

## Original Article

# Function and subsets of dendritic cells and natural killer cells were decreased in gastric cancer

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**Abstract:** Dendritic cells (DCs) and natural killer (NK) cells initiate specific immune responses against tumor cells. The aim of the present study was to determine the cytotoxicity and the subsets of the DC and NK cells and the cytokines level of DC and NK cells from cancer tissue and peripheral blood in the gastric cancer patients. Cytotoxicity of DC and NK was determined using the Cytotox non-radioactive assay. The cytotoxic activity of DC or NK isolated from cancer tissue and peripheral blood was attenuated in gastric cancer patients. CD11c, CD80, CD83, CD16, CD57 and CD69 were decreased in the cancer tissue and peripheral blood in the gastric cancer patients. CD86, CCR7 and CD59 were no significance in the cancer tissue and peripheral blood from gastric cancer patients. Tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-2, T-bet and IL-15R $\beta$  levels were decreased in DC and NK from the gastric cancer tissue and peripheral blood in the gastric cancer patients. IL-15 and IL-15R $\alpha$  level were no significance in DC and NK in the gastric cancer tissue and peripheral blood in the gastric cancer patients. These results indicate that the cytotoxic activity and subsets and cytokines of DC and NK cells in the cancer tissue and peripheral blood in the gastric cancer patients were decreased. The decrease of subsets content and cytokines of DC and NK may contribute to a decrease in the function of DC and NK in the tissue and peripheral blood in the gastric cancer patients.

**Keywords:** Dendritic cell, natural killer cell, cytotoxicity, cytokine

## Introduction

Dendritic cells (DCs) are recognized as being specialized appropriate T-antigen presenting cells which play a key role in generating primary and secondary immune responses against specific antigens [1]. Two peripheral blood DC subsets have been described: myeloid-derived CD11c+CD123-DCs (DC1) and lymphoid-derived CD11c-CD123+ DCs (DC2) [2, 3]. Natural killer (NK) cells contribute to the innate immune defense against tumors and microbial pathogens. Human NK cells make up approximately 10% to 15% of total blood lymphoid cells and perform two major functions: the first is recognizing and lysing tumor cells and virally infected cells [4, 5]; the second is regulating the innate and adaptive immune responses [6, 7].

It has been shown that DC vaccine transfected with gastric cancer cell total RNA carrying the 4-1BB/4-1BB ligand gene has a stronger ability

to kill gastric cancer cells through promoting T cell proliferation and enhancing the ability of cytotoxic T lymphocytes to kill gastric carcinoma cells [8]. Circulating lymphocyte subsets in gastric cancer patients are significantly changed such as CD3+, CD8+ CD4+, CD19+, CD44+, CD25+, NK cells [9]. The levels of CD3+T, CD4+T, NK cell and CD4+T/CD8+T in gastric cancer patients were lower as compared to healthy volunteers [10]. CD81 mRNA levels were found to be low in primary tumors, and these low expression levels were found to correlate with the stage and grade of the tumors [11]. However, the cytotoxic activity and subsets content of DC and NK in the cancer tissue and peripheral blood in the gastric cancer patients are not very clear.

It has been demonstrated that impaired DC function contributes to the less effective innate and adaptive immune responses against *H. pylori* seen in gastric cancer patients and *H.*

*pylori* can regulate interleukin (IL)-10 production [12]. The IL-12 and tumor necrosis factor (TNF)-alpha level was much higher in trans-gene-d DC cells than blank DC cells [13]. IL1 $\beta$  physiologically induced by helicobacter pylori infection enhanced gastric carcinogenesis by affecting both inflammatory and epithelial cells [14]. Mammalian IL-15 plays an important role in the activation of DC and NK cells along with its receptors named ILR [15]. However, the levels of inflammatory cytokines in DC and NK in the cancer tissue and peripheral blood in the gastric cancer patients are not very clear. The present study was designed to determine the cytotoxic activity and the subsets content of DC and NK and the levels of inflammatory cytokines in DC or NK isolated from cancer tissue and peripheral blood in the gastric cancer patients.

