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ORIGINAL

Spaska A. Stanilova
Zhivko T. Karakolev
Gospodin S. Dimov
Zlatka G. Dobreva
Lyuba D. Miteva
Emil S. Slavov
Chavdar S. Stefanov
Noyko S. Stanilov

High interleukin 12 and low interleukin 10 production after in vitro stimulation detected in sepsis survivors

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S. A. Stanilova (✉) · Z. G. Dobreva · L. D. Miteva · E. S. Slavov · N. S. Stanilov
Department of Molecular Biology, Immunology, and Genetics, Faculty of Medicine, Thrakia University, Armeiska 11 St., 6000 Stara Zagora, Bulgaria
e-mail: stanilova@mf.uni-sz.bg
Tel.: +359-42-600675
Fax: +359-42-600705

Z. T. Karakolev · G. S. Dimov
Department of Intensive Medicine and ICU, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

C. S. Stefanov
Department of Anesthesiology and Intensive Care, Medical University, Plovdiv, Bulgaria

Abstract *Objective:* To evaluate viability of isolated peripheral blood mononuclear cells (PBMC) and production of cytokines in vitro after stimulation as prognostic factors for survival in sepsis patients. *Design:* Prospective study of the biological response of PBMC in the onset of severe sepsis. *Setting:* Research laboratory of molecular biology and immunology and university hospital ICU, Faculty of Medicine, Trakia University. *Patients:* Twenty-three patients meeting the criteria for severe sepsis, and 14 control subjects. *Interventions:* Isolated PBMC were stimulated in vitro with: C3-binding glycoprotein (C3bgp; 30 μg), lipopolysaccharide (30 μg), phytohemagglutinin (20 μg), pokeweed mitogen (30 μg), and dexamethasone (500 μg). *Measurements and results:* We measured the levels of interleukins (IL) 6, 10, and 12 in culture supernatants. Stimulation with C3bgp and phytohemagglutinin led to significantly lower PBMC secretion of IL-6 in nonsurvivors than in survivors and healthy donors. Stimulation with C3bgp, lipopolysaccharide, and pokeweed mitogen considerably reduced IL-12 production in nonsurvivors. Stimulation with lipopolysaccharide and pokeweed mitogen caused immune cells in nonsurvivors to produce higher levels of IL-10 than in survivors. Survival of PBMC reduced viability for nonsurvivors' PBMC, both spontaneously and as induced by lipopolysaccharide or pokeweed mitogen. *Conclusions:* The viability of PBMC at the onset of sepsis and enhanced production of IL-12 and diminished production of IL-10 after stimulation with all stimuli used may be a favorable prognostic factor in sepsis.

Keywords Severe sepsis · Peripheral blood mononuclear cell · Interleukin 6 · Interleukin 12 · Interleukin 10 · Cell viability

Introduction

Altered host defense mechanisms are considered important for the development of systemic inflammatory response syndrome (SIRS) and sepsis. Advances in unraveling the pathophysiology and genetic basis for the host response to sepsis have changed the prevailing understanding of sepsis as an uncontrolled inflammatory response [1]. It is evident that infection or trauma induces septic response by triggering the release of several molecules, which are injurious to various tissues and organs. SIRS, sepsis, and multiple organ failure are associated with exacerbated production of proinflammatory cytokines as assessed by their increased level in the blood [2, 3, 4]. Paradoxically, immune depression compared to healthy controls has been observed with reduced production of interleukins (IL) 1 and 6 and of tumor necrosis factor (TNF) α by circulating leukocytes in whole blood upon lipopolysaccharide (LPS) stimulation [5, 6]. Moreover, persistence of depressed cytokine secretion is correlated with mortality. The immune dysfunction which occurs in sepsis patients is related to a modified state of

P23)

Il-10 AND IL-12 RELATED CYTOKINE GENE EXPRESSION IN COLORECTAL CARCINOMA

Miteva L (1), Stanilov N (2), Mintchev N (1), Stanilova S (1).

(1) Department of Molecular biology, Immunology and Medical Genetics, Medical Faculty; (2) Department of Neurosurgery, Surgery and Urology, 2nd Surgery clinic, University Hospital; Trakia University, Armeiska 11 str; Stara Zagora; Bulgaria. e-mail: lmiteva@mf.uni-sz.bg

Colorectal carcinoma (CRC) is a common malignancy with high mortality rates worldwide. It is generally believed that cancer development requires the suppression of host-immune response and that cytokines play a central role in regulation of the anti-tumor immune response. IL-12p70 (IL-12p35/p40) exerts antitumor activity through activation of innate and Th1 adaptive immunity, while the effect of IL-23p19/40 on tumor development and progression is still in debates. On the other hand, IL-10 is the most potent inhibitor of IL-12 and IL-23 synthesis and suppresses the Th1 immune response.

Therefore, the aim of our study was to characterize the IL-10, IL-12p40, IL-12p35 and IL-23p19 cytokine expression in human normal and tumor tissues and their expression in patients' blood.

Paired human tumor and normal adjacent tissues were obtained from six patients undergoing routine therapeutic surgery. A venous blood sample from the same six patients was taken before surgery.

Blood samples from nine healthy donors were used as controls for cytokine expression. After total RNA extraction from tissue and blood, the samples were reverse transcribed and the level of gene expression was detected by quantitative real-time polymerase chain reactions (qRT-PCR) using TaqMan assay. qRT-PCR data were analyzed using the Relative Expression Software Tool (REST).

We didn't observe any significant differences in the relative expression of detected cytokine genes in peripheral blood between CRC patients and control group. These results indicate that expression of IL-10, IL-12 and IL-23 mRNA on systemic levels are not indicative for colon cancer development in humans. However, we found substantial upregulation of the mRNAs encoding IL-10 and both subunits of IL-23 (p40/p19), but not of mRNA encoded IL-12p35, in tumor tissue. The expression of IL-23p19 mRNA was increased in the highest level (20.706 fold; p=0.002), followed by IL-10 mRNA (6.634 fold; p=0.002) and IL-12p40 mRNA (3.933; p=0.038) in tumor tissue compared to normal adjacent tissues. No significant difference of IL-12p35 mRNA expression in tumor compared to normal tissue was detected.

Our results indicated that local IL-10 and IL-23 expression probably regulates the tumour immune surveillance and had significant impact in promoting development of colorectal carcinoma.

P24)

AUTOANTIBODIES TO CYCLIC CITRULLINATED PEPTIDE AND RHEUMATOID FACTORS IN THE DIAGNOSIS OF RHEUMATOID ARTRITIS

Ylli Z., Mone I., Shyti E., Sulcebe G.

