Trakia Journal of Sciences, Vol. 8, Suppl. 2, pp 132-136, 2010

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

ROLE OF IL-12P40 AND IL-10 IN PROGRESSION OF COLORECTAL CANCER

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ABSTRACT

The aim of the study was to investigate the association between serum levels of IL-12p40 and IL-10 and progression of the colorectal cancer (CRC).

An association between severity of CRC and serum levels of investigated cytokines was observed. IL-12p40 was in the highest level in stage-I (423.6±224.7pg/ml) compared to more severe stages. In the opposite direction were the data for immunosuppressive cytokine, IL-10. Patients with stage-IV had the highest level of IL-10 (36.02±9.5pg/ml).

On the basis of our results we could conclude that IL-12p40 and IL-10 serum levels were inversely associated with tumour progression. More severe stages of CRC are characterized by low IL-12p40 and high IL-10 serum levels.

Key words: cytokine, IL-12p40, IL-10, colorectal carcinoma

ITRODUCTION

Colorectal carcinoma is one of the most common and aggressive tumor in humans. It is known that development and progression of tumors is associated with deviations in the human immune system. Cytokines have a key function in realization and regulation of an adequate anti-tumor immune response (1).

Interleukin-(IL)-12p70 and IL-10 are regulatory cytokines with an antagonistic effect. IL-12p70 is a heterodimeric cytokine with well known anti-tumor activity, and is composed of two subunits – p40 and p35. IL-12p70 directs the polarization of naïve T-lymphocytes towards Th1 phenotype and promotes the cell mediated immune response (2). There is data which shows significantly decreased production of IL-12p70 from *in vitro* stimulated mononuclear cells from patients with CRC compared to healthy donors (3, 4), as well as lower cytokine serum levels (5), reduced number of IL-12-positive cells in advanced stages of CRC (6) and patients with stomach carcinoma (7). In addition, it is known that IL-12p40 subunit is a part of another heterodimeric cytokine IL-23 (p40 and p19), as well as it exists in the homodimeric IL-12p80 and in the monomeric IL-12p40 form (2). The role of IL-12p40 and/or IL-12p80 for the development and progression of human colorectal carcinoma at this time is poorly understood.

Functional antagonist of IL-12p70 is the IL-10. IL-10 is Th2-cytokine, which increases antibody synthesis, promotes the humoral immune response and suppresses the anti-tumor immunity. It is well known that IL-10 inhibits the expression of p40, and both p35 and p19 subunits in macrophages and dendrite

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cells (8, 9). In spite of this data today a dual role of IL-10 for the immune response in cancer development is being discussed. From one side IL-10 has been shown as immune suppressive cytokine, which aids the development of tumors through oppression of the anti-tumor cell mediated immune response. From another side, number of pre-clinical and clinical experiments with both animal models and human cell populations show regression of the tumor after IL-10 application. This data shows IL-10 as a cytokine with an anti-tumor effect (10, 11).

Keeping the presented facts in mind the aim of our study is to determine the serum levels of IL-12p40 and IL-10 in patients with CRC as well as to find an association between the levels of both cytokines and the progression of colorectal carcinoma in humans.

MATERIALS AND METHODS

Subjects

A total of 48 Bulgarian patients with CRC and 27 age and sex matched healthy donors were recruited for the study. The patients group was composed of 26 (54%) male and 22 (46%) female. The mean age at diagnosis of male versus female among the cases was 64±11years versus 66±12 years (p=0.31; t-test). The mean age of total group of CRC patients was 65±11 years. Tumor grading and staging was performed according to the tumor–node–metastasis (TNM) classification. Seven (14.6%) of the patients had tumors in stage I, 18 (37.5%) in stage II, 16 (33.3%) in stage III, and the rest 7 (14.6%) patients had tumors in stage IV. Different stages of CRC were found in both sex groups in approximately equal proportion (χ^2=2.698, df=3, p=0.44). The histopathological examination confirmed the diagnosis of cancer. According to the tumor location, 11 (50%) female and 16 (62%) male of patients were with rectal and 11 (50%) female and 10 (38%) male of patients were with colon cancer. Patients were excluded if they had clinical evidence of infections, fever, autoimmunity diseases; had immunostimulatoty therapy, or were subjects to radiotherapy.

