclear cells from healthy volunteers and cancer patients, and NK^- fractions were used as feeder cells. Purified NK cells were co-cultured with feeder cells in AIM-V medium (Invitrogen, Carlsbad, CA, USA) supplemented with 5% human serum and 1000 units/mL human interleukin-2. *Results.* NK cells co-cultured with feeder cells from healthy volunteers (feeder-HV) expanded more than NK cells co-cultured with feeder cells from cancer patients (feeder-CP). During the 14-day culture period, NK cells from patients with advanced cancer co-cultivated with feeder-HV expanded on average 300-fold. NK cells co-cultivated with feeder-CP expanded on average 169.4-fold. Cultures grown in the presence of feeder-HV contained 93.8 ± 7.0% (mean ± standard deviation; n = 6) $CD3^-CD56^+$ NK cells, and cultures grown in the presence of feeder-CP contained 83.6 ± 15.9% $CD3^-CD56^+$ NK cells. Feeder-HV caused a relative increase in $CD3^+CD4^+$ T cells, whereas feeder-CP did not induce changes. Interleukin-15, a cytokine that induces NK-cell proliferation, was detected in the culture supernatants of feeder-HV but not in those of feeder-CP. *Conclusions.* Feeder cells obtained from healthy volunteers have the potential to expand and activate NK cells from patients with advanced cancer. The novel NK-cell expansion method described here provides a technique for acquiring the large numbers of highly active NK cells from patients with cancer for autologous adoptive immunotherapy.

Key Words: *advanced cancer, autologous cell therapy,* ex vivo *expansion, feeder cells, NK cells*

Introduction

Natural killer (NK) cells are important for effective immune responses against transformed or virally infected cells and against certain pathogens. NK cells constitute 5–20% of human peripheral blood lymphocytes and are derived from $CD34^+$ hematopoietic progenitor cells (1). These large, granular lymphocytes are phenotypically defined by their expression of CD56 and lack of CD3 and T-cell receptor molecules (2). Because NK cells are able to lyse target cells without prior priming as is needed for T lymphocytes, NK cells are particularly attractive for use in adoptive cellular immunotherapy (3).

Several methods for NK-cell expansion for their use in adoptive cancer immunotherapy have been reported (4–7). Interleukin (IL)-2 can stimulate NK-cell proliferation, but only a few NK cells maintain their ability to proliferate after the initial response (4–6). IL-4, IL-7 and IL-12 also can induce proliferation but are overall less potent than IL-2 (7). In addition, IL-15 alone or in combination with IL-2 induces minimal NK-cell expansion (8). Cytokines may be necessary but are not sufficient for expansion of NK cells.

Most investigators believe that sustained proliferation of NK cells requires additional stimulatory signals, such as the presence of monocytes (9) or B-lymphoblastoid cells (10,11). Miller *et al.* (9) reported an approximate 30-fold expansion of NK cells after 18 days in culture with 1000 IU/mL IL-2 and monocytes. Perussia *et al.* (12) found that irradiated B-lymphoblastoid cells in direct contact with NK cells induced a 25-fold expansion of NK cells after 2 weeks of stimulation. Ohno *et al.* (13) reported that co-culturing NK cells with HFWT, a Wilm tumor-derived cell line, results in a 400-fold

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(Received 12 July 2012; accepted 12 October 2012)

http://dx.doi.org/10.1016/j.jcyt.2012.10.019

Table I. Patient characteristics[a] at the time of blood sampling for this study.

Patient no.	Sex/age (y)	Cancer	Status at sampling	Stage at sampling	Treatment after sampling	Current status
1	M/68	DLBCL	Initial diagnosis	IIIB	Chemotherapy	NED
2	F/54	DLBCL	Initial diagnosis	IVA	Chemotherapy	NED
3	M/53	Mantle cell lymphoma	Initial diagnosis	IVA	Chemotherapy	NED
4	F/60	NK/T-cell lymphoma	Initial diagnosis	IVB	Chemotherapy	Death
5	M/18	NK/T-cell lymphoma	Initial diagnosis	IVA	Chemotherapy	Relapsed, alive with disease
6	M/18	Hydroa vacciniforme-like lymphoma	Initial diagnosis	IVB	Chemotherapy	Death
7	M/67	DLBCL	Initial diagnosis	IE	Chemotherapy+ radiation therapy	NED
8	F/67	DLBCL	Initial diagnosis	IVA	Chemotherapy	NED
9	M/79	Follicular dendritic cell sarcoma	Initial diagnosis	IV	Chemotherapy	Alive with disease
10	M/70	NSCLC (SCC)	Relapsed	IV	Chemotherapy+ radiation therapy	Death
11	M/66	Cholangiocarcinoma	Relapsed	IV	Supportive care	Death
12	M/56	NSCLC (SCC)	Metastatic	IV	Chemotherapy	Death
13	F/69	Chondrosarcoma	Relapsed	IV	Supportive care	Alive with disease
14	M/80	Nasopharyngeal cancer	Initial diagnosis	IV	Supportive care	Alive with disease

DLBCL, diffuse large B-cell lymphoma; F, female; M, male; NSCLC, non-small cell lung cancer; NED, no evidence of disease; SCC, squamous cell carcinoma.
[a]Patients with lymphoma (n = 9) and patients with solid cancer (n = 5).

expansion of NK cells after 2 weeks. Other investigators have used allogeneic mononuclear cells (14), autologous lymphocytes (15) and umbilical cord mesenchymal cells (16). In addition, Imai *et al.* (17) and Fujisaki *et al.* (18) showed that the median NK-cell recovery rate after NK-cell stimulation with genetically modified K562 was 152-fold after 14 days. This proliferation was significantly higher than the proliferation observed when purified NK cells were unstimulated or stimulated with IL-2 alone.

Tumor-infiltrating NK cells have a different phenotype and reduced cytotoxicity compared with NK cells from healthy donors. NK cells from peripheral blood of patients with some types of cancers also demonstrate reduced cytotoxicity. In a previous study of patients with metastatic melanoma, NK cells had decreased activity and interferon-γ production compared with healthy donors. These cells showed an increase in non-cytotoxic $CD56^{bright}CD16^{dim}$ and a reduction in cytotoxic $CD56^{dim}CD16^{bright}$ NK cell subsets (19). In addition, the frequency of NK cells expressing the activating receptors NKp30, NKp44, NKG2D and NKG2C was significantly decreased in patients with acute myeloid leukemia compared with normal controls (20).

NK cells are potential tools for cancer immunotherapy because of their cytotoxic abilities that are enhanced by antibodies and the possibility of *ex vivo* expansion and adoptive transfer. The difficulty of culturing NK cells from patients with advanced cancer has been the limiting factor for generating sufficient cell numbers for autologous adoptive NK-cell therapy. The aim of this study was to optimize a method for *ex vivo* NK-cell expansion and to determine the role of feeder cells in expanding NK cells from cancer patients.

Methods

Blood samples and cell lines

This study included 14 patients with cancer, nine with lymphomas and five with terminal solid tumors (Table I). Blood samples from healthy volunteers were obtained from the leukocyte reduction system chamber. Consent forms and approval for this study were obtained from the donors and the institutional review board of Seoul National University Hospital of Korea, College of Medicine.

K562 (chronic myelogenous leukemia), Daudi (Burkitt lymphoma) and BJAB (Burkitt lymphoma) cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The human lymphoma cell lines SU-DHL6 and U-2932 were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). These cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco-BRL, Gaithersburg, MD, USA), 2 mM glutamine and 1% gentamicin solution (Gibco-BRL).

Isolation of NK cells

Human peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque (Amersham