Laboratory of Immunology and Tissue Typing, University Hospital Center " Mother Teresa" in Tirana

Introduction: Rheumatoid arthritis (RA) is a common rheumatic disease of uncertain etiology. The rheumatoid factor (RF) that is one of the American College of Rheumatology classification criteria for RA, has a low sensitivity and specificity for this disease. Many studies have recently shown that the antibodies to cyclic citrullinated peptides (CCP) are highly specific serological markers for RA and are thought to be directly involved in the disease pathogenesis. Material and Methods: In order to evaluate the diagnostic value of anti-CCP and RF in RA we examinated 49 patients sera coming from university rheumatological clinics with clinical diagnosis of RA, 3 with other rheumatic diseases and 11 controls. The RA patients were regrouped in

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ORIGINAL ARTICLE

High expression of Foxp3, IL-23p19 and survivin mRNA in colorectal carcinoma

Noyko Stanilov · Lyuba Miteva · Nikolay Mintchev · Spaska Stanilova

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Abstract
Introduction Cytokines have been suggested to both modulate anti-tumor responses and promote tumor growth.
Materials and methods We analyzed the expression of pro-inflammatory IL-12p35, IL-12p40, IL-23p19, anti-inflammatory IL-10, antiapoptotic factor survivin, and transcription factors—RelA, c-Jun, and Foxp3 mRNA in patients' blood, colon carcinoma tissue, and in normal mucosal tissue by real-time polymerase chain reaction. The quantity determination of serum IL-12p40, IL-23, and IL-10 was performed by enzyme-linked immunosorbent assay.
Results We observed significantly higher levels in patients for all three analyzed cytokines, with IL-23 concentration change being the highest. We detected the greatest upregulation of IL-23p19, Foxp3 and survivin mRNA in colorectal carcinomas than normal mucosa. A statistically significant upregulation of IL-12p40, IL-10, and c-Jun mRNA but not for IL-12p35 and RelA mRNA in tumor tissue comparing to normal tissue was also established.
Conclusions In conclusion, we show a characteristic gene expression profile combining markers associated with inhibition of anti-tumor immune response (Foxp3, IL-10), inhibition of apoptosis (survivin), and induction of the cytokines with protumoral activity as IL-12p40 and IL-23p19 (IL-23) in the colorectal tumor tissue but not in peripheral blood of patients.

Keywords Colorectal carcinoma · Cytokine · qRT-PCR · IL-12p40 · IL-23

N. Stanilov
Department of Neurosurgery, Surgery and Urology,
2nd Surgery clinic, University Hospital, Faculty of Medicine,
Trakia University,
Stara Zagora, Bulgaria

L. Miteva · N. Mintchev · S. Stanilova (✉)
Department of Molecular Biology, Immunology and Genetics,
Faculty of Medicine, Trakia University,
Armeiska 11 St.,
6000 Stara Zagora, Bulgaria
e-mail: stanilova@mf.uni-sz.bg

Introduction

It is well established that cancer arises in chronically inflamed tissue, and similarities in the regulatory mechanisms have been suggested [1, 2]. The same holds true in colorectal cancer cells. The main regulator of inflammation is the activity of immune system that is affected by pro- and anti-inflammatory cytokines. At the other side, the tumor growth is under immune surveillance and depends on cytokines secreted from tumor-infiltrating immune cells [3–5].

Focusing on individual cytokines has generated evidence that pro-inflammatory cytokine IL-12 and IL-23 and anti-inflammatory cytokine IL-10 may have a complex role in gastrointestinal carcinogenesis. The proper balance between IL-12-related cytokines (IL-12p70, IL-12p40; IL-23) and IL-10 produced from antigen presenting cells controls the appearance of normal Th1- and Th2-mediated immune response. Bioactive heterodimer IL-12p70, composed of p35 and p40 subunits, is a potent Th1-related cytokine clearly involved in the anti-tumor immune response [6, 7]. IL-12p70 and IL-23 are heterodimers which share the same subunit—IL-12p40, encoded by IL-12B gene (IL12B). IL-12p70 and IL-23 contain the unique subunits p35 and p19, encoded by IL12A and IL23A gene, respectively [8].

IL-10 is a Th2 cytokine that suppresses Th1-mediated immune response, reciprocally enhances antibody-mediated responses, and suppresses antitumor immunity in hosts [9].

Медицински преглед

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Medical Review

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ОБЗОРИ
REVIEWS

СЪВРЕМЕННА ДИАГНОСТИКА И ЛЕЧЕНИЕ НА ПАЦИЕНТИ СЪС СПОНТАНЕН БАКТЕРИАЛЕН ПЕРИТОНИТ

Й. Йовчев, Ст. Николов, Н. Станилов и Ив. Овчаров
Втора хирургична клиника, УМБАЛ – Стара Загора

Резюме. Бактериалната транслокация е ключовият механизъм в патогенезата на спонтанния бактериален перитонит и е възможна само при намалени защитни сили на организма, наблюдаващи се при цироза. Днес клинико-лабораторните варианти на това заболяване са известни и благодарение на това се въвеждат различни нови диагностични методи. От друга страна, антибиотичното лечение, базирано на познанията за заболяването, е с добри клинични резултати, получени чрез приложението на трето поколение цефалоспорини. Множество клинични проучвания по въпросите за бъбречната дисфункция установиха, че албуминът е способен да намали риска от бъбречно увреждане, като подобри ефективния интраваскуларен обем. Така пациентите с единствен епизод на инфекция следва да провеждат антибиотична профилактика и в същото време се налага необходимостта от преценка за последваща чернодробна трансплантация.

***Ключови думи**: инфекция, асцит, спонтанен бактериален перитонит*

Y. Yovtchev, St. Nikolov, N. Stanilov, Iv. Ovtcharov. CONTEMPORARY DIAGNOSIS AND TREATMENT OF PATIENTS WITH SPONTANEUS BACTERIAL PERITONITIS

Summary. The key mechanism in the pathogenesis of the spontaneous bacterial peritonitis is the bacterial translocation. It is possible only when patients are with decreased immune resistance, which is observed in cirrhosis. Now the clinical-laboratory versions of this disease are known and different new diagnostic methods are introduced. On the other hand, the antibiotic treatment based on the knowledge of the disease shows good clinical results achieved by the usage of third generation cephalosporins. Clinical studies on the renal dysfunction established that albumin is capable to decrease the risk of renal injury by improving the effective intravascular volume. That's why patients with single episode of infection should undergo antibiotic prophylaxis. At the same time, it is necessary to consider the need of subsequent hepatic transplantation.