Blood specimens from the patients and healthy donors were collected in sterile tubes for serum isolation and stored at -70°C until use.

Informed consent was obtained from all subjects and authorization was given by the Ethics Review Board of the Faculty of Medicine, Trakia University, Stara Zagora and Medical University, Pleven

Cytokine determination.

The quantity determination of IL-12p40 and IL-10 in sera was performed by ELISA kits (BioSource, Austria for IL-12p40; R&D Systems, France for IL-10), following the manufacturers' protocol. The concentration of cytokines was determined by using standard curve constructed with kit's standards. The minimum detectable dose of the IL-12p40 ELISA kit is <2.0 pg/ml, and of the IL-10 ELISA kit is less than 3.9 pg/ml.

Statistical analysis.

Differences in IL-12p40 and IL-10 serum level in patients with different stages of colorectal carcinoma and healthy donors were calculated by Mann – Whitney U test of StatSoft6. Differences were considered significant when the P value was less than 0.05.

RESULTS

Serum levels of IL-12p40 in CRC patients:

The serum levels of IL-12p40 in total group of patients' were significantly higher than those in healthy donors (224.5±146.5pg/ml *vs*. 79.4±43pg/ml; p=0.000004; **fig. 1**). It was found no differences of IL-12p40 serum levels between male and female patients (197.85±11.6pg/ml *vs*. 266.76±167.5pg/ml; p=0.177), and between patients with rectum (211.2±111.1pg/ml) and colon cancer as well (230.9±166.9pg/ml; p=0.815). Results presented in **fig.2** demonstrated an reverse correlation between IL-12p40 serum levels and progression of CRC: the highest level of IL-12p40 was detected in stage I of CRC (423.6±224.7pg/ml); followed by stage II - (214.9±134 pg/ml; p=0.02), stage III - (191.1±107.1 pg/ml; p=0.013) and the lowest level of IL-12p40 was detected in stage IV of CRC patients (176.9±81.2pg/ml; p=0.002).

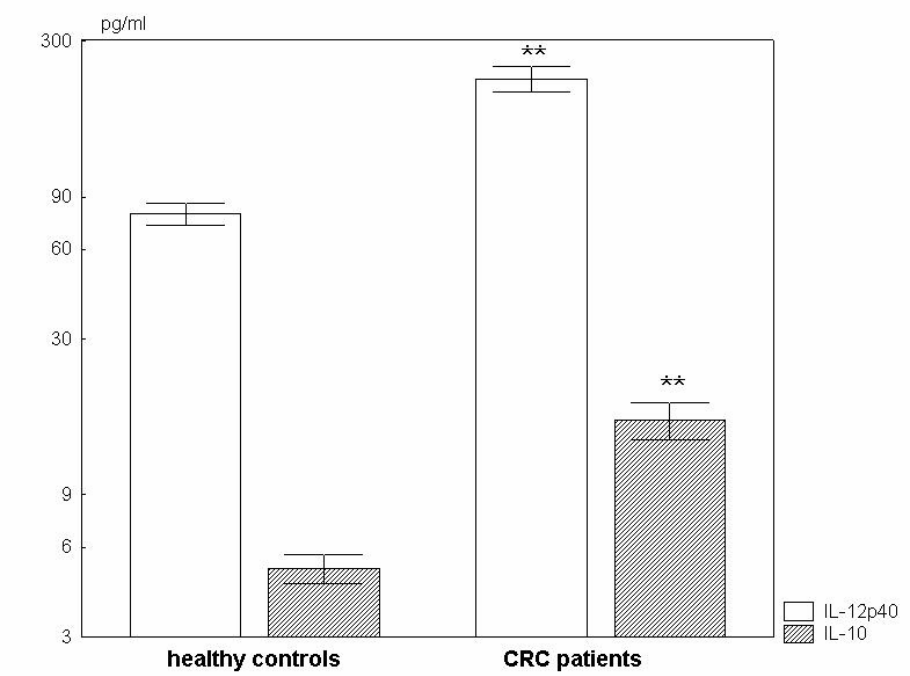

Figure 1. IL-10 and IL-12p40 serum levels in CRC patients and healthy donors. The results are presented as mean value ± SE. ** p<0.01, CRC patients vs. healthy donors.

