Immunobiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

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

Generation of cancer-specific CD8$^+$ CD69$^+$ cells inhibits colon cancer growth

Bin Lan[a,*], Jian Zhang[b], Dong Lu[a], Wenqin Li[a]

[a] Department of Gastroenterological Surgery, The First Affiliated Hospital, Fujian Medical University, 20 Chazhong Rd., Fuzhou 350005, Fujian, China
[b] Department of Neurology, The Affiliated Union Hospital, Fujian Medical University, 29 Xinquan Rd., Fuzhou 350001, Fujian, China

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 13 August 2015
Accepted 13 August 2015
Available online xxx

Keywords:
Colon cancer
CD8 T cell
Apoptosis
Antitumor immunity
Cancer mouse model

ABSTRACT

The therapy of colon cancer (Cca) is poor currently. The abnormality of immune function is recognized in cancer-bearing hosts. This study aims to inhibit Cca growth with the *in vivo* induction of Cca-specific CD8$^+$ CD69$^+$ T cell polarization. In this study, a mouse tumor model was created with a mouse colon cancer (Cca) cell line. The Cca-bearing mice were immunized with Cca extracts and alum (using as an adjuvant). The CD8$^+$ CD69$^+$ cell polarization status was analyzed by flow cytometry and enzyme-linked immunosorbent assay. The results showed that a Cca antigen-specific CD8$^+$ CD69$^+$ cells were induced in the Cca-bearing mice. The Cca growth was efficiently inhibited by the Cca-specific CD8$^+$ CD69$^+$ cell response. The Cca-specific CD8$^+$ CD69$^+$ cells were detected in the Cca mass and spleen, which had the ability to induce Cca cell apoptosis. In conclusion, the Cca-specific CD8$^+$ CD69$^+$ cell polarization can be induced in Cca-bearing mice, which can efficiently inhibit the Cca growth.

© 2015 Elsevier GmbH. All rights reserved.

1. Introduction

The therapeutic effect of colon cancer (Cca) relies on the early diagnosis and early treatment. The radical removal of Cca by surgical operation is to obtain the ideal therapeutic results currently given the cancer metastasis has not occurred (Lykoudis et al., 2014). However, once the metastasis occurs, additional treatments are to be applied in spite of removing the cancer in situ by surgical operation (Saif et al., 2007). Chemotherapy may inhibit cancer cells in situ as well as in remote sites (Bonetti et al., 2014); the specificity of chemotherapy is to be improved in spite of tremendous advance has been made in the recent years (Curtin, 2013).

The tumor specific immune tolerance indicates that the immune system does not respond to the stimulation of the existing tumor cells, which facilitates tumor cells to escape from the immune surveillance and grow out into tumor (Quezada et al., 2011). The dysfunction of the immune effector cells on attacking tumor cells is another pathological feature of tumors (Wang et al., 2011). Thus, it is expected to inhibit the tumor growth by breaking down the established tumor specific immune tolerance (Dougan and Dranoff, 2009) or restore the tumor-inhibitory function of immune effector cells.

CD8$^+$ T cells are a fraction of T lymphocyts; it is also called cytotoxic T cells that kill cancer cells. CD8$^+$ T cells express T cell receptors that recognize specific antigens. The antigens must be presented by the class I MHC molecules from antigen presenting cells. In order for the TCR to bind to the class I MHC molecule, TCR must be accompanied by a glycoprotein called CD8, which binds to the constant portion of the class I MHC molecule. Upon activation, CD8$^+$ T cells release cytotoxic mediators to kill cancer cells. Yet, the underlying mechanism is not fully understood.

Cumulative evidence from clinical studies and laboratory studies demonstrates that the abnormality of the immune balance plays a critical role in the pathogenesis of a large number of human diseases, such as in the T helper (Th) 1/Th17 polarization-related chronic inflammatory diseases (Allenbach et al., 2014; Neuman, 2007) or in the Th2 polarization-related allergic diseases (Kaur and Brightling, 2012; Mantel et al., 2007). Animal models of these diseases have been created to study the pathogenesis of human diseases. To date, some of the animal models can successfully mimic the immune cell polarization such as Th1 or Th2 polarization. Since the polarized immune effector cells may inhibit cancer cell growth (Chang et al., 2007; Muller et al., 2002), we hypothesize that to generate polarized Cca-specific immune effector cells in the Cca-bearing mice can inhibit Cca growth. Thus, we created a Cca mouse model. The Cca-bearing mice were immunized with Cca cell extracts as an immunogen and a mixture of adjuvant of alum. The results showed that a fraction of Cca-specific CD8$^+$ CD69$^+$ cells was

* Corresponding author.
E-mail address: binrlan@outlook.com (B. Lan).

http://dx.doi.org/10.1016/j.imbio.2015.08.010
0171-2985/© 2015 Elsevier GmbH. All rights reserved.

Please cite this article in press as: Lan, B., et al., Generation of cancer-specific CD8$^+$ CD69$^+$ cells inhibits colon cancer growth. Immunobiology (2015), http://dx.doi.org/10.1016/j.imbio.2015.08.010

generated in the Cca-bearing mice after the immunization. These cells expressed tumor necrosis factor (TNF)-α, interferon (IFN)-γ, perforin and granzyme B, which efficiently induced Cca cell apoptosis and inhibited Cca growth in mice.