***Key words**: infection, ascites, spontaneous bacterial peritonitis*

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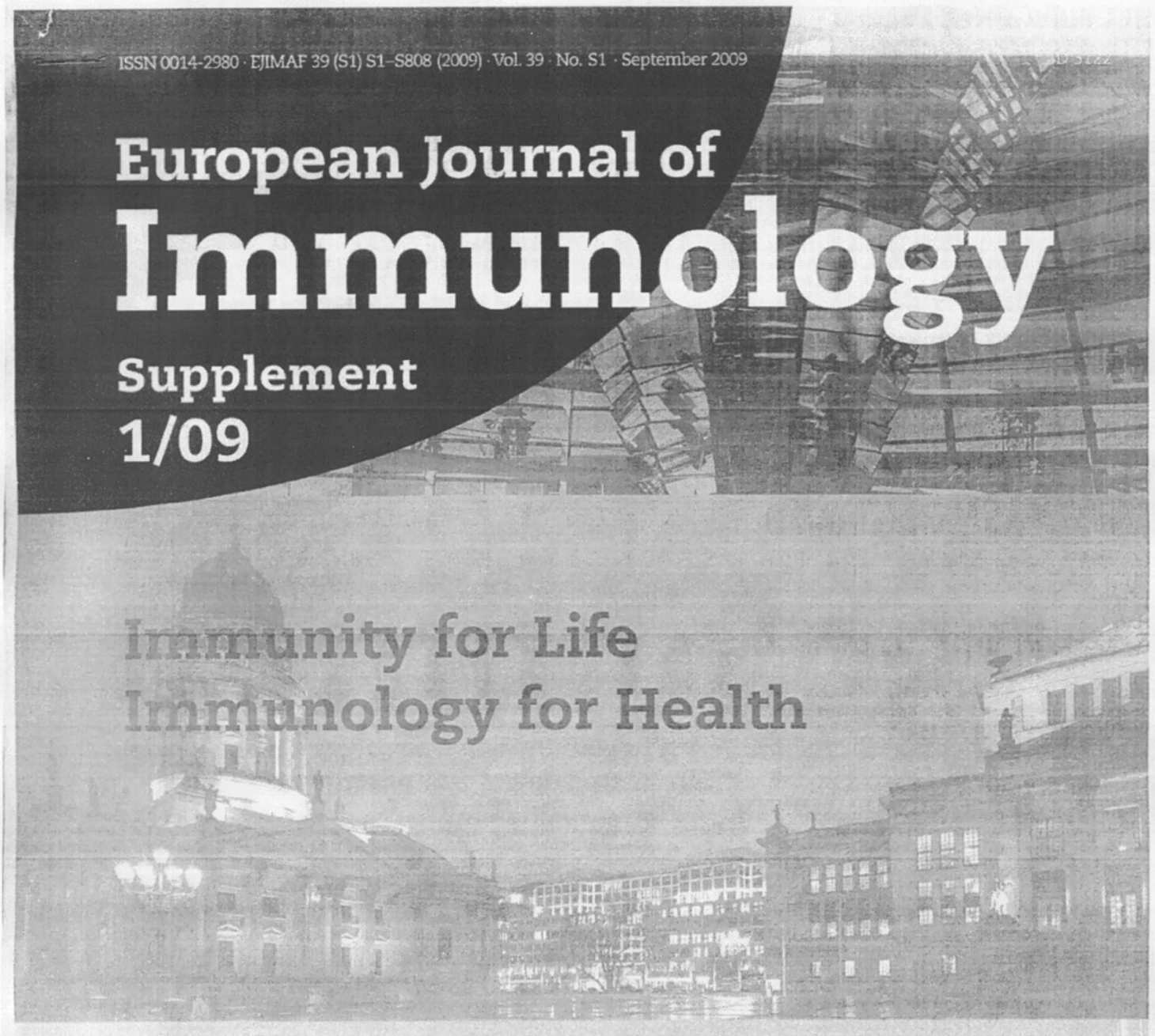

Abstracts

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PD10/18 *IN VITRO* INDUCTION OF MYELOID-DERIVED SUPPRESSOR CELLS FROM HUMAN PROGENITOR CELLS

S. Solito[1], E. Falisi[1], F. Bastianelli[2], S. Francescato[2], G. Basso[2], P. Zanovello[1], V. Bronte[1], S. Mandruzzato[2]
[1]University of Padova, Department of Oncology and Surgical Sciences, Oncology Section, Padova, Italy, [2]University of Padova, Department of Pediatrics, Padova, Italy

Growing tumors exploit different strategies to overcome the immune attack. One of this escape mechanism appears to involve the progressive accumulation of myeloid-derived suppressor cells (MDSC). MDSC comprise a phenotypically heterogeneous population of myeloid cells that exert a powerful inhibitory activity on antitumor lymphocytes. We recently described a subset of mielomonocytes with immunosuppressive properties found in tumor-bearing mice, which express the alpha chain of the receptor for IL-4 (IL4Rα). This phenotypic marker is useful since its presence correlates with immunosuppressive activity of the mononuclear cell fraction of MDSC. Human MDSC equivalent were described in cancer patients, but their characterization in still elusive.
Objectives: To evaluate the phenotype and the suppressive activity of leukocyte subsets freshly isolated from the blood of melanoma and colon cancer patients and to define growth factors able to generate MDSC *in vitro* from human bone marrow precursors.
Methods: Blood from patients was collected during surgery from melanoma metastases and colon carcinoma in order to evaluate the phenotype and the suppressive activity. Fresh bone marrow aspirates samples with normal cytological characteristics were obtained from tumor-free patients as a part of the diagnostic follow-up and cultured *in vitro* with different cytokine cocktails.
Results: We identified myeloid cells with immunosuppressive properties among monocyte and neutrophils circulating in peripheral blood of patients with colon and melanoma. IL4Rα is up-regulated in both myeloid populations, but its presence correlates with an immunosuppressive phenotype only when PBMC, but not PMN are considered. Also we defined a cocktail of cytokines that induces the expansion of bone-marrow immature myeloid populations expressing IL4Rα, with phenotype and inhibitory activity closer to patients's MDSC. Bone marrow-derived MDSC are able to suppress lymphocyte proliferation by decreasing CD3ζ chain expression and by impairing IFN-γ production of T cells.
Conclusion: Our results indicate that cells with characteristics of MDSC can be found in both mononuclear and polymorphonuclear fraction, and that a useful marker for their identification is the alpha chain of IL4R. Moreover, we identified growth factors that are able to derive *in vitro* myeloid cells from bone marrow precursors with analogous phenotype and function of MDSC identified in cancer patients.

PD10/19 CYTOKINES AND DOWN-STREAM SIGNALING PATHWAYS INVOLVED IN GLIOBLASTOMA-INDUCED INHIBITION OF HUMAN DC DIFFERENTIATION

D. Oosterhoff[1], H. van Cruijsen[1], J.J. Lindenberg[1], R. van Boerdonk[2], C. Fehres[1], S.M. Lougheed[1], R.J. Scheper[2], T.D. de Gruijl[1]
[1]VU University Medical Center, Medical Oncology, Amsterdam, Netherlands, [2]VU University Medical Center, Pathology, Amsterdam, Netherlands

Glioblastoma-derived cytokines can interfere with DC development and function. Here, we set out to identify the responsible cytokines and down-stream signaling events, as abrogation of their effects might increase the efficacy of immunotherapeutic strategies. We found U373 glioblastoma-conditioned medium to inhibit DC and LC differentiation, resulting in maintained CD14 and reduced CD1a expression. This phenotypic inhibition coincided with functional DC impairment as determined by allogeneic MLR and cytokine production profiles. High levels of IL-6 were found in the U373-conditioned media, and addition of hrIL-6 during DC differentiation mimicked the observed inhibitory effects on DC phenotype and function. To identify the signaling pathways responsible for these effects western blots were performed showing that both U373-conditioned medium and hrIL-6 induced activation of the STAT3 pathway in DCs. Addition of neutralizing antibodies against IL-6 during DC differentiation reduced the levels of activated STAT3 and reversed the glioblastoma-induced suppression of DC differentiation. To further delineate the down-stream signaling events responsible for the observed inhibitory effects we made use of small-molecule inhibitors and found evidence to suggest a differential involvement of the STAT3 pathway depending on the affected DC precursor (monocytes vs CD34+ progenitors). Currently, we are investigating the effects of an extended panel of supernatants from primary glioma cells on MoDC differentiation. Our data suggest that suppressive cytokines secreted by glioblastoma cells can activate multiple signaling pathways in DCs and in order to completely overcome cytokine induced DC suppression, multiple strategies are warranted.

