Chinese-German Journal of Clinical Oncology
DOI 10.1007/s10330-011-0710-1
February 2011, Vol. 10, No. 2, P105–P107

Clinical study on IL-8, TNF-α, T-cell subgroup in serum of patients with rectal cancer

Jian Chen[1], Fang Xiang[2], Xiaojun Tang[3]

[1] Department of General Surgery, the 81st Hospital of PLA, Nanjing 210002, China
[2] Department of Pharmacy, the 81st Hospital of PLA, Nanjing 210002, China
[3] Department of General Surgery, Huaian First Hospital Affiliated to Nanjing Medical University, Huai'an 223300, China

Received: 18 September 2010 / Revised: 30 September 2010 / Accepted: 5 November 2010

Abstract ***Objective:*** The aim of our study was to investigate the immune status of patients with rectal cancer and its relationship with clinicopathological features. ***Methods:*** The serum levels of interleukin-8 (IL-8), tumor necrosis factor (TNF-α) and T-cell subgroup contents were measured using a double-antibody sandwich assay of ELISA in 43 patients with rectal cancer, and compared with the normal health adults. ***Results:*** In patients with rectal cancer, the serum levels of CD4, CD4/CD8 of T-cell subgroup in peripheral blood were significantly lower than the control group ($P < 0.01$), which gradually decreased with increase of Dukes stage; but the levels of CD8, IL-8 and TNF-α were higher than the control group, which gradually increased with increase of Dukes stage. ***Conclusion:*** The immunocompromice exists in patients with rectal cancer, there is a correlation between the contents of T-cell subgroup, IL-8 and TNF-α in serum and the Dukes stage of rectal cancer. Therefore immunotherapy can be used in patients with rectal cancer.

Key words rectal cancer; T-cell subgroup; interleukin-8; tumor necrosis factor

The current study has confirmed that the occurrence, development and treatment effects of cancer are closely related to the body immune suppression and immune defects. Usually patients with cancer manifested the immunocompromice, poor body power of resistance, limitation of the ability to removal of carcinogenic factors; Once tumor formed, tumor cells could secrete immunosuppressive factors, so that the body of the anti-tumor immune response would be suppressed, leading to growth of tumor cells by escaping immune [1]. T cell subgroups, tumor necrosis factor (TNF-α) and interleukin 8 (IL-8) are directly or indirectly involved in tumor occurrence, development and immune processes, we have detected them in the serum of patients with rectal cancer, and investigated its clinical significance.

Patients and methods

Population

Forty-three patients with rectal cancer were entered into the study group. There were 32 men, 11 women, with a mean age of 54.2 years (range, 35 to 70 years). Among them, 4 patients had metastasis. Histologically, 22 cases were well differentiated adenocarcinoma, 15 cases were moderately differentiated adenocarcinoma, and 6 cases were poorly differentiated adenocarcinoma. According to the Ducks stage system, tumor stage was A in 3 patients, B in 23 patients, C in 11 patients, and D in 6 patients. All cases were confirmed by clinical and postoperative pathological examination. Patients at Dukes D stage were conducted palliative surgery, other patients underwent the radical operation. In addition, 24 eligibles of the healthy examination were used as normal controls, 16 men and 8 women, with a mean age 48 years (range, 24 to 68 years), and no gender and age differences compared with patients.

Methods

The detection of T-cell subgroups were conducted in patients of the two groups before treatment. The CD4, CD8 cell counts and CD4/CD8 ratio in the mononuclear cells were analyzed by flow cytometry, using monoclonal antibody-alkaline phosphatase method, with the healthy adults as normal controls, the anti-human CD4, CD8 monoclonal antibody which can be bound to the mononuclear cells in human peripheral blood. TNF-α, IL-8 were measured by double antibody sandwich assay of ELISA.

Correspondence to: Jian Chen. Email: chenbond@126.com

Operations were strictly carried out according to instructions. All specimens were tested at the same batch. Mouse anti-human monoclonal antibodies were purchased from Academy of Military Science (China); The kits of IL-8 and TNF-α were purchased from the Shenzhen Jingmei biotechnology company (China).