2. Materials and methods

2.1. Reagents

The antibodies for flow cytometry were purchased from BD Bioscience (Beijing, China). The reagents for Western blotting and RT-qPCR were purchased from Invitrogen (Shanghai, China). The fluorochrome-labeled antibodies of CD3, CD8, CD69 TNF-α, IFN-γ, perforin and granzyme B were from BD Biosciences (Shanghai, China). The ELISA kits of TNF-α, IFN-γ, perforin and granzyme B were purchased from R&D Systems (Beijing, China). The immune cell isolation reagent kits were purchased from Miltenyi Biotech (Shanghai, China). The neutralizing anti-CD69 mAb and anti-TNF-α mAb were from Biomart (Shanghai, China). The Annexin V kit was from Sigma–Aldrich (Shanghai, China).

2.2. Cell lines and cell culture

Mouse colon cancer cell line (CT26.CL25 cells; Cca cells) was purchased from ATCC (Beijing, China). CT26.CL25 cells were cultured with RPMI1640 medium; The media were supplemented with fetal bovine serum (10%), 0.1 mg/ml streptomycin, 100 U/ml penicillin and 2 mM glutamine. The medium was changed in every 3 days. The cell viability was assessed by the Trypan blue exclusion assay.

2.3. Cca mouse model

Male BALB/c mice (6–8 week old) were purchased from the Beijing Experimental Animal Center. The mice were maintained in a pathogen free environment. The using mouse in the present study was approved by the Animal Ethic Committee at Fujian Medical University. Each mouse was injected with 10^6 Cca cells in 0.3 ml saline into the groin of one side. The mice were monitored daily for the Cca mass growth. The Cca mass size was measured with a slide caliper and calculated to mm^3.

2.4. Preparation of Cca extracts

CT26.CL25 cells were harvested; the total proteins were extracted from the cells by the routine procedures of protein extraction. The proteins were quantified with the Bio-Rad protein assay kit. The Cca extracts were used in the immunization.

2.5. Immunization

A Cca-specific immune response mouse model was created using alum as an adjuvant, which is extensively used to create allergy models (Allen et al., 2012). Briefly, an immunogen mixture was made of 0.3 ml alum and 0.1 ml Cca extracts (containing 0.1 mg protein), which is one dose for one mouse. The Cca-bearing mice were subcutaneously injected with one dose of the immunogen on day 10 and day 17, respectively.

2.6. Flow cytometry

Cells were stained with the fluorochrome-labeled antibodies (or isotype IgG) of interest for 30 min on ice and fixed with 2% paraformaldehyde containing 0.5% saponin for 1 h. After washing, the cells were stained with fluorochrome-labeled antibodies (or isotype IgG) for 1 h at room temperature. The cells were analyzed by flow cytometry (FACSCanto II, BD Bioscience, Shanghai, China). The data were analyzed with software Flowjo using the data of isotype IgG staining as a gating guidance.

2.7. Cca tissue processing

Cca tissue was excised from mice and cut into small pieces, and incubated in culture medium containing collagenase IV (0.5 mg/ml) for 2 h at 37 °C. The cells were filtered through a cell strainer (70 μm) and cultured for further experiments.

2.8. Immune cell isolation

The immune cells are isolated by the magnetic cell sorting (MACS) with commercial reagent kits following the manufacturer's instructions. The purity of the isolated cells was checked by flow cytometry.

2.9. Assessment of Cca-specific $CD8^+$ $CD69^+$ cell proliferation

$CD8^+$ $CD69^+$ cells were isolated by MACS, labeled with carboxyfluorescein succinimidyl ester (CFSE), and cultured with DC at a ratio of 10:1 in the presence of the Cca extracts (using as a specific antigen; 1 μg/ml). The cells were collected 3 days later and analyzed by flow cytometry.

2.10. Assessment of Cca apoptosis

$CD8^+$ $CD69^+$ cells were isolated from the spleen of Cca-bearing mice after the immunization. Cca cells and $CD11c^+$ DCs were cultured in Transwell basal chambers with the $CD8^+$ $CD69^+$ cells in the apical chambers (T cell:DC:Cca cell = 10^6:10^5:10^6/ml). Cells were harvested from the basal chambers, stained with anti-CD11c antibody, the Annexin V reagents and propidium iodide. The cells were analyzed by flow cytometry. The $CD11c^+$ DCs were gated out first. Cells with Annexin V^+ or Annexin V^+/propidium $idodide^+$ were regarded as apoptotic.

2.11. Enzyme-linked immunosorbent assay (ELISA)

Cytokines in the supernatant were analyzed by ELISA with commercial reagent kits following the manufacturer's instructions.

2.12. Statistics

The data are presented as mean ± SD. Differences between groups were determined by ANOVA with $p < 0.05$ as a significant criterion.

3. Results

3.1. Specific immunization inhibits Cca growth

A Cca mouse model was created. The tumor mass grew to about 2 cm in all mice on day 10 after Cca cell transplantation. BALB/c mice were immunized with Cca extracts (using as a Cca specific antigen) plus an adjuvant (alum) on day 10 and 17. The Cca mass size was monitored in the following 2 weeks. The tumor size records showed that in the immunized mice, the tumor size gradually shrank after the immunization, while the tumor in control mice (treated with saline) kept growing (Fig. 1A). The Cca-bearing mice died between day 30 to day 36; none of the immunized mice died during the 50-day observation period (Fig. 1B). The results indicate that the specific immunization can inhibit Cca cell growth in mice.

Please cite this article in press as: Lan, B., et al., Generation of cancer-specific $CD8^+$ $CD69^+$ cells inhibits colon cancer growth. Immunobiology (2015), http://dx.doi.org/10.1016/j.imbio.2015.08.010

Download English Version:

https://daneshyari.com/en/article/10940789

Download Persian Version:

https://daneshyari.com/article/10940789

Daneshyari.com