### Materials and methods

#### *Tissue and blood specimens*

Forty-eight gastric cancer and corresponding normal gastric tissue samples (more than 10 cm away from the edge of the gastric cancer) were taken from gastric cancer patients (the Third Affiliated Hospital of Suzhou University and the Drum Tower Hospital). Blood samples were collected from cancer patients and control into a tube containing EDTA-K<sub>2</sub>. No patients had received chemotherapy or radiotherapy before surgery.

#### *Measurement of cytokine production*

DC and NK cells isolated from cancer tissue and blood were cultured for 5 days and the culture media were then collected. The total protein was extracted and measured using a protein assay kit (BCA; Pierce, Santa Cruz, CA, USA). The levels of cytokines release were determined by enzyme-linked immunoassay (ELISA) kit (R & D Systems; Oxfordshire, UK) according to the manufacturer's instructions. Briefly, a 96-well microplate was coated with an antibody specific for TNF- $\alpha$ , T-bet, IL-2 or IL-15. We added 100  $\mu$ l of sample and 100  $\mu$ l of standard diluent buffer to each well in duplicate, incubated it for 90 min at 37°C, and then washed it five times. Subsequently, 100  $\mu$ l of biotinylated anti-TNF- $\alpha$ , IL-2, IL-15, T-bet or antibody solution were added, incubated for 60 min at 37°C, and then washed. 100  $\mu$ l of streptavidin-horserad-

ish peroxidase conjugate solution were added, incubated for 30 min at 37°C, and washed. 100  $\mu$ l of chromagen solution were added and incubated in the dark for 15 min at 37°C. The reactions were stopped with HCl and read at 450 nm using an ELISA plate reader. Standardization curves were made with known concentrations of TNF- $\alpha$ , IL-2, IL-15, T-bet.

#### *Western blotting*

NK cells and DC from cancer and control were cultured for 5 days and the culture media were then collected and lysed in modified RIPA or lysed directly in 1  $\times$  SDS loading buffer. After process of electrophoresis and transmembrane, proteins on nitrocellulose membrane were probed with the IL-15R $\alpha$  or IL-15R $\beta$  primary antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by incubation with the secondary antibodies (1:5000; Immunology Consultants Lab, USA). The bands were visualized by enhanced chemiluminescence using ECL (Pierce Chemical) and captured on X-ray films. GAPDH (Bioworld Technology Inc., USA) protein was used as a loading control. The total IL-15R $\alpha$  or IL-15R $\beta$  protein level were normalized to the GAPDH protein level.