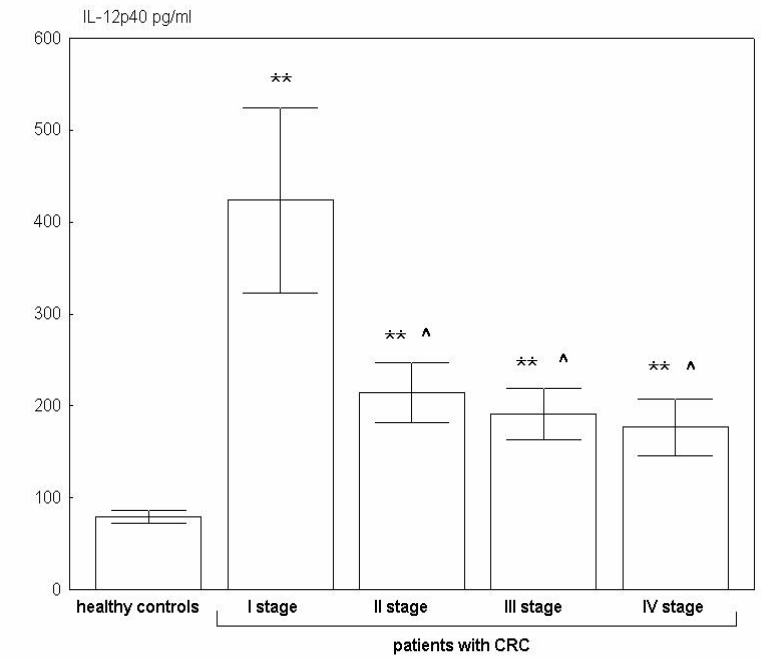

Figure 2. IL-12p40 serum levels in CRC patients categorized based on stage of cancer according to the TNM classification. The results are presented as mean value ± SE. ** p<0.01, CRC patients vs. healthy donors; ^p<0.05 – vs stage I of CRC

Serum levels of IL-10 in CRC patients:
Serum concentrations of IL-10 in CRC patients were significantly elevated compared to healthy donors (16.09±12.5 vs. 5.1±2.1pg/ml;p=0.0006) **(figure 1)**. It was found no differences of IL-10 serum levels between male and female patients (18.84±14.7 pg/ml vs. 12.5±8.1pg/ml; p=0.315), and between patients with rectum (16.5±11.3 pg/ml) and colon cancer (16.17±14.1; p=0.663)

also. Further, we investigated the association of IL-10 serum levels and severity of CRC. The results are presented in **fig.3**. After grading and staging the cases according to TNM classification, it was established an association between IL-10 serum levels and severity of CRC. The highest level of IL-10 was detected in patients with stage IV of CRC patients - 36.02±9.5pg/ml compared to earlier stages as followed: 17.07±11.3pg/ml for stage III (p=0.02); 8.55±5.35pg/ml for stage II (p=0.002) and11.0±5.7pg/ml for stage I (p=0.009). In addition, we demonstrated that even stage I serum levels of IL-10 were significantly enhanced than healthy donors (p<0.05) as shown in **fig.3**.

