Immunology Letters 127 (2009) 33–38

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

LIGHT protein suppresses tumor growth by augmentation of immune response

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ARTICLE INFO

Article history:
Received 22 June 2009
Received in revised form 19 August 2009
Accepted 20 August 2009
Available online 27 August 2009

Keywords:
Cancer immunotherapy
Cytokine
Tumor necrosis factor
Protein therapy
Recombinant protein

ABSTRACT

The tumor necrosis factor (TNF) superfamily member LIGHT has potent anti-tumor activities through activation of the immune response, and it is a promising candidate for use in cancer immunotherapy. However, there are no reports of the anti-tumor effects of LIGHT protein *in vivo* because of the lack of easy, efficient methods of manufacturing this protein. Here, we developed a method of manufacturing recombinant LIGHT protein using *Escherichia coli* through refolding of inclusion bodies; we then evaluated the anti-tumor activity of the protein. LIGHT protein expressed in *E. coli* showed the same biological activities and binding affinities to its receptors as did LIGHT expressed in mammalian cells. In addition, intratumoral injection of LIGHT significantly suppressed tumor growth, with augmentation of antigen-specific IFN-γ-producing cells in the regional lymph nodes and spleen. These results indicate that LIGHT protein efficiently evokes the systemic tumor-specific immune response, and thus induces tumor suppression.

1. Introduction

Immunotherapy is now gaining recognition as a valid approach for the treatment of cancer, in addition to the conventional modalities of surgery, chemotherapy, and radiotherapy [1–3]. Cytokine-based immunotherapy, in particular, has currently attracted a great deal of attention [4–8]. Several cytokines, including IL-2 [9], IL-12 [10] and interferon (IFN) [11], have been investigated for the treatment of patients with advanced malignancy. However, response rates using these therapies are generally low and tumors can relapse. One of the main reasons for these poor response rates is that a basic lack of interaction between the tumor and the host's immune system can lead to poor T cell priming, improper localization of anti-tumor T cells, and, ultimately, abrogation of anti-tumor immunity [12–14]. To overcome these obstacles in anti-tumor immunity, it is necessary to develop an approach that recruits naive T cells into tumors and then activates them through robust co-stimulation.

The tumor necrosis factor (TNF) superfamily member LIGHT (from "homologous to Lymphotoxins, shows Inducible expression, and competes with herpes simplex virus Glycoprotein D for Herpesvirus entry mediator, a receptor expressed by T lymphocytes") has attracted considerable interest as a novel cancer immunotherapy agent [15]. LIGHT is produced as a glycosylated 29 kDa type-II transmembrane protein by activated T cells, monocytes, granulocytes, and immature dendritic cells [15]. LIGHT binds to two functional cellular receptors, lymphotoxin β receptor (LTβR) and herpes virus entry mediator (HVEM) [16,17]. On engagement of LTβR, LIGHT can induce the release of various chemokines from stromal cells [18,19]. In addition, triggering of HVEM on T cells by LIGHT co-stimulates T cell proliferation and IFN-γ secretion, leading to enhanced T cell immunity [19,20]. A recent study has shown that transgene expression of LIGHT in tumors induces the production of chemokines and adhesion molecules in the tumor tissue, resulting in the recruitment of naive T cells that are then efficiently activated and expanded inside the tumor, leading to the rejection of established, highly progressive tumors in mice [6,7,18]. These findings suggest that LIGHT is a superior candidate for cytokine-based cancer immunotherapy to overcome the above-mentioned obstacles in anti-tumor immunity. However, because of the lack of an efficient method of manufacturing recombinant LIGHT pro-

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doi:10.1016/j.imlet.2009.08.010

tein, there have been no reports evaluating the potential of LIGHT protein for cancer immunotherapy.

In this study, we attempted to establish a simple, easy, and highly efficient method of manufacturing recombinant human LIGHT protein by using *E. coli.* In addition, we evaluated the *in vivo* anti-tumor activity of LIGHT and examined the mechanisms of tumor suppression induced by LIGHT.

2. Materials and methods

2.1. Cells and animals

B16-OVA (ovalbumin) cells (OVA cDNA transfectants of murine melanoma B16 cells) were kindly provided by Dr. Y Nishimura (Kumamoto University, Japan). The cells were cultured in RPMI 1640 (Sigma, St. Louis, MO) medium supplemented with 10% FBS, 50 μM 2-mercaptoethanol (Nacalai Tesque, Kyoto, Japan), and 200 μg/mL hygromysin B (Invitrogen, Carlsbad, CA). The HT29.14S cell line, a clone of the HT29 colon adenocarcinoma sensitive to the pro-apoptotic activity of LIGHT [21], was kindly provided by Dr. C.F. Ware (La Jolla Institute for Allergy and Immunology, La Jolla, CA) and cultured in Dulbecco's Modified Eagle's Medium (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% FBS. Female C57BL/6 mice were purchased from Nippon SLC (Shizuoka, Japan) and used at 6–8 weeks of age. All of the animal experimental procedures were performed in accordance with Osaka University's guidelines for the welfare of animals.