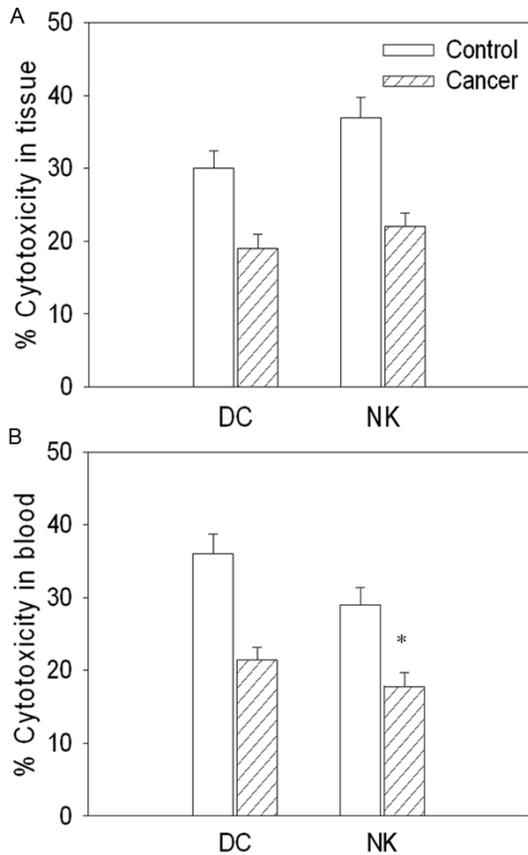
#### *Flow cytometry*

Cellular phenotyping was performed on a FACS Cantoll flow cytometer (Becton Dickinson, San Jose, CA, USA) as described with minor modifications. The following fluorochrome labelled monoclonal antibodies conjugated to FITC, PE, PETxR, PeCy7, PerCPCy5.5, APC, APC-Cy7, Pacific Blue and appropriate isotype controls were used for surface staining according to the manufacturer's instructions: CD11c, CD16, CD56, CD57, CD59, CD69, CD83, CD80, CD86, CCR7 (all mabs from Biolegend, Germany), Siglec-F (BD Biosciences, Germany), Langerin (CD207; 929F301, Imgenex, San Diego, USA) and 120g8 (Dendritics, France). Absolute leukocyte numbers were determined by using trucoount beads (Becton Dickinson, Germany) according to the manufacturer instructions.

#### *Cytotoxicity of DC and NK*

Cytotoxicity of DC and NK towards TRAMP-C1 target cells was determined using the Cytotox 96 non-radioactive assay. Briefly, after 5 days

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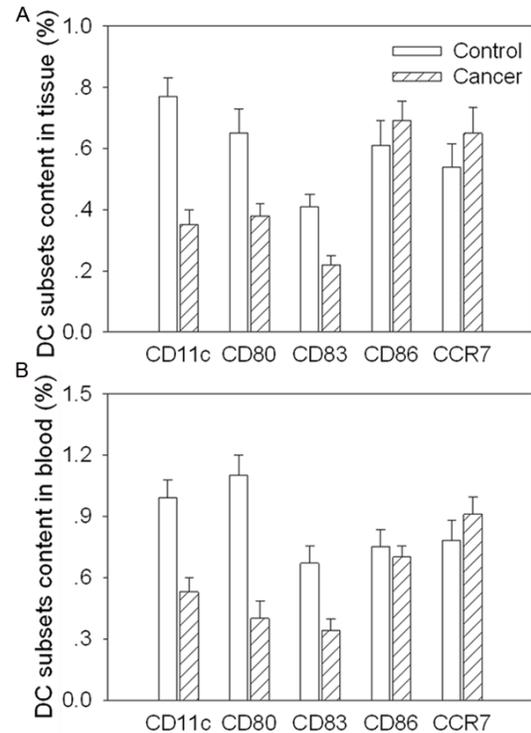


**Figure 1.** Cytotoxicity of dendritic cells (DCs) and natural killer (NK) cells isolated from cancer tissue and peripheral blood in gastric cancer patients. A. The cytotoxicity of DC and NK isolated from cancer tissue; B. The cytotoxicity of DC and NK isolated from peripheral blood. Values are mean  $\pm$  SE. \* $P < 0.05$  versus Control. N = 8 for each group.

of culture, NK and DC cells were plated in 96-well plates. After 24 h, TRAMP-C1 target cells were added to the wells. After 24 h, plates were spun down and supernatant was collected for analysis of cytotoxicity using the Cytotox 96 non-radioactive assay (Promega) which determines release of lactate dehydrogenase.

### Statistical analysis

Data were analyzed by using SPSS 18.0 (IBM, Armonk, NY, USA). Comparisons between 2 observations were assessed by Student's paired *t*-test. One-way or two-way ANOVA was used followed by the Bonferroni test for post hoc analysis when multiple comparisons were made. All of the data were expressed as the mean  $\pm$  SE. A value of  $P < 0.05$  was considered statistically significant.