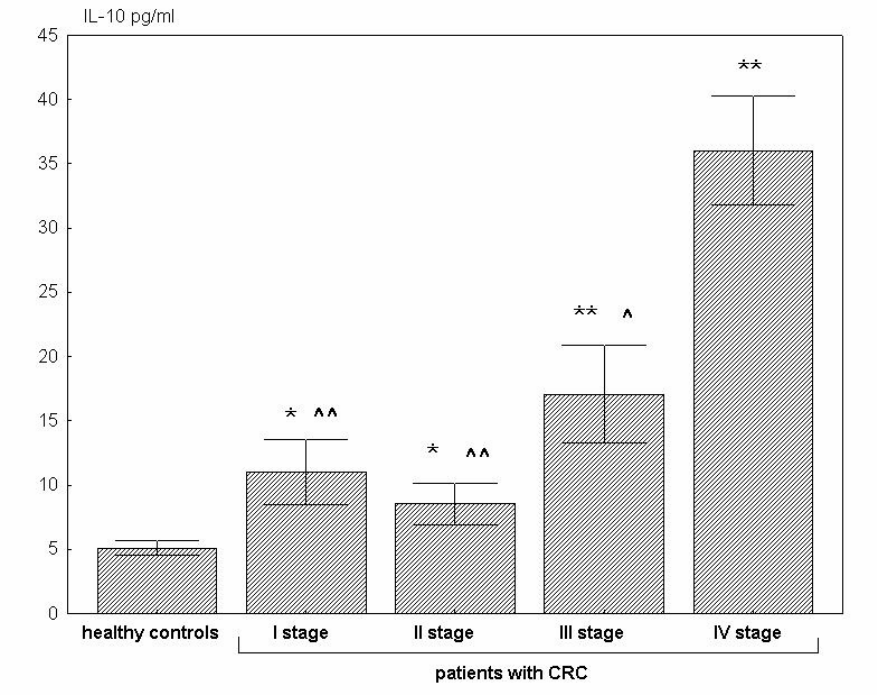

Figure 3. IL-10 serum levels in CRC patients categorized based on stage of cancer according to the TNM classification. The results are presented as mean value ± SE. *p<0.05; **p<0.01, CRC patients vs. healthy donors; ^p<0.05 – vs stage IV of CRC

DISCUSSION:

Our results demonstrate an association between serum levels of IL-12p40 and IL-10 and the progression of CRC. Elevated levels of IL-10 and low levels of IL-12p40 were found in advanced stages of CRC. This data correlates with earlier studies demonstrating a decrease of IL-12 levels in advanced stages of CRC (3, 4, 6, 15). It is necessary to point out that these studies were focused on the heterodimeric form of IL-12, IL-12p70, while the focus of our research is IL-12p40. The anti-tumor property of IL-12p70 is well known and has been demonstrated in numerous studies, while the independent role of IL-12p40 is still not clear. It is known that IL-12p40 is been secreted in larger quantities compared to IL-12p70, and that it exists as IL-23 (p40/p19), homodimeric IL-12p80 and monomer IL-12p40 [2], which role for the tumor genesis and progression is not known. Contradictory data exists, which shows IL-23 as an anti-tumor (12) as well as a pro-tumor cytokine (13). Our previous study also shows increased expression of IL-23p19 mRNA in tumor tissue (14) In addition, IL-12p40/p80 presents as an antagonist for IL-12p70 receptor in mice experiments. In humans it is known that IL-12p70 competes with IL-12p40/p80 for the same receptor, but the binding of IL-12p40/p80 to this receptor does not induces intracellular signals (2). From our current study is not possible to determine whether low serum levels of IL-12p40 in advanced stages of CRC correlate with low levels of IL-12p70 or IL-23, but we can accept that low serum levels of IL-12p40 are indicative for disruptions in the anti-tumor immune response, and are associated with poor CRC outcome.

Our research on IL-10 shows that it appears to have more of a pro-tumor than anti-tumor properties. The highest levels of IL-10 we have found in the most severe of CRC stages (stage IV). The pro-tumor properties of IL-10 can be

explained with the inhibitory effect on the Th1-cytokine production, in particular IL-12p70 (8), the inhibitory effect on the engaging of apoptosis and stimulation of cell proliferation (10). It is known that apart from molecular mechanisms with which IL-10 aids the tumor progression remain uncertain, we present additional evidence in support of the hypothesis that IL-10 has pro-tumor properties in development of CRC.

In conclusion, the obtained results demonstrated the IL-12p40 and IL-10 are associated with CRC progression in humans. Serum levels of IL-10 and IL-12p40 can be used as a serological marker for CRC severity. Severe stages of CRC are demonstrated with high levels of IL-10 and low levels of IL-12p40, while in the first stages of the disease are associated with low levels of IL-10 and high levels of IL-12p40.

ACKNOWLEDGMENTS

This study was funded by scientific projects: 4/2008 and 4/2009 at Medical Faculty, Trakia University, Stara Zagora, Bulgaria.

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