Veterinary Immunology and Immunopathology 165 (2015) 1–13

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Generation of recombinant canine interleukin-15 and evaluation of its effects on the proliferation and function of canine NK cells

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ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 31 March 2015
Accepted 1 April 2015

Keywords:
Canine
Interleukin-15
Recombinant protein
NK cells
Immunotherapy

ABSTRACT

Interleukin-15 (IL-15) is a pleiotropic cytokine that plays a pivotal role in both innate and adaptive immunity. IL-15 is also a promising cytokine for treating cancer. Despite the growing importance of the clinical use of IL-15 for immunotherapy, no attempts have been made to generate a recombinant canine IL-15 (rcIL-15) and to examine its effects on the antitumor activities of immune effector cells in dogs. Here, we generated an rcIL-15 protein consisting of Asn-49–Ser-162 with a C-terminal His tag and examined its functions ex vivo in terms of the proliferation and antitumor effects on canine non-B, non-T, large granular natural killer (NK) cells. Non-B, non-T, large granular NK cells rapidly expanded in response to stimulation with rcIL-15 in the presence of IL-2, and a majority of the cells that selectively expanded over 21 days exhibited a $CD3^{-}CD5^{-}CD4^{-}CD8^{+/-}CD21^{-}$ phenotype. Purified rcIL-15 significantly enhanced the expansion rate of canine NK cells derived from peripheral blood mononuclear cells compared to human IL-15, or culture in the absence of IL-15 for 21 days ($p < 0.05$). Purified rcIL-15 was superior at enhancing the effector function of NK cells compared to human IL-15. The cytotoxic activity against canine thyroid adenocarcinoma (CTAC) cells, interferon-γ production, and the mRNA expression levels of perforin and granzyme B of expanded NK cells cultured with rcIL-15 were significantly elevated compared to those cultured with human IL-15 or without IL-15 ($p < 0.05$). Intravenous administration of rcIL-15 significantly increased the numbers of lymphocytes in the peripheral blood of dogs on days 6, 8, and 11 after injection compared to numbers before administration ($p < 0.05$). The results of this study suggest that the rcIL-15 protein, consisting of Asn-49–Ser-162, enhanced the proliferation and antitumor effects of canine NK cells and promoted the generation of lymphocytes in dogs.

1. Introduction

Interleukin-15 (IL-15) is a member of the γ-chain cytokine family, which plays important roles in the regulation of immune responses (Fehniger and Caligiuri, 2001). Binding of IL-15 to its receptor IL-15Rα/IL-2Rβ/γ-chain

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http://dx.doi.org/10.1016/j.vetimm.2015.04.002
0165-2427/

(γ_c) generates an immune-enhancing signaling cascade through the JAK/STAT, PI3K/Akt, and Ras/MAPK pathways (Budagian et al., 2006; Lin et al., 1995; Miyazaki et al., 1994; Zambricki et al., 2005; Zhang et al., 2008). These signaling pathways promote the activation, proliferation, and survival of natural killer (NK) cells and T cells (Imada et al., 1998; Kelly et al., 2003). More importantly, IL-15 produced by bone marrow and thymus stromal cells stimulates the generation, maintenance, and homeostasis of NK cells (Kennedy et al., 2000). Since IL-15 is essential for generating and activating lymphocyte subsets involved in antitumor immunity, it is considered a promising anticancer immunotherapy drug (Jakobisiak et al., 2011).

IL-15, a critical cytokine for NK cell development, survival and activation, possesses superior advantages for tumor immunotherapy compared to that of IL-2, which also potentiates NK cell function and has been used as an immunotherapeutic agent to promote NK cell antitumor activity (Miller et al., 2014; Pillet et al., 2009). IL-15 has a lower toxicity than IL-2 (Munger et al., 1995), and it supports the survival of NK cells by inhibiting activation-induced cell death (Marks-Konczalik et al., 2000). Unlike IL-2, IL-15 has little effect on the expansion of regulatory T (T_{reg}) cells, which exert an immunosuppressive effect (Berger et al., 2009; Cornish et al., 2006; Zorn et al., 2006). In addition, IL-2, but not IL-15, activated NK cells were shown to increase their sensitivity to apoptosis when these cells come into contact with the vascular endothelium (Rodella et al., 2001). Based on these beneficial features, IL-15 is currently being developed for clinical use as a cancer therapy in humans. This cytokine is also utilized in the ex vivo expansion and activation of NK cells for use in adoptive immunotherapy, and to support the in vivo expansion and function of NK cells after infusion. IL-15 is currently in clinical trials (Miller et al., 2014; Romee et al., 2014).