**Figure 2.** Subsets content of dendritic cells (DCs) isolated from cancer tissue and peripheral blood in gastric cancer patients. A. The subsets content of DC isolated from cancer tissue; B. The subsets content of DC isolated from peripheral blood. Values are mean  $\pm$  SE. \* $P < 0.05$  versus Control. N = 8 for each group.

## Results

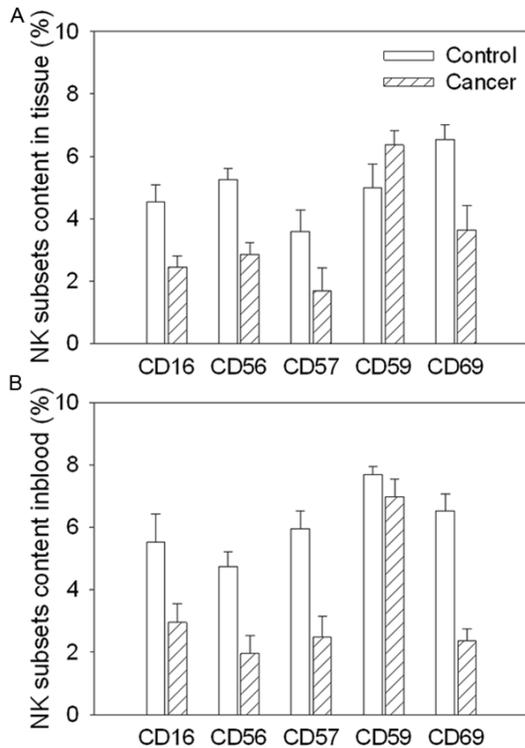
### Cytotoxic activity of DC and NK

The cytotoxic activity of DC and NK isolated from cancer tissue was attenuated in gastric cancer patients compared with controls. The cytotoxicity of DC and NK isolated from peripheral blood was also attenuated in cancer patients (**Figure 1**).

### DC subsets in tissue and blood

CD11c content was decreased in the cancer tissue and peripheral blood in the gastric cancer patients compared with controls. CD80 and CD83 were also decreased in the cancer tissue and peripheral blood in the gastric cancer patients compared with controls. In the cancer tissue and peripheral blood, CD86 and CCR7 were no significance in the gastric cancer patients compared with controls (**Figure 2**).

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**Figure 3.** Subsets content of natural killer (NK) cells isolated from cancer tissue and peripheral blood in gastric cancer patients. A. The subsets content of NK isolated from cancer tissue; B. The subsets content of NK isolated from peripheral blood. Values are mean  $\pm$  SE. \* $P < 0.05$  versus Control. N = 8 for each group.

### NK subsets in tissue and blood

CD16 was decreased in the cancer tissue and peripheral blood in the gastric cancer patients compared with controls. CD56, CD57 and CD69 were also decreased in the cancer tissue and peripheral blood in the gastric cancer patients. However, CD59 was no significance in the cancer tissue and peripheral blood in the gastric cancer patients compared with controls (Figure 3).

### Cytokines production in DC and NK in the tissue

TNF- $\alpha$  level was decreased in DC and NK in the gastric cancer tissue compared with controls. IL-2 and T-bet levels were also decreased in DC and NK isolated from gastric cancer tissue. IL-15 and IL-15R $\alpha$  level were no significance in DC and NK in the gastric cancer tissue compared with controls. However, IL-15R $\beta$  protein

level was decreased in the DC and NK isolated from cancer tissue in the gastric cancer patients (Figure 4).