PD10/20 CORRELATION BETWEEN IL12B PROMOTER POLYMORPHISM AND IL12B EXPRESSION IN COLORECTAL CANCER

L. Miteva[1], N. Stanilov[2], N. Mintchev[1], T. Deliysky[3], S. Stanilova[1]
[1]Trakia University, Medical Faculty, Department of Molecular Biology, Immunology & Genetics, Stara Zagora, Bulgaria, [2]Trakia University, University Hospital, 2nd Surgery Clinic, Department of Neurosurgery, Surgery and Urology, Stara Zagora, Bulgaria, [3]University School of Medicine, Oncology Center, Pleven, Bulgaria

Objectives: Members of the IL-12 family, including IL-12p80, IL-12p70, and IL-23 share a common subunit, IL-12p40 encoded by IL12B gene. Although several studies reported an association between IL12B promoter polymorphism (CTCTAA/GC) and different immune-mediated diseases, the relationship of this polymorphism with colorectal cancer (CRC) has not been explored. The aim of the study was to examine the possible influence of IL12B promoter polymorphism in the susceptibility and severity of CRC, and to assess its effect on IL-12p40 mRNA expression in tumor tissue.
Methods: A total of 85 CRC patients and 134 healthy donors were genotyped for the IL12Bpro polymorphism using amplification refractory mutation system (ARMS) – PCR. Paired human tumor and normal adjacent tissues were obtained from 12 patients and were used for RNA extraction. IL12B gene expression assay was performed using TaqMan based real time-PCR. Relative quantification was performed using the comparative ΔΔCt method. The mean ΔCt values obtained after amplification of cDNA from normal mucosa of each CRC patient were used as calibrators.
Results: The distribution of the IL12Bpro genotypes among 85 patients with CRC was similar to that observed in the control group, although there was a trend of higher frequency of genotype-22 among cases (25%) compared to controls (19%) and similarly of allele-2 (49% vs. 42.5%) but without statistical significance ($p=0.168$ and $p=0.159$, respectively). The grading and staging of the cases according to TNM classification did not reveal any significant association with studied polymorphism. However, we observed that IL12Bpro polymorphism had an important functional effect on IL-12p40 expression in tumor tissue. IL12B expression was significantly elevated in tumor samples with genotype-22 (RQ=46.964) compared to genotype-11 (RQ=1.9395; $p=0.037$), which showed the lowest level of IL12B expression. The increase of IL-12p40 mRNA level was smaller (RQ=29.543) among samples with heterozygous genotype compared to genotype-22, but the differences did not rich statistical significance. **Conclusion:** We demonstrated that carrying of genotype-22 was associated with elevated expression of IL12B in tumor tissue. On the basis of these preliminary results we could conclude that IL12Bpro polymorphism has an impact on the pathogenesis of colorectal tumor mediated by the expression of IL12p40 mRNA.

PD10/21 NATURAL CD8$^+$ T-CELL RESPONSE AGAINST MHC CLASS I EPITOPES OF TUMOR ANTIGENS (MUC1, CEA AND HER-2) IN PERIPHERAL BLOOD OF GASTRIC CANCER PATIENTS

J.T. Baran[1], A. Szczepanik[2], J. Kulig[2], A. Czupryna[2], M. Zembala[1]
[1]Polish-American Institute of Paediatrics, Jagiellonian University Medical College, Clinical Immunology, Krakow, Poland, [2]Jagiellonian University Medical College, First Department of General and Gastrointestinal Surgery, Krakow, Poland

The increased number of tumor infiltrating T lymphocytes in the tumor bed has been shown to be associated with a favorable prognosis in different types of tumors. HLA-peptide multimeric complexes allow the direct ex vivo visualization of antigen specific CD8$^+$ T cells by flow cytometry. Tumor associated antigens (TAA) e.g. carcinoembryonic antigen (CEA), human epidermal growth factor receptor-2 (HER-2) and mucin-1 (MUC-1) are broadly expressed in gastrointestinal tumors and are attractive candidates as targets for T cell-based adoptive immunotherapy. In this study we evaluated a pentamer technology to detect CD8$^+$ T cells specific against MUC1, CEA or HER-2 in the blood of HLA-A2$^+$ gastric cancer patients. The results show that majority of the patients have detectable peripheral CD8$^+$ T cells specific for $MUC1_{12-20}$ (LLLLTVLTV) peptide and $HER\text{-}2_{369-377}$ (KIFGSLAFL) but only some of them have CD8$^+$ T cells directed against $CEA_{571-579}$ (YLSGANLNL) epitope. These data demonstrate that MUC1 and HER-2 proteins represent dominant targets for CD8$^+$ T cells in gastric cancer, and MUC1 and/or HER-2 positive CD8$^+$ T cells could be used in adoptive transfer immunotherapy protocols of gastric cancer patients.

PD10/22 MODULATION OF B7-H EXPRESSION ON RCC CELLS UPON TREATMENT WITH TNF-α AND IL-4

D. Quandt[1], U. Müller[1], B. Seliger[1], Tumorimmunology
[1]Martin-Luther University, Institute for Medical Immunology, Halle (Saale), Germany

The tumor microenvironment comprises of non-malignant supporting tissue, blood vessels and inflammatory immune cells. The later predominantly produce cytokines, which can act on a huge variety of cell types bearing the right cytokine receptor. Although it has recently been shown that members of the B7-H family are differentially expressed on tumor cells there exist with the exception of the regulation of B7-H1 by IFNγ little information on their effects on tumor cells. Employing renal cell carcinoma (RCC) tumor cell lines the role of IL-4 and TNF-α treatment on B7-H molecule expression was studied. Upon confirmation of the respective IL-4Rα and TNF receptor I expression on RCC cell lines phosphorylation of the key signaling components STAT6 and NFκB as well as HLA class I upregulation, determined by intracellular and/or surface flow cytometric analysis, was found upon stimulation of RCC cells with IL-4 and/or TNF-α, which was associated with reduced RCC cell growth. Interestingly, the mRNA and protein expression pattern of members of the B7-H family varied upon treatment with IL-4 and TNF-α. IL-4 or TNF-α alone induced a moderate upregulation of B7-H1 and B7-H3, whereas only TNF-α strongly increased B7-H2 surface expression. Most important the combined IL-4/TNF-α treatment showed additive effects resulting in a high upregulation of both B7-H1 and B7-H3, whereas B7-H4 was not induced upon cytokine treatment. Time kinetic analysis revealed that B7-H2 expression is already increased 8 hrs post stimulation resulting in the highest increase after 72hrs. In contrast B7-H1 and B7-H3 upregulation occurred at 24 hrs with the highest difference 72 hrs post stimulation. The proliferative response of allo CD8+ T cells cocultured with combined IL-4/TNF-α treated tumor cells was not altered. This is may be explained by a balance resulting from the concomitant upregulation of molecules, which promote T cell responses like HLA class I, B7-H2 and B7-H3 and a strong upregulation of B7-H1 proposed to block T cell responses.

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Mini-Review

RELAPAROTOMY-DEFINITION AND ATTEMPT FOR A NEW CLINICAL CLASSIFICATION

Y. Yovtchev*, St. Nicolov, N. Stanilov, Iv. Ovcharov, Al. Petrov

Department of Surgical Diseases, University Hospital –Stara Zagora

ABSTRACT

In the last years as a result of heaping of clinical database connected with adequately and timely operative treatment in patents with postoperative complications existed a series of questions. First of all it was connected with the way of classification on the reasons that demanded new laparotomies. At the moment the offered classification for laparotomy shows our understanding for the leading causal connection to a certain degree. Although its priorities, there are some contradictions in combined cases.

This makes us doing a review of the adopted classification and to try to bring her clinical meaning up to date. We hope it would help and facilitate the daily surgical work if, of course, it will be good received in medical surgical community.

