Clinical significance of regulatory B cells in the peripheral blood of patients with oesophageal cancer

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Abstract

B cell subsets have been found to exhibit a negative regulatory function, like Tregs. The present study investigates the effects of CD5+CD19+ interleukin (IL)-10 (B10) on the occurrence and development of oesophageal carcinoma by analysing B10 levels in the peripheral blood of patients with oesophageal carcinoma. Peripheral blood of 120 oesophageal cancer patients and 120 healthy controls were collected, and regulatory B cell counts were determined by flow cytometry. The level of B10 cells in the peripheral blood of patients with oesophageal carcinoma was significantly higher than that in healthy controls ($p < 0.05$). In addition, B10 levels in stage III-IV patients (3.5 ±0.7%) were higher than those in stage I-II patients (2.5 ±0.6%), which were in turn higher than those in the healthy controls (1.3 ±0.3%). The level of B10 increased with clinical progression of oesophageal cancer, suggesting that B10 cells may influence the development or progression of oesophageal cancer.

Key words: flow cytometry, blood, cancer.

Introduction

Oesophageal carcinoma is a common malignant tumour, accounting for 1/8th of all cancer mortality [1, 2]. Previous studies have shown that subsets of regulatory T cells (Tregs) play important roles in immune suppression in cancer patients [3]. In recent years, B cell subsets, specifically regulatory B cells (Bregs), have also been found to exhibit a negative regulatory function, like Tregs. Research has shown that this group of cells are B cells (CD5+CD19+) that express CD5+ [4]. CD5+ B cells produce autoantibodies and pro-inflammatory cytokines, increase antigen presentation, and play an important role in autoimmune diseases [5]. Based on the production of different cytokines, CD5+B cells are divided into Br1, Br3, and Breg, of which those responsible for the production of interleukin (IL)-10 and for exerting a negative regulatory effect on the immunity are referred to as Br1 (B10) [6, 7]. Recently, Noh *et al.* found that CD5+CD19+ Bregs secrete IL-10 in peripheral blood of patients with allergic diseases [8]. Chen found that expression of CD5+CD19+ IL-10 Bregs in the peripheral blood of patients with hepatocellular carcinoma was higher than that in healthy controls, suggesting that Bregs play a crucial role in the develop-ment and prognosis of carcinogenesis [9]. To investigate the relationship between B10 and oesophageal cancer by defining the role of B10 in the diagnosis, treatment, and prognosis of patients with oesophageal carcinoma, levels of B10 were examined at different stages in oesophageal cancer patients in this study.

Material and methods

Ethics statement

This study was approved by the Ethics Committee of The Second People's Hospital of Lianyungang, China. The participants provided their verbal informed consent to participate in this study, and this consent was recorded in an electronic spreadsheet as approved by the Ethics Committee of The Second People's Hospital of Lianyungang, China.

Subjects

We collected peripheral blood from 120 oesophageal cancer patients from the departments of thoracic surgery and radiotherapy of Lianyungang Hospital Affiliated to

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Bengbu Medical College between December 2011 and December 2013. All patients had no other complications and did not accept operations before admission or chemotherapy. The patients (69 males, 51 females; age range: 50-70 years old, average age: 63.5 years) were grouped according to the standard of staging set by the American Cancer Coalition (Edge and Compton, 2010): I-II stage: 58 cases; III-IV stage: 62 cases. A total of 120 healthy age- and sex-matched control subjects from the hospital medical centre were included in the study.

Sample collection

The venous peripheral blood from patients (5 ml per person) were extracted between 6 a.m. and 8 a.m. by using heparin lithium anticoagulant tubes. The bloods were tested within three hours after collection.

Extraction and culture of peripheral blood mononuclear cells

Two millilitres of whole blood was uniformly mixed with 2 ml of phosphate buffered saline (PBS) and added to a 3 ml lymphocyte extraction Ficoll centrifugal tube (GC, America). The mixture was centrifuged at $1,500 \times g$ for 30 minutes. After centrifugation, the contents of the tube were divided into five layers: the top layer was a plasma layer, followed by a mononuclear cell layer, separation liquid layer, granule cell layer, and red cell layer. The mononuclear cells were transferred into another centrifuge tube and 6 ml of PBS was added. The mixture was centrifuged at $1,500 \times g$ for 10 minutes and the supernatant was discarded. This operation was repeated twice. The washed mononuclear cell number was adjusted to 2×10^6/ml by adding RPMI1640. The mononuclear cell solution (0.5 ml) was transferred into a Falcon flow tube (GC, America), into which 2 µl of the stimulator (consisting of concanav-

alin A, lipopolysaccharide, ionomycin, and staphylococcal enterotoxin B) was then added. The mixture was incubated at 37°C in a 5% CO_2 incubator for 5 hours.