### Cytokines production in DC and NK in blood

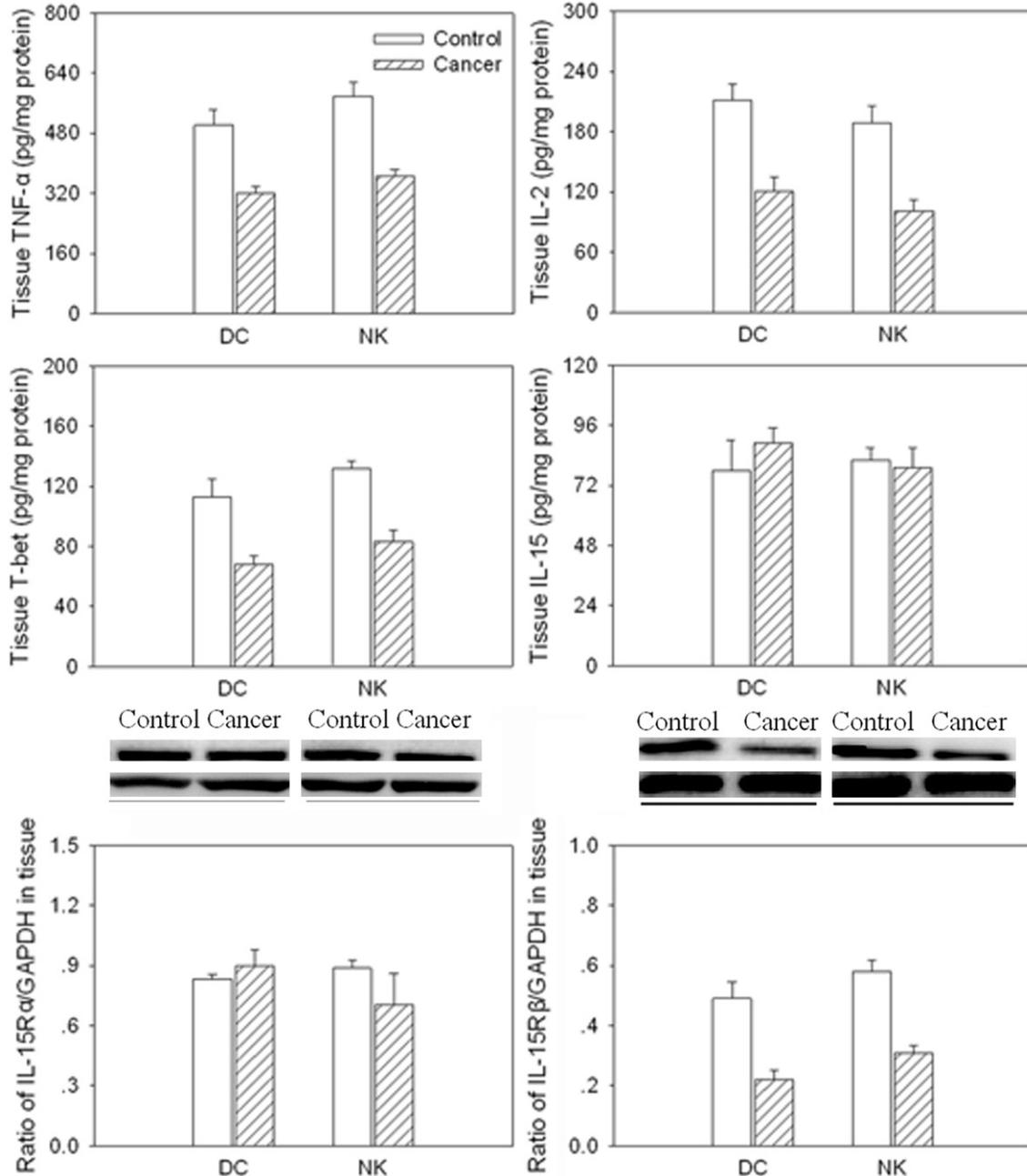
TNF- $\alpha$  level was decreased in DC and NK in the peripheral blood from gastric cancer patients compared with controls. IL-2 and T-bet levels were also decreased in DC and NK isolated from blood in the gastric cancer patients. IL-15 and IL-15R $\alpha$  was no significance in DC and NK in blood from the gastric cancer patients compared with controls. However, IL-15R $\beta$  protein level was decreased in the DC and NK isolated from blood in the gastric cancer patients (Figure 5).

