Successful adoptive immunotherapy with OK432-inducible activated natural killer cells in tumor-bearing mice

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Abstract

We had demonstrated that the NK cell mediated cytotoxicity of murine spleen cells could be augmented by in vivo priming and subsequent in vitro challenge with a streptococcal preparation OK432, and the cell surface phenotype of induced killer cells was Thy-1+, asialo GM1+, suggesting that the activated cells were of NK lineage (OK-NK cell). We had also clarified that IL-2 played a major role in inducing the OK-NK cells via the production of IFN-γ. In this study, we examined the effect of adoptive transfer of OK-NK cells on syngeneic tumors in mice. Mice were implanted with SP2 myeloma cells intraperitoneally (i.p.), or C26 colon adenocarcinoma cells subcutaneously to make the models of peritonitis carcinomatosa or solid tumor, and the OK-NK cells were transferred i.p. or intratumorally, adoptively. By the adoptive transfer of OK-NK cells, 92% of mice bearing SP2-tumor had been cured. The tumor growth of C26-solid tumor was inhibited, and the survival rate of mice bearing C26-tumor was significantly increased. The intratumoral remnants of 125I-labelled OK-NK cells were 61, 27 and 8% at 4, 12 and 36h after intratumoral transfer, respectively. By multiple transfer of OK-NK cells, the antitumor effect was more effectively augmented than that of a single transfer. Results in this study suggested that OK-NK cells could be useful for the therapy of cancer patients.

Abbreviations: IFN-γ: interferon γ; IL-2: interleukin 2; LAK cells: lymphokine activated killer cells; NK cells: natural killer cells; OK-NK cells: OK432-inducible activated natural killer cells.

Introduction

A streptococcal preparation OK432, a heat- and penicillin-treated lyophilized powder of the SU strain of Streptococcus pyogenes, has been used for the therapy of cancer because of its activity as a biological response modifier [1]. There are many reports that natural killer (NK) cell activity is augmented by OK432 in vivo or in vitro [2-4].

We have reported that in vivo priming and in vitro rechallenge with OK432 maximally enhance natural cytotoxicity of murine spleen cells, and that the surface phenotype of killer
cells activated with OK432 is Thy-1\(^+\) and asialo-GM1\(^+\), suggesting that the killer cells were activated NK cells (OK-NK cells) [5]. We clarified that interleukin 2 (IL-2) played a major role in inducing OK-NK cells in spleen cells primed \textit{in vivo} and subsequently challenged \textit{in vitro} with OK432, through the production of interferon \(\gamma\) (IFN-\(\gamma\)) [6].

Recently, lymphokine activated killer (LAK) cells have been used for the therapy of cancer patients, and there are many reports that the LAK cells exert the therapeutic effects on cancer patients [7–9]. However, usefulness of the LAK therapy combined with IL-2 administration has been limited due to its side effects.

In this study, the salient therapeutic effect was observed \textit{in vivo} when the OK-NK cells with broad spectrum of cytotoxicity against various tumor cells were transferred into tumor-bearing mice adoptively, and the organ-distribution of transferred OK-NK cells was reported.

**Materials and methods**

BALB/c female mice, 8–10-week-old, were used for the experiments, SP2 syngeneic myeloma cells were used as an NK-sensitive target, and syngeneic C26 colon adenocarcinoma cells were used as an NK-resistant target [5].

Tumor cell lines were maintained in RPMI-1640 medium (Nissui Co., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (GIBCO, Grand Island, New York, USA), 2 mM L-glutamine, 100 U penicillin, 100 \(\mu\)g streptomycin/ml and 50 \(\mu\)M 2-mercaptoethanol (complete medium).

**Priming and challenge with OK432**

OK432 (Chugai Pharmaceutical Co., Tokyo) was suspended in physiological saline at a concentration of 0.1 mg dry weight/0.5 ml. Mice were given subcutaneous injections of 0.1 mg OK432 for \textit{in vivo} priming. On days 4–8 after priming \textit{in vivo} with OK432, spleen cells were harvested to be challenged \textit{in vitro}, because stronger cytotoxicity was observed against NK-sensitive and -resistant targets on day 4–14, as compared with that induced by \textit{in vitro} OK432 challenge alone [5]. For \textit{in vitro} challenge, spleen cells at a concentration of 2.5 \(\times\) 10\(^6\)/ml in complete medium were incubated with an optimal dose of OK432 (12.5 \(\mu\)g/ml) for various incubation times at 37\(^\circ\)C in a humidified 5% CO\(_2\) atmosphere, because this dose of OK432 could maximally augment the NK activity [5]. After incubation, the cells were harvested, washed and suspended in complete medium.

**Cytotoxicity assay**

A 4 h \(^{51}\)Cr-release assay was performed as previously described elsewhere [5, 6]. Briefly, target cells were labeled with 100 \(\mu\)Ci Na\(_2\)^{51}\)CrO\(_4\), washed three times. \(^{51}\)Cr-labeled target cells (100 \(\mu\)l; 1 \(\times\) 10\(^5\)/ml) were added in triplicate to 100 \(\mu\)l effector cells (effector to target ratios varied from 100:1 to 25:1) in round-bottom microtiter plates (Corning No. 25850). After 4 h incubation at 37\(^\circ\)C, the supernatants were counted using a gamma counter. The spontaneous release did not exceed 10\% for SP2 and 25\% for C26 of maximum release that was obtained by 1 M HCl. The percentage cytotoxicity was calculated as follows (all \(^{51}\)Cr values in cpm).

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\text{Cytotoxicity} = \frac{\text{test}^{51}\text{Cr release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100
\]

**Tumor-bearing mice**

Mice implanted with 8 \(\times\) 10\(^6\) of SP2 tumor cells intraperitoneally (i.p.) and mice implanted with 1 \(\times\) 10\(^6\) of C26 tumor cells subcutaneously were used as the models of peritonitis carcinomatosa and solid tumor, respectively. A larger amount of SP2 tumor