Statistical analysis

The SAS 13.0 statistical software was used for analysis. All data were expressed as mean ± standard deviation ($\bar{\chi} \pm s$), and the difference between the two groups was analyzed by the *t* test; $P < 0.05$ was considered as statistically significant difference.

Results

Changes of T-cell subgroups in patients with rectal cancer and controls group

As shown in Table 1, patients with Ducks B, C, D stages, the CD4 cell counts of T-cell subgroup in peripheral blood and CD4/CD8 ratio were significantly lower than the control group, with the increasing of CD4 cells ($P < 0.01$). The T-cell changes of patients at Dukes A stage were not significant ($P > 0.05$); the cell counts of CD4, CD8 and the CD4/CD8 ratio had both the significant differences ($P < 0.05$).

The detection results of IL -8, TNF-α in patients with rectal cancer and the control group

As shown in Table 2, The expression levels of IL-8 increased significantly with Dukes' A–D stage, and compared with the control group, there were significant differences. The level of TNF-α at each stage of rectal cancer significantly increased, except Dukes A stage, the rest of the stage, compared with the control group, there were significant differences.

Table 1 T-cell subgroups detection results in each group ($\bar{\chi} \pm s$)

Group	CD4 (%)	CD8 (%)	CD4/ CD8 (%)
Dukes A	42.63 ± 3.28▪	28.34 ± 2.76▪	1.68 ± 0.26▪
Dukes B	38.42 ± 3.14#	29.87 ± 3.18#	1.51 ± 0.28#
Dukes C	33.26 ± 2.62▲	31.25 ± 2.57▲	1.25 ± 0.17▲
Dukes D	29.13 ± 3.04▲	33.32 ± 3.64▲	1.03 ± 0.18▲
Control group	43.52 ± 4.68	25.47 ± 3.56	1.85 ± 0.32

Compared with the control group, ▪$P > 0.05$, #$P > 0.05$, ▲$P < 0.01$

Table 2 The detection results of interleukin -8, TNF-α in each group

Group	IL-8 (pg/mL)	TNF-α (pg/mL)
Dukes A	76.43 ± 12.26#	106.11 ± 21.84▪
Dukes B	79.12 ± 11.34#	118.63 ± 20.16#
Dukes C	81.03 ± 12.34▲	128.77 ± 24.66▲
Dukes D	84.18 ± 11.31▲	187.43 ± 32.27▲
Control group	47.17 ± 10.41	76.13 ± 21.26

Compared with the control group, ▪$P > 0.05$, #$P < 0.05$, ▲$P < 0.01$

Discussion

IL-8 is a member of the CXC chemokine subfamily, produced by the monocyte-macrophage cells, vascular endothelial cells, with extremely complex and diverse functions. The study has found IL-8 has the effects of regulating blood vessel proliferation and inducing tumor angiogenesis, IL-8 and its corresponding receptors can promote the vascular endothelial cell proliferation and division, and can promote tumor progression and metastasis when being bound to the receptors on cancer cells. Li *et al* [2] confirmed that IL-8 can promote the growth, infiltration, metastasis and diffusion of multiple tumors, and the malignant tumors with IL-8 and its mRNA positive expression has a rapid progression, high microvessel counts, high incidence of metastasis and poor prognosis. The studies of Brew *et al* [3] have shown that for the *in vitro* experiments, the tumor cell of rectal cancer has autocrine growth effect; this effect can also to some extent improve the growth and metastasis of colorectal neoplasm in the body. The study results showed that serum level of IL-8 in patients with rectal cancer was significantly higher than the normal adults, and increased significantly with the elevated clinicopathological staging of rectal cancer, which was consistent with report of Terada *et al* [4], indicating that IL-8 may be the important chemokine reflecting the progress, metastasis and prognosis of rectal cancer.