## Discussion

Gastric cancer is the second most common cancer worldwide and the second most common cause of cancer-related deaths [16] and has a strong link with chronic inflammation [17, 18]. Immune dysfunction may contribute to the tumor progression in the gastric cancer [19]. Impaired function of immune cells such as CD8(+) T cells, NK cells, and DCs results in tumor progression in cancer patients. The present study demonstrates new findings that the cytotoxic activity of DC or NK isolated from cancer tissue and peripheral blood was decreased in gastric cancer patients. The subsets content and inflammatory cytokines of DC and NK cells in the cancer tissue and peripheral blood in the gastric cancer patients were decreased. The decrease of subsets content and cytokines of DC and NK may contribute to a decrease in the function of DC and NK in the tissue and peripheral blood in the gastric cancer patients.

DCs are potent professional antigen-presenting cells with the ability to prime naïve T cells, and play an important role in the initiation and regulation of immune responses. The NK cells play an important role in immune surveillance during tumorigenesis [20, 21]. Gastric patients with low levels of circulating mature DCs had significantly lower values of T lymphocytes, T helper lymphocytes and NK cells than those with normal mature DC levels [22]. In the present study, we show that the cytotoxic activity of DC or NK isolated from cancer tissue and peripheral blood was attenuated in gastric cancer patients. The subsets of DC such as CD11c,

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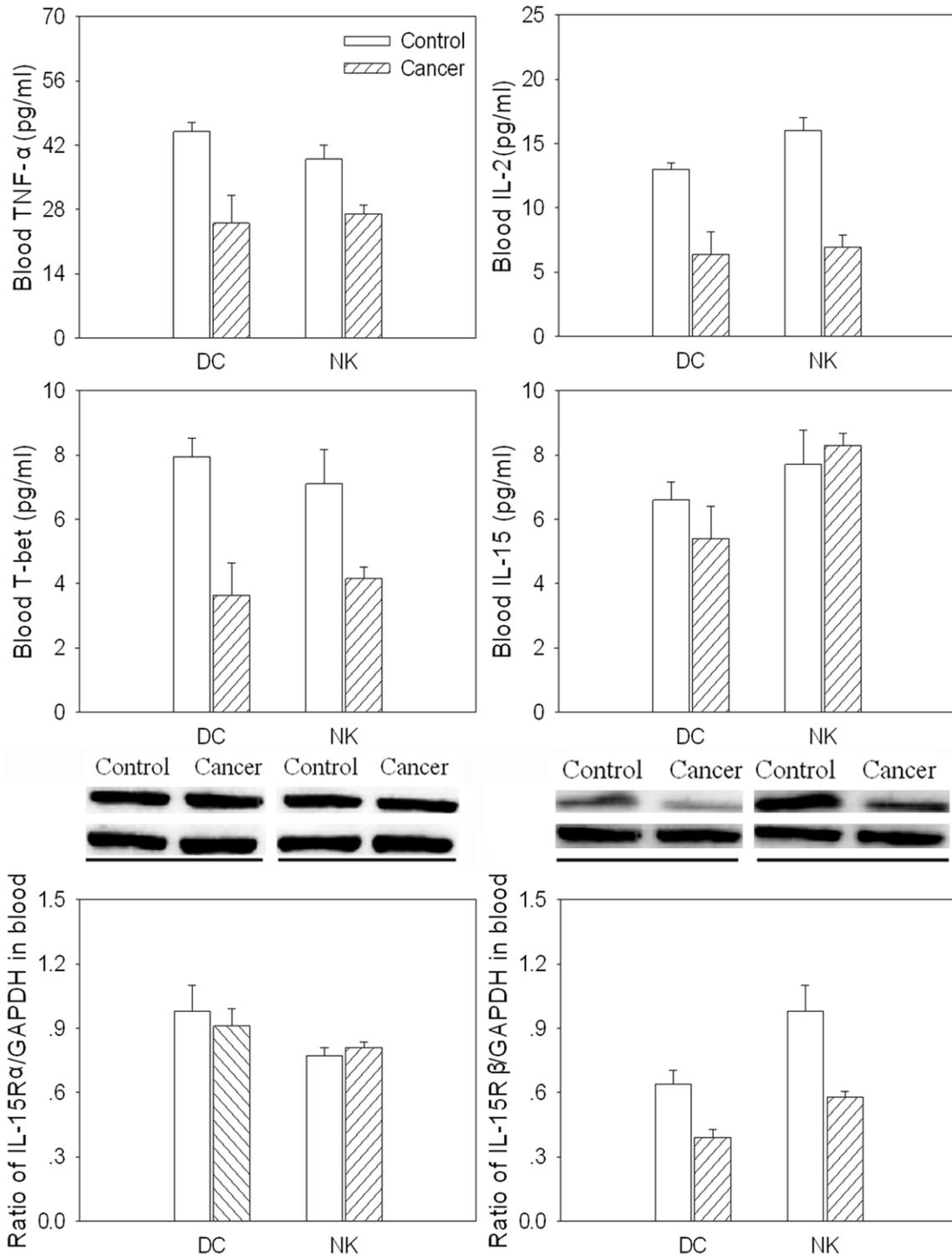