Key words: re-laparotomy, classification

Under the name of the relaparotomy we understand doing of repeated laparotomy after run through a stomach operation before that.This term has a Greek origin and it has three parts in it re-repeated, lapara-stomach, tomie-cut up, i.e. a repeated exploratory operation of the stomach cavity.

Some authors try to put the relaparotomy in a straight relation with the first operation. They explain the surgical treatment of the complications, which are arisen after the operation of the abdominal organs, or the part of this treatment when it is not factually finished. It exists some misunderstanding about this definition. Thus, the relaparotomy can be done and for removing some diagnostically and tactical mistakes from the first operation and also in a new disease.

According to (1) under the relaparotomy we understand "a new opening of the abdominal cavity in postoperative period, independently of the reason of the operation. Of course, this definition is also not a completely clear about what is this a relaparotomy, because it doesn't point the duration of postoperative period and it doesn't fix the time after the first operation.

Mamich V. I. and co. (2) offers in the definition for a relaparotomy to consist the time of hospitality in surgical clinics – "an interference, which accomplish in the time of hospitality in a surgical ward, after the first operation". The reason that the patents are kept in the ward for different periods of time, often for months, is unclear and inaccurate in many cases.

It doesn't exist yet an exact distinction of the terms "repeated operation" and "relaparotomy". The world famous surgeons like (3, 4), even they mix the term "relaparotomy" with a reoperation in the early postoperative period. These authors think that reoperation as "early" until 14th and "lately" after 14^{th} from the first abdominal operation. They define as "lately" one and the reoperation which is done 30 days after the first one on purpose closure of intestinal fissures, colostomies or reconstructive operations of gastrointestinal or urogenital tract. As itself the term "relaparotomy" means the next intervention in the abdominal cavity, so that the reoperation is combined definition and it can be also used for other types of the repeated operations in the stomach.

* **Correspondence to**: *Y. Yovtchev, Department of Surgical Diseases, University Hospital –Stara Zagora, E-mail: yovtchev@abv.bg*

Interleukin-12b Polymorphisms in Association with Susceptibility to Severe Sepsis

Spaska Angelova Stanilova, PhD, DSc[1] Lyuba Dineva Miteva, PhD,[1] Noyko Stefanov Stanilov, MD,[2] Chavdar Stefanov Stefanov, MD, PhD,[3] Zhivko Todorov Karakolev, MD, PhD[4]

([1]Department of Molecular Biology, Immunology & Genetics, Faculty of Medicine, Trakia University, Stara Zagora, [2]Department of Surgery, Faculty of Medicine, Trakia University, Stara Zagora, [3]Department of Anaesthesiology and Intensive Care, Medical University, Plovdiv, [4]Department of Intensive Medicine and IC,; Trakia University, Stara Zagora, Bulgaria)

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Abstract

Background: This study investigates the allele and genotype distribution of a single nucleotide polymorphism located at position +16974A/C in 3'-untranslated region (UTR) and a complex polymorphism (IL-12B*pro*) within the promoter region of IL-12B gene in association with susceptibility to severe sepsis.

Methods: A total of 130 healthy volunteers and 43 patients, meeting the criteria for severe sepsis, were included. The polymorphism in 3'-untranslated region of the IL-12B was detected by restriction fragment length polymorphism assay. The allele-specific polymerase chain reaction was used for IL-12B*pro* polymorphism detection.

Results: The results showed that healthy controls had significant elevation of IL-12B*pro*-1 allele frequencies, compared to patients with sepsis (60% vs 44%; OR=1.895; 95% CI 1.157÷3.102; *P*=0.011). Homozygous genotype-11 was also more frequent in healthy than in the sepsis group (35% vs 16%; OR=0.358; 95% CI, 0.150÷0.855; *P*=0.02).

Conclusions: We established that the IL-12B*pro*-1 allele is associated with sepsis susceptibility, and genotype IL-12B*pro*-11 could have a protective effect to the development of severe sepsis.

After reading this article, readers should be able to discuss the role of IL-12 in the pathogenesis of sepsis, the functional effect of polymorphisms in regulatory regions of the IL12B gene on cytokine production, sepsis susceptibility, and the outcome of the disease.

Molecular Diagnostics exam 81001 questions and corresponding answer form are located after this CE Update on page **XX.**

Altered host defense mechanisms are considered important for the development of sepsis and septic shock. Pro- and anti-inflammatory responses contribute to the development and outcome of severe sepsis.[1] Cytokines play a pivotal role in the regulation of the type and magnitude of the immune response, and the polymorphic nature of the cytokine genes may confer flexibility on the immune response. Therefore, all genes encoding cytokines involved in the modulation of inflammatory responses are candidate genes for determining the human genetic background, which is responsible for interindividual differences in susceptibility and outcome of sepsis.[2,3]

Growing evidence suggests that immunoregulatory cytokine interleukin (IL)-12 plays a role in the pathogenesis of severe sepsis. IL-12p40 is known as a component of the bioactive cytokines IL-12 (IL-12p70) and IL-23. It is produced by antigen-presenting cells, and IL-12 plays an important role in interferon-gamma production by T cells and NK cells.[4] Some studies have reported that impaired IL-12 production increases the risk of infection after surgery.[5,6] Moreover, recent evidence suggests a selective preoperative defect in monocyte IL-12 production as a predictive factor for the lethal outcome of postoperative sepsis in humans and mice.[7,8] Previous reports by us as well as others have indicated that increased IL-12p40 production is associated with a favorable outcome of the sepsis.[9,10]

Several polymorphisms have been described in the IL-12B gene, encoded IL-12p40 subunit, including a single-nucleotide polymorphism (SNP) in 3'-untranslated region (UTR) of IL-12B with number rs3212227 and a complex polymorphism (-6415CTCTAA/GC) in promoter region of the IL-12B (IL-12B*pro*), resulting from 4bp microinsertion combined with an AA/GC transition (rs17860508).[11,12] Moreover, several studies have demonstrated that these 2 polymorphisms affect gene expression and IL-12 production.[13-16] These data suggest that both polymorphisms in the IL-12B, associated with different cytokine production, may have an important impact on both susceptibility and severity of sepsis.

In review of the literature data cited above for the role of IL-12 in sepsis, the aim of the present study was to investigate the association between I-12B*pro* polymorphism and SNP in 3'UTR of I12B in relation with susceptibility to severe sepsis.

Patients and Methods

Patients

Forty-three consecutively admitted adult patients meeting the criteria for sepsis with a verified infection in 1 or more major organs, and 125 healthy volunteers (control group)

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Advanced Colorectal Cancer Is Associated With Enhanced Il-23 and IL-10 Serum Levels

Noyko Stanilov MD,[1] Lyuba Miteva PhD,[2] Tashko Deliysky MD, PhD, DSc,[3] Jovcho Jovchev MD, PhD,[1] Spaska Stanilova PhD, DSc[2]

([1]Department of Neurosurgery, Surgery, and Urology, 2nd Surgery Clinic, University Hospital, Stara Zagora, [2]Department of Molecular Biology, Immunology and Medical Genetics, Trakia University, Medical Faculty, Stara Zagora, [3]Oncology Center, University School of Medicine, Pleven, Bulgaria)

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Abstract

Objective: Cytokines play a pivotal role in the induction of host immune responses against tumor growth and are involved in the development and progression of colorectal cancer in humans.