Cell surface and intracellular staining and flow cytometry

Ten microlitres of CD5-FITC (BD, America) and 10 µl of CD19-PE (BD, America) were added to a Falcon tube, mixed, and allowed to stand in the dark at room temperature for 15 minutes. Then, 100 µl of FIX & RERM Reagent (A, ADG, Australia) was added, mixed, and left in the dark at room temperature for 15 minutes. Three millilitres of PBS was added to the Falcon tube and the mixture was centrifuged at $1,500 \times g$ for 5 minutes, after which the supernatant was discarded. Then, 5 µl of IL-10 APC (BD, America) was added, mixed, and left in the dark at room temperature for 15 minutes. Next, 3 ml of PBS was added to the Falcon tube and the mixture was centrifuged at $1,500 \times g$ for 5 minutes, discarding the supernatant at the end. Finally, 500 µl PBS was added, and the stained cells were detected by flow cytometry (BD, America).

Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using Statgraphics Centurion XV version 15.1.02. Multifactor ANOVA with posterior multiple range test was used to determine significant differences between groups.

Results

The CD5+B cell ratio in the peripheral blood of patients with stage III-IV oesophageal cancer (3.49 ±1.08%, Fig. 1) was higher than that of oesophageal cancer patients at stages I-II (2.53 ±0.86%, $p < 0.05$, Fig. 1), which was higher
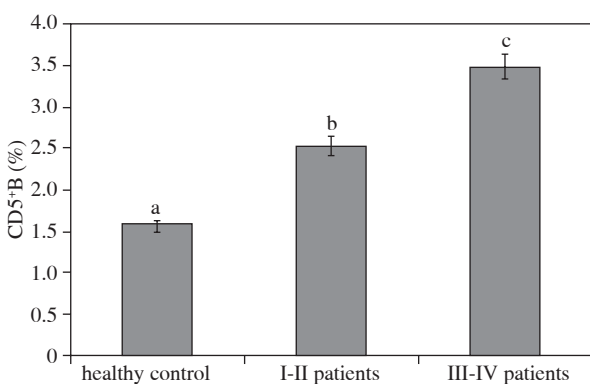

Fig. 1. The expression of CD5+B in the peripheral blood of patients with oesophageal carcinoma and in the healthy group. The different letters on the bars indicate significant differences ($p < 0.05$). Data are shown as mean ± SD ($n = 3$)
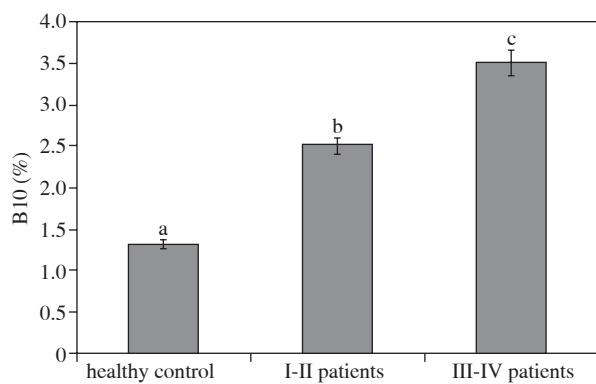

Fig. 2. The levels of B10 in the peripheral blood of patients with oesophageal carcinoma and in the healthy group. The different letters on the bars indicate significant differences ($p < 0.05$). Data are shown as mean ± SD ($n = 3$)

still than that of healthy controls ($1.57 \pm 0.81\%$, $p < 0.05$, Fig. 1). The ratio of B10 subsets in the peripheral blood of stage III-IV oesophageal cancer patients ($3.5 \pm 0.7\%$) was also higher than that of stage I-II oesophageal cancer patients ($2.5 \pm 0.6\%$, $p < 0.05$, Fig. 2), which was in turn higher than that of healthy controls ($1.3 \pm 0.3\%$, $p < 0.05$, Fig. 2).

Discussion

The main functions of B10, which have recently attracted the interest of many scholars at home and abroad, are inhibition of inflammation in autoimmune diseases and anti-tumour activity [9-12]. In this study, B10 ratios in the peripheral blood of oesophageal cancer patients and healthy controls were compared. The results showed that the B10 ratio in the peripheral blood between oesophageal cancer patients increased significantly compared with the healthy control group. These results are similar to a previous finding that the proportion of CD19+ IL-10B cells in patients with liver cancer was higher than that in healthy controls [9]. Inoue *et al.* proposed that the increased antitumor immune response in mice lacking B cells is related to reduced secretion of IL-10 [13]. In addition, research has shown that expression of CD19+ IL-10 gradually increases in patients with hepatocellular carcinoma on the first day and one week after surgical operation, probably due to removal of the suppressive effect of B10 by surgical operation [9]. Furthermore, experiments have shown that removal of Bregs is important for tumour clearance induced in mice [14]. The current study found that expression of CD5+ B cells is higher in oesophageal carcinoma patients than in healthy controls. The results also showed that with stronger inhibition of CD5+ B in oesophageal carcinoma patients, expression of CD5+ B also became higher, and this correlated positively with the stage of oesophageal carcinoma. This may be related to the amount of inhibition of IL-10 secretion. We also found that as the amount of IL-10 increased, the inhibition became stronger.

In summary, the results showed that with higher clinical staging of oesophageal cancer, the immune function of patients was lower, and B10 expression was higher. Thus, our results suggest that B10 may be related to the development of oesophageal cancer.

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The authors declare no conflict of interest.

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