**Figure 4.** Levels of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-2, T-bet, IL-15, IL-15R $\alpha$  and IL-15R $\beta$  in dendritic cells (DCs) and natural killer (NK) cells isolated from cancer tissue in gastric cancer patients. Values are mean  $\pm$  SE. \* $P < 0.05$  versus Control. N = 8 for each group.

CD80 and CD83 were decreased in the cancer tissue and peripheral blood in the gastric cancer patients compared with controls, CD86 and CCR7 were no significance in the gastric cancer patients compared with controls. The subsets of NK cell such as CD16, CD56, CD57 and CD69 were decreased in the cancer tissue and peripheral blood in the gastric cancer patients,

CD59 was no significance in the tissue and peripheral blood in the gastric cancer patients compared with controls. These results show that the function of DC and NK cell was attenuated. The decrease of subsets content of DC and NK contribute to a decrease in the cytotoxic activity of DC and NK in the tissue and peripheral blood in the gastric cancer patients.

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**Figure 5.** Levels of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-2, T-bet, IL-15, IL-15R $\alpha$  and IL-15R $\beta$  in dendritic cells (DCs) and natural killer (NK) cells isolated from peripheral blood in gastric cancer patients. Values are mean  $\pm$  SE. \* $P$  < 0.05 versus Control. N = 8 for each group.

It has been shown that NK cells from peripheral blood of gastric cancer patients have a severely

suppressed ability to produce IFN- $\gamma$  after stimulation with *H. pylori* lysate [23]. T-bet, as a key

marker for type 1 immune responses, was significant increases in T-bet(+) lymphocytes in tumor tissues as compared with normal stomach tissues, gastritis tissues or gastric polyp specimens and mainly consisted of CD4(+), CD8(+) and CD56(+) tumor-infiltrating lymphocytes [24]. In the present study, tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-2, T-bet and IL-15R $\beta$  levels were decreased in DC and NK in the gastric cancer tissue and peripheral blood in the gastric cancer patients. IL-15 and IL-15R $\alpha$  level were no significance in DC and NK in the gastric cancer tissue and peripheral blood in the gastric cancer patients. The decrease of cytokine levels of DC and NK contribute to a decrease in the function of DC and NK in the tissue and peripheral blood in the gastric cancer patients.

In conclusion, the cytotoxic activity of DC and NK cell from cancer tissue and peripheral blood in the gastric cancer patients was attenuated. The decrease of subsets content and inflammatory cytokine levels of DC and NK may contribute to a decrease in the function of DC and NK in the tissue and peripheral blood in the gastric cancer patients.

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### Disclosure of conflict of interest

None.

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