Only a few attempts have been made to develop a cytokine-based immunotherapy against canine cancer cells, although the incidence of cancers in dogs has increased recently (Chou et al., 2009; Hsiao et al., 2008; Lin et al., 2008). In combination with IL-6 or IL-6 and IL-12, IL-15 has been examined in terms of the antitumor effects exerted through the augmentation of NK cytotoxicity against canine transmissible venereal tumor (CTVT) (Chou et al., 2009; Chuang et al., 2009; Lin et al., 2008). However, most the cytokine genes examined in their reports were from humans. In the present study, we generated an rcIL-15 protein consisting of 114 amino acids (positions 49–162 of the IL-15 preproprotein) for clinical use, and confirmed its biological activity by evaluating its ability to stimulate the proliferation and function of canine non-B, non-T, large granular NK lymphocytes in vitro, which are defined as NK cells in dogs (Knapp et al., 1993). We also determined the ability of rcIL-15 to promote the generation of lymphocytes in vivo after intravenous administration. The results of this study indicated that the purified rcIL-15 could be utilized as a valuable cytokine for cancer immunotherapy, and to support NK cells following adoptive transfer in dogs.

2. Materials and methods

2.1. Animals

A total of eleven healthy male beagles (3–6 years old) were used in this study. Peripheral blood was obtained from six of these dogs to conduct in vitro experiments. A further five dogs were assessed for leukocyte responsiveness after intravenous administration of rcIL-15. These dogs were undergoing regular health and blood checkups for hematological investigations. All dogs had previously received routine vaccinations and were dewormed regularly. Blood collection did not exceed 10 ml/kg of body weight and the samples (about 60 ml) from each dog were drawn into tubes containing sodium-heparin (BD Vacutainer Systems, Franklin Lakes, NJ, USA) without anesthesia. The use of animals was in accordance with the guidelines of the Institutional Animal Care and Use Committee of Kongju National University.

2.2. Cytokines and antibodies

Recombinant human IL-2 and IL-15 (rhIL-2 and rhIL-15) (PeproTech, Rocky Hill, NJ, USA) were used to expand canine NK cells. FITC-conjugated mouse anti-dog CD3 (CA17.2A12), APC-conjugated rat anti-dog CD5 (YKIX322.3), RPE-conjugated rat anti-dog CD4 (YKIX302.9), RPE-conjugated rat anti-dog CD8 (YCATE55.9), and RPE-conjugated mouse anti-canine CD21 (CA2.1D6) antibodies (all from Bio-Rad, Hercules, CA, USA) were used for flow cytometry.

2.3. Cell culture and expansion of canine NK cells

Canine thyroid adenocarcinoma (CTAC) cells were purchased from the European Collection of Cell Cultures (Porton Downingtown, UK), and K562 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen, Carlsbad, CA, USA) at 37 °C in a humidified 5% CO_2 incubator. Peripheral blood mononuclear cells (PBMCs) were isolated by discontinuous Hypaque–Ficoll density gradient (Histopaque 1.119, Sigma, St. Louis, MO; Lymphoprep™ 1.077, Axis-Shield PoC AS, Helsingfors, Norway). For ex vivo expansion of canine NK cells, isolated PBMCs (3.5×10^6) were incubated with 100 Gy-irradiated K562 cells (0.5×10^6) at 37 °C in an atmosphere containing 5% CO_2 for 21 days in 24-well tissue culture plates. Cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) supplemented with 100 IU/ml human IL-2 (PeproTec, Rocky Hill, NJ, USA), 10 IU/ml human IL-15 (PeproTec) or rcIL-15 (10 IU/ml). Fresh medium containing IL-2 and IL-15 was provided every 2–3 days. To evaluate the rate of cell expansion and purity of NK cells, the absolute number of NK cells was calculated by multiplying the total viable number of cells by the percentage of $CD3^-CD5^-CD21^-$ cells