TNF-α is by far a cytokine with the strongest anti-tumor effect, mainly produced by monocytes/macrophages, with a variety of biological functions, on the one hand involving in the regulations of cell growth, proliferation and differentiation; on the other hand having an important immune function. It has direct cytotoxic effects to a variety of tumor cells and normal cells. It also can induce immune responses [5], and plays a very important role in tumor occurrence and development. The data in this group confirmed that the expression level of patients at each stage was significantly higher than the normal control group, but there was no significant difference in each stage. This indicated that no matter the stage of tumor is, the increase is non-specific. At the same time, the results also showed that serum level of TNF-α in patient with rectal cancer was significantly higher than the normal group. The reason of serum level increase of TNF-α may be that the high levels of TNF-α stimulated the secretions of monocytes/macrophages, and also be related with the tumor autocrine.

T lymphocyte subsets are the most important cell population in the body function within immune system to maintain a normal body immune function. The different

variations of T lymphocyte subsets have closed relationship with tumor formation, expansion and metastasis [6–8]. The immune regulation effects of T cell subgroups complete mainly by T helper cells (CD4), T suppressor cell (CD8), CD4 can help B cell differentiation produce antibodies, CD8 can inhibit the synthesis of antibodies. Tumor patients showed reduced CD4, increased CD8, decreased of CD4/CD8 ratio; Recently studies of many scholars have found that with the gradual development of tumor, the CD4/CD8 ratio of tumor patients decreased or inverted, probably because in the process of tumor development, with the increased of tumor, TNF levels can be increased, thereby inducing tumor cells to produce large amounts of inhibitory factor, which can further inhibit the activity of T cells, so that the local immune functions of tumor were suppressed; which showed decreased of CD4/CD8 ratio. The results of data in this group were similar, indicating that in patients with rectal cancer, when the tumor was confined to the wall of rectum, the body has a certain immune function. The inhibitory effects on CD8 can be reduced by immune regulation, and the CD4/CD8 ratio can also be close to normal. After the impingement of the pathological changes was beyond the muscular layer, the apparent immunosuppressive could be appeared in the body, and the CD4/CD8 ratio was significantly lower than the normal control group. However, at Dukes' B, C, D stages, there were no statistic significance between the detection results in each stage of T lymphocyte subsets. It indicated that in colorectal cancer, when tumor invasion was not beyond the muscular layer, the body has still a certain immune regulatory capacity, and after entering the Dukes' B stage, the immune suppression status of body was significant [9].

The combined detection and analysis of T cell subgroups, IL-8 and TNF-α in peripheral blood in patients with rectal cancer indicate that they are the effective indicators associated with progression of rectal cancer, and the decreased levels of systemic immune function of patients with rectal cancer, combined with the changes of different Duke stage, will provide valuable reference information for the choice of clinical treatment program. As for the actual significance, further information accumulation and follow-up are required.

References

1. Engelhorn ME, Guevara-Patino JA, Noffz G, *et al*. Autoimmunity and tumor immunity induced by immune responses to mutations in self. Nat Med, 2006, 12: 198–206.
2. Li A, Dubey S, Varney ML, *et al*. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. J Immunol, 2003, 170: 3369–3376.
3. Brew R, Erikson JS, West DC, *et al*. Interleukin-8 as an autocrine growth factor for human colon carcinoma cells *in vitro*. Cytokine, 2000, 12: 78–85.
4. Terada H, Urano T, Konno H. Association of interleukin-8 and plasminogen activator system in the progression of colorectal cancer. Eur Surg Res, 2005, 37: 166–172.
5. Fukui T, Matsui K, Kato H, *et al*. Significance of apoptosis induced by tumor necrosis factor-alpha and/or interferon-gamma against human gastric cancer cell lines and the role of the p53 gene. Surg Today, 2003, 33: 847–853.
6. Chattopadhyay S, Chakraborty NG, Mukherji B. Regulatory T cells and tumor immunity. Cancer Immunol Immunother, 2005, 54: 1153–1161.
7. Yu P, Lee Y, Liu W, *et al*. Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. J Exp Med, 2005, 201: 779–791.
8. Ercolini AM, Ladle BH, Manning EA, *et al*. Recruitment of latent pools of high-avidity CD8(+) T cells to the antitumor immune response. J Exp Med, 2005, 201: 1591–1602.
9. Cao J, Chen XP, Li WL, *et al*. Absence of FHIT expression is associated with apoptosis inhibition in colorectal cancer. Chinese-German J Clin Oncol, 2007, 6: 44–45.