Methods: A group of 48 patients and 27 healthy volunteers were included in the study. The quantitative determination of IL-23, IL-17, IL-10, and IFN-α serum levels were performed by ELISA.

Results: We observed significantly enhanced IL-23 in CRC patients compared to the controls. The serum level of IL-10 was significantly higher in CRC patients than healthy donors. The highest level of IL-10 was detected in patients with stage IV, which was significantly greater than those in stages I, II, and III.

Conclusions: We found that IL-23 and IL-10 levels were significantly elevated in patients' sera, in contrast to IL-17 and IFN-α, and this serum cytokine profile may play a role in tumor development. In addition, an increased level of IL-10 was strongly associated with the progression of colorectal carcinoma (CRC).

Colorectal carcinoma (CRC) is 1 of the most common and aggressive types of cancers. The anti-tumor immune response is regulated by numerous factors, including cytokines produced by the tumor-infiltrating lymphocytes and activated immune cells (ie, macrophages, dendritic cells, and lymphocytes). The locally and systemically produced mixture of cytokines plays a pivotal role in the induction of host immune response, which can either block or facilitate tumor growth.[1,2]

Cytokine regulation of human CRC is not clearly understood. The systemic and local cytokine environment may modulate the immunogenicity of colorectal cancer cells and affect anti-tumor immune function of tumor-infiltrating lymphocytes.[3] Focusing on individual cytokines has generated evidence that pro-inflammatory cytokine IL-23 and IL-17 and anti-inflammatory cytokine IL-10 may have a complex role in gastrointestinal carcinogenesis.

Interleukin-23 is a heterodimeric cytokine (p40/p19) consisting of a novel p19 subunit and the p40 subunit of IL-12p70. Studies have defined IL-12p70 as an important factor for the differentiation of naive T cells into IFN-α producing Th1 cells and exhibits anti-tumor activity.[4-6] IL-23 has similar but discrete functions from IL-12p70,[7] and IL-23 induces activated memory T cells to proliferate and produce IFN-α. In addition, IL-23 induces the production of a proinflammatory cytokine, IL-17, from activated T cells, namely Th_{IL-17}.[8] Moreover, IL-17 has been shown to promote angiogenesis and regulate production of a variety of proangiogenetic factors, including vascular endothelial growth factor.[9,10] Although the antitumor activities of IL-12p70 are well characterized, studies of the role of IL-23 and IL-17 in development of CRC in humans are still limited.

The activities of IL-12 family cytokines are antagonized primarily by IL-10. IL-10 is a Th2 cytokine suppressing Th1-mediated immune response, reciprocally enhancing antibody-mediated responses, and suppressing anti-tumor immunity in hosts.[11] Previous studies demonstrated that advanced colorectal cancer is associated with impaired IL-12 production from stimulated peripheral blood mononuclear cells and enhanced serum IL-10 levels.[12]

However, the role of IL-10 in cancer remains ambiguous. Some studies show that IL-10 plays a dual, controversial role in carcinogenesis in humans, as a tumor promoting and tumor inhibiting factor.[13,14]

On the other hand, IFN-α, the favor cytokine of innate immunity, can modulate both the tumor cells and the immune system, including cytokine production.[15] Moreover, IFN-α has been demonstrated to exhibit significant anti-tumor activity and has been effectively used in the anti-tumor therapy, including colon carcinoma.[16] No association between serum concentration of IFN-α and progression of colorectal cancer has been reported.

To determine the systemic activity of immune status in CRC patients, we analyzed the serum level of some pro-inflammatory and anti-inflammatory cytokines implicated in the host immune response. Thus, the aim of the study was to determine whether IL-23, IL-17, IL-10, and IFN-α levels are involved in the development and progression of colorectal cancer in humans.

Materials and Methods

Subjects

A total of 48 Bulgarian patients with CRC were recruited for the study. Cases with a diagnosis of CRC visiting the University Hospital in Stara Zagora and an Oncology Center in Pleven, Bulgaria, between January 2007 and April 2008 were selected. All patients had no previous diagnosis of inflammatory bowel disease, Crohn's disease, or any of the known hereditary cancer syndromes. The histopathological examination confirmed the diagnosis of cancer. Patients were operated on in both the University Hospital, Stara Zagora,

Original Article

POLYMORPHIC VARIANTS OF THE INSULINE-LIKE GROWTH FACTOR I RECEPTOR GENE IN BULGARIAN POPULATION

Iliya Karakolev,
Noyko Stanilov[1],
Spaska Stanilova

Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora
[1]Department of Neurosurgery, Surgery and Urology, Medical Faculty, and "St. Ivan Rilski" Hospital, Stara Zagora
Bulgaria

Corresponding Author:
Iliya Karakolev
Department of Molecular Biology, Immunology and Medical Genetics
Medical Faculty
Trakia University
11, Armejska str.
6000, Stara Zagora
Bulgaria
e-mail: ilya.karakolev@gmail.com

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Summary

An attenuation of the insulin-like growth factor I (IGF-I) signaling has been associated with elongation of lifespan. In humans, IGF-I level has an age-related modulation with a lower concentration in the elderly, depending on hormonal and genetic factors affecting the IGF-I receptor gene (IGF-IR). The aim of our study was to survey the genotype and allele frequency of +3179G>A (rs2229765) SNP in Bulgarian population according to age and gender. For this purpose, we used a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. We genotyped 246 healthy donors distributed in two groups: elderly and youth (n=108, age range 30-81 years and n=138, age 18 years, respectively). Genotype distribution in the group of elderly was: AA (21%), AG (44%) and GG (35%), and in the group of youth was: AA (19%), AG (44%) and GG (36%). The observed frequency of AA genotypes was slightly higher in males than in females in both groups. Based on the experimental data, we could conclude that there is no statistically significant differences in the distribution of genotypes in the +3179G>A polymorphism of *IGF-IR* between both age subgroups from the Bulgarian population we studied.

Key words: single nucleotide polymorphism, insulin-

Introduction

Insulin-like growth factors (IGF-I and IGF-II), their binding proteins (IGFBPs) and the receptors mediating their signaling (types I and II IGF-R) play critical roles in normal development, growth, metabolism, and homeostasis [1, 2]. The IGF-I pathway exerts such diverse influence on mammalian biology that the scope of its function is only now beginning to be understood. It has been insinuated in fundamental processes such as determining life span and coping with oxidative stress in rodents [3]. IGF-IR bears both structural and functional resemblance to other closely related tyrosine kinase receptors, such as InR in *Drosophila melanogaster* [4, 5] and DAF-2 in *Caenorhabditis elegans* [6, 7]. It starts functioning during fetal development and retains its importance throughout life, although the consequences of its normal or abnormal activation change with aging. IGF-IR and

АЛГОРИТЪМ ЗА РЕОПЕРАЦИЯ ПРИ ПАЦИЕНТИ С ПЕРИТОНИТ

Йовчо Йовчев[1], Татяна Влайкова[2], Николай Недков[1], Стоян Николов[1], Нойко Станилов[1], Ален Петров[1], Георги Минков[1]

[1]Тракийски Университет, Медицински Факултет – катедра "Хирургия, неврохирургия и урология" - 6000 Стара Загора, България, e-mail: dr.delon81@gmail.com

[2] Тракийски Университет, Медицински Факултет – катедра „Химия и биохимия"

APPROACH FOR RELAPAROTOMY IN PATIENTS WITH PERITONITIS

[1]Y. Yovchev, [2]T. Vlaykova, [1]N. Nedkov, [1]St. Nikolov, [1]N. Stanilov, [1]A. Petrov, [1]G. Minkov

[1]Trakia University, Medical Faculty, Department of Surgery, Neurosurgery and Urology - 6000 Stara Zagora, Bulgaria

[2]Trakia University, Medical Faculty, Department of Chemistry and Biochemistry ", 6000 Stara Zagora, Bulgaria

ABSTRACT

Today there are still many unsolved problems when an early postoperative complication is established in abdominal cavity after surgery intervention.