2.2. Expression and purification of LIGHT protein from E. coli

Human LIGHT cDNA was kindly provided by Dr. K. Tamada (University of Maryland, Baltimore, MD), and we cloned the extracellular domain region of LIGHT sequence (amino acids Gly66-Val240) into pET15b (Novagen, Darmstadt, Germany). Production of LIGHT protein using *E. coli* BL21(DE3) (Stratagene, Cedar Creek, TX) was performed similarly as described previously [22]. In brief, BL21(DE3) harboring the expression plasmid pET15b-LIGHT was incubated in Terrific Broth including 1 mM isopropyl β-D-1-thiogalactopyranoside, and the resultant inclusion bodies were washed, solubilized, and reduced by the methods previously described [23]. Then, the solubilized LIGHT was refolded by 100-fold dilution in a refolding buffer (100 mM Tris–HCl, 2 mM EDTA, 1 M arginine, and 551 mg/L of oxidized glutathione; pH 9.5) for 36 h at 4 °C. After dialysis against a buffer containing 20 mM Tris–HCl (pH 7.4) and 100 mM urea, active trimeric LIGHT proteins were purified by HiPrep Sephacryl S-100 HR column (GE Healthcare, UK) following the ion exchange chromatography (Q Sepharose Fast Flow; GE Healthcare). We used recombinant LIGHT protein generated by mammalian cells (mamLIGHT) as a control (R&D Systems, Minneapolis, MN).

2.3. Cytotoxicity assay

HT29.14S cells (5000 cells/well) were incubated for 12 h at 37 °C. Then the cells were incubated at 37 °C with serial dilutions of LIGHT protein in the presence of 10 units/mL human IFN-γ (R&D Sys-

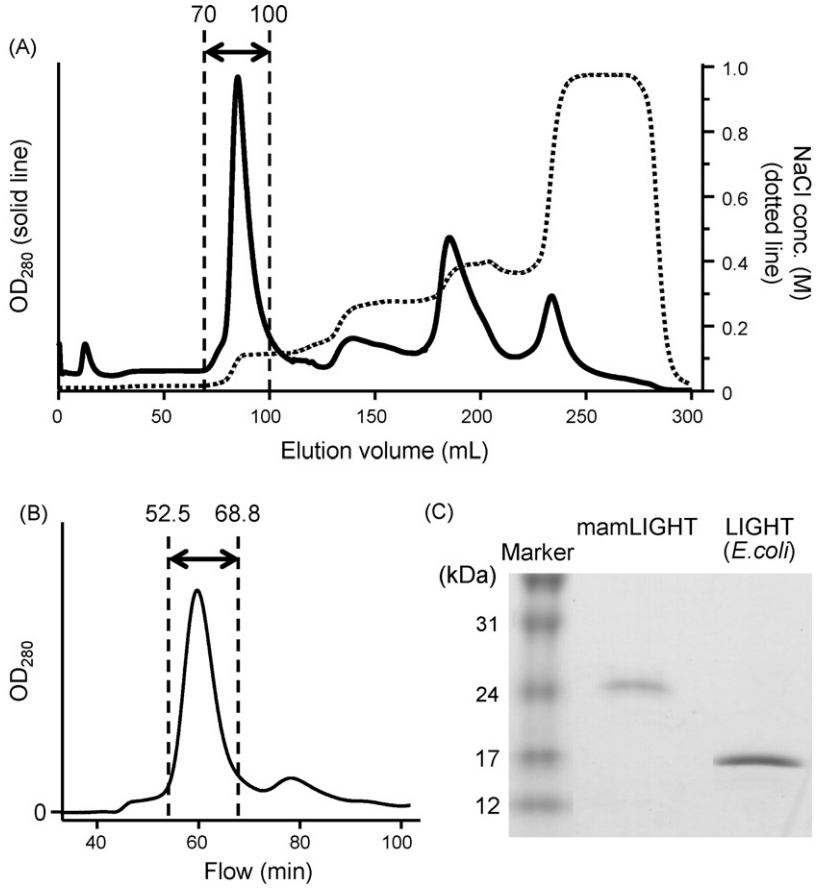

Fig. 1. Purification of LIGHT protein expressed in *E. coli*. (A) Purification of LIGHT by ion exchange chromatography. Refolded LIGHT was purified by ion-exchange chromatography on a Q-Sepharose column at a flow rate of 10 mL/min in stepwise elution, and the fractions indicated (between 70 mL and 100 mL in elution volume) were collected. Absorbance at a wavelength of 280 nm is shown as the solid black line. The salt gradient utilized is shown as the dashed black line. (B) Purification of LIGHT by size-exclusion chromatography. LIGHT was purified by gel filtration chromatography on a Sephacryl S-100 HR column at a flow rate of 0.8 mL/min in PBS; the fractions indicated (between 52.5 min and 68.8 min) were collected. (C) SDS-PAGE analysis of LIGHT. Purified LIGHT protein was analyzed by SDS-PAGE, and stained with Coomassie Blue.