Remain unclear the answers relating to determining the severity of postoperative deviation or worsening of intestinal paresis. Early diagnosed clinically and instrumentally symptoms and syndromes in some cases compounding or confused the operator in his assessement and decision for reoperation.

We set the goal to offer a clinical scale for determining severity of postoperative complication which is already diagnosed. We hope that will contribute to more easily assess the need for second surgery intervention

***Keywords**: clinical symptoms and syndromes after surgery, reoperation*

Един от основните двигатели и едно от условията за успешното комплексно лечение е деконтаминацията на перитонеалната кухина от развиващата се там патогенна флора. Водещ момент в това е своевременното оперативно лечение по време на което, се отстранява първоизточника на инфекцията с последваща санация на коремната кухина. Само извършването на санацията не е условие за успешно лечение. Една част от пациентите в следоперативния период, продължават да произвеждат токсини и токсичен ескудат, съдържащ бактерии и тъканни детритни маси, които поддържат инфекцията. [1,3]

В някои от случаите, поставените тръбни дренажи бързо се ограничават от фибринови налепи и се превръщат в неефиктивен метод за елиминиране на токсичните продукти. Това налага възприемането на повторна ревизия на коремната кухина (релапаротомия).[2]

До настоящият момент, показанията за програмираната релапаротомия са формулирани недостатъчно ясно. Релапаротомията, като хирургична процедура е показана при пациенти, при които съществуват клинични симптоми за нарастваща абдоминална токсикоза или развиващата се полиорганна недостатъчност. [3] Този подход противоречи на термина програмирана релапаротомия, тъй като се намесва и чисто субективният фактор за преоценка на състоянието. Стига се до парадокса за отлагане на операцията, до нейното забавяне или в повечето случаи се извършва, когато вече се е развила клиниката на полиорганните нарушения.[2]

Ние проведохме анализ и преоценка на наблюдаваните в следоперативния период симптоми и синдроми с цел подготовка и осъществяване на програмираната релапатотомия. Този анализ включва следните показатели: изходно състояние, оперативна находка, следоперативно протичане обхващащо първите 72 часа след края на операцията. Критериите, които бяха отчитани при статистическата обработка бяха:

Poster Session: Biomarkers & Disease Profiling 1: Learning from Genetics

P1544

A study of a highly conserved intronic region's polymorphism of TIGIT costimulatory receptor in T1D

M. Shunina, K. Kisand, K. Kisand & R. Uibo
Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia

Purpose/Objective: TIGIT (T cell immunoglobulin and ITIM domain) is a recently discovered receptor, which share the same ligands with costimulatory receptor CD226 and is expressed on regulatory, memory and activated T cells. In this receptor pair TIGIT pathway leads to T cell inactivation while CD226 transmits activating signal. Genome-wide association study suggested strong association of CD226 locus with type 1 diabetes (T1D), but there are some evidences, that TIGIT can also play an important role in the development of autoimmune diseases as a negative regulator of immune responses. Currently very little is known about CD226 and TIGIT genetic polymorphisms and their significance in T1D. The aim of the study is to examine genetic polymorphisms of one of these receptors – an inhibitory receptor TIGIT in Estonian population, and look for their association with T1D.
Materials and methods: We compared human and mouse TIGIT gene sequences and found 10 highly conserved intronic regions within the human TIGIT gene. Then we screened these regions for SNP with MAF (minor allele frequency) value over 0.15 and found two SNPs suitable for further investigation. We carried out restriction analysis of one of them: rs62265703 SNP (MAF = 0.1526) located in the second conserved region.
Results: We showed that the SNP MAF is higher in Estonian population (0.254) compared to predicted frequency (0.233) for HapMap CEU population. Statistical analysis revealed no association between the rs62265703 genotype and T1D diagnosis. However in our study population we confirmed that the CD226 polymorphism rs763361 (Gly307Ser) is associated with increased T1D susceptibility.
Conclusions: A further study could assess a functional role of the CD226 polymorphism in the control of T cell activation in both healthy individuals and T1D patients and the TIGIT's impact on an impediment of auto-reactive T cell activation.

P1545

Association of +3179G/A IGF-1R polymorphism and IGF-1 serum level with colorectal cancer development

I. Karakolev,* N. Stanilov† & S. Stanilova‡
**Molecular Biology Immunology and Medical Genetics, Medical Faculty of Trakia University, Stara Zagora, Bulgaria, †Department of Surgery, Medical Faculty of Trakia University, Stara Zagora, Bulgaria, ‡Department of Molecular biology Immunology and Medical genetics, Medical Faculty of Trakia University, Stara Zagora, Bulgaria*

Purpose/Objective: The insulin-like growth factor (IGF) system plays a prominent role in cancer development. The IGF-1 receptor (IGF-1R) and its associated signalling pathway is an important growth regulatory pathway that has been implicated in tumour genesis, angiogenesis, metastasis and autoimmune diseases. Through IGF-1R, the proliferative activity of IGF-1 is mainly regulated by the MAPK signalling pathway and its antiapoptotic activity by the PI-3 kinase pathway. It has been demonstrated a significant elevated risk for colorectal cancer (CRC) in relation to increased IGF-1 serum level. Recently, single nucleotide polymorphism located in exon 16 (+3179G/A; rs2229765) of *IGF-1R* gene was found associated with serum levels of IGF-1 and human longevity.
Materials and methods: This study was designed to compare +3179G/A *IGF-1R* genotype distribution in 110 CRC patients to a group of 220 healthy controls. We also investigated serum IGF-1 levels in CRC patients and healthy controls in an association to genotype. IGF-1 serum levels were measured by ELISA and genotyping for the +3179G/A polymorphism was performed by RFLP-PCR assay.
Results: In the study population no significant differences in allele frequencies of investigated polymorphism of IGF-1R between CRC and healthy controls were observed. The higher frequency of heterozygous AG genotype (53% versus 45%; OR = 1.43, 95%CI = 0.82 ÷ 2.50) and lower frequency of GG genotype (29% versus 36%; OR = 0.88, 95%CI = 0.43 ÷ 1.83) was seen in cases versus controls. When CRC patient's group was divided into stages of disease by TNM classification we observed statistically significant increased frequency of AG genotype in III stage compared to controls: 62% versus 45%; OR = 2.81, 95%CI = 1.09 ÷ 7.57; $P = 0.019$. Moreover, AG genotype was overrepresented in advanced (III-IV) CRC compared to early (I-II) stages: 63% versus 43%; OR = 3.37, 95%CI = 1.21 ÷ 9.54; $P = 0.009$, demonstrating that the AG genotype is a risk factor for progression of the disease. According to genotype serum IGF-1 levels decreased depending on number of G allele. Patients with AA and AG genotypes had significantly increased IGF-1 level then patients with GG genotype regardless of disease stage.
Conclusions: Our results showed a relationship between the +3179G>A polymorphism of the IGF-1R and serum IGF-1 with the progression of colorectal carcinoma.

P1547

Deficiency of cyclin D3 a cell cycle gene contributes to development of type 1 diabetes in NOD mice

N. S. Avila,* U. S. Gupta,* E. S. Solé,* D. Mauricio,† J. Verdaguer,† C. Mezquita,‡ M. Roussel,§ P. Sicinski¶ & C. Mora Giral*
**Experimental Medicine, Institut de Recerca Biomèdica de Lleida, Lleida, Spain, †Endocrinology and Nutrition Department, Arnau de Villanova University Hospital, Lleida, Spain, ‡Laboratory of Molecular Genetics, Physiology Department, University of Barcelona, Barcelona, Spain, §Tumor Cell Biology, St. Jude Research Hospital, Memphis, TN, USA, ¶Cancer Biology, Harvard University, Boston, MA, USA*

Purpose/Objective: To find a new target gene that help to develop a new treatment for type 1 diabetes.
Materials and methods: Mice: KO CcnD3 C57BL6 mice were developed in the laboratory of Peter Siscinski . The mice were then backcrossed 13 times with NOD or NOD/SCID mice to generate aKO micein a NOD background.

Immunofluorescence (IF) and immunohistochemistry (IHC).

Pancreatic sections were incubated with (GP anti mouse INS, IgG anti-Cyclin D3, DAPI) antibodies, and as secondary antibodies anti GP Cy2 and anti IgG Cy5.

Pancreatic islet studies.

Pancreas were digested by collagenase (1.5 mg ml * 1) and isolated in Hanks Buffer salt solution (HBSS). For Cyclin D3 expression, islets per condition were handpickedand digested with trypsin, intensity media of fluorescence (IMF) of Cyclin D3 in CD45-Glut2+ cells were observed by flow cytometry.

Cell culture, transient transfections.

NIT-1 cultures were grown in DMEM supplemented with 25 mM glucose and 10% fetal bovine serum. The cDNA of Cyclin D3 was subcloned under the rat insulin promoter (RIP) in a cassette derived from pBluescript SK (Stratagene, USA) named pBSK-Neo, which confers resistance to Neomycin. The NIT-1 cell line was stably transfected and the resistant clones were selected by Geneticin (G418).
Results: Using the Microarray technology we have identified genes that have downregulation in pancreatic islet endocrine cells during the autoimmune attack progression, one of which encodes for cyclin D3.

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EXPRESSION OF INSULINE-LIKE GROWTH FACTOR-1 RECEPTOR mRNA IN COLORECTAL CARCINOMA PATIENTS

I.K arakolev[1], N. Stanilov[2], L. Miteva [1], J. Jovchev[3], Z. Dobreva[1], S.Stanilo va[1]
[1]Trakia University, Medical Faculty, Department of Molecular Biology,
Immunology and Medical Genetics, Stara Zagora,B ulgaria
[2]"St. Ivan Rilski" Hospital, Medical Faculty, Department of Neurosurgery, Surgery and Urology,
Stara Zagora, Bulgaria
[3]University Hospital,D epartment of Neurosurgery, Surgery and Urology, Stara Zagora,B ulgaria
Correspondence to: Iliya Karakolev
E-mail: ilya.karakolev@gmail.com

The IGF-1R signalling pathway can positively regulate cell-cycle progression and thus play a critical role in cancer development. Although recent studies provide sufficient evidence supporting the functional importance of IGF-1R in cancer, the prognostic significance of IGF-1R expression levels to colorectal cancer (CRC) remains obscure. Expression of IGF-1R mRNA was examined in paired samples of CRC and adjacent normal mucosa, as well as in CRC patients' venous blood. We also investigated the effect of two monocytes stimulus - C3bgp and LPS on induced mRNA of IGF-1R from normal and colorectal human monocytes. The role of a member of MAPK signal transduction pathways - JNK in IGF-1R expression was assessed. The expression of IGF-1R mRNA was measured by relative RT-PCR. The results demonstrated that expression of IGF-1R mRNA in venous blood from CRC was down-regulated compared to venous blood from healthy donors (1.426 vs. 0.645; p=0.024). Mean IGF-1R mRNA level was found to be approximately 5.8 fold higher in tumour tissue compared to adjacent normal mucosa (p=0.02). Strong IGF-1R mRNA expression was found for early stages of CRC. The results showed that both stimuli used, strongly up-regulated mRNA expression for IGF-1R in CRC monocytes, then in monocytes from healthy donors. The highest level of IGF-1R expression was detected after C3bgp stimulation, regardless of JNK inhibitor presence. The significance for increasing expression of IGF-1R was observed for early CRC when patients were divided according to the tumour stage. We can conclude that down-regulation of mRNA expr ession of IGF-1R in peripheral blood cells and stimulated monocytes from patients with advanced stages of colorectal cancer are a hallmark of tumour progression and can be used as a prognostic factor.

Keywords: colorectal cancer, IGF-1R, qRT-PCR

Introduction

Colorectal cancer (CRC) is the second most common cancer as it is responsible for 20% of all cancer deaths in the developed countries. In Bulgaria, the incidence of colorectal cancer is in the top third within the European Union and the second most common cancer-related death in men and women nationwide (Ministry of Health, Bulgaria; WHO). Although the primary therapy of CRC is surgical, the elucidation of different novel prognostic markers that could also serve as therapeutic targets is necessary to better understand this cancer entity and to improve outcome. The very early events that rescue cells from cell cycle arrest are mediated through signals transmitted by a group of peptides, collectively known as growth factors (5, 9). These molecules can be classified into two subgroups, namely the "competence" factors, such as the platelet-derived growth factor that enable cells to enter into the G_1 phase, and the "progression" factors, such as the insulin-like growth factor (IGF) that are required for progression from G1 into the S phase and, ultimately, cell division (7).

The IGF system is comprised of ligands (IGF-1 and IGF-2), receptors (IGF-1R and IGF-2R), and a family of six binding proteins. The IGF1R is activated by binding of either IGF1 or IGF2. Binding of ligand induces a conformational change and autophosphorylation of key residues in the β subunits of the receptor, and docking proteins then interact with the phosphorylated residues of the activated receptor. Activation of the receptor and transduction of the intracellular signalling kinase cascades culminates in cell proliferation and anti-apoptotic effects (2,1 6).

The IGF-1R and its associated signalling pathway is an important growth regulatory pathway

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HIGHER TNF-ALPHA PRODUCTION DETECTED IN COLORECTAL CANCER PATIENTS MONOCYTES

N.S. Stanilov[1], Z.G. Dobreva[2] and S.A.S tanilova[2]
[1]Medical Faculty and "St. Ivan Rilski" Hospital, Department of Neurosurgery, Surgery and Urology, Stara Zagora, Bulgaria
[2]Trakia University, Faculty of Medicine, Department of Molecular Biology, Immunology and Medical Genetics, Stara Zagora,B ulgaria
Correspondence to: Spaska Stanilova
E-mail: stanilova@mf.uni-sz.bg

ABSTRACT

Many studies have shown that TNF-α is critically involved in tumor promotion and progression. We examined the ability of monocytes from patients with colorectal cancer in different stages of the disease to produce TNF-α after stimulation and the involvement of JNK signal transduction pathway in TNF-α synthesis. Purification of monocytes was done by plastic adherence of PBMC. Monocytes were stimulated with LPS or C3bgp. The involvement of JNK MAP kinase in TNF-α secretion was evaluated by using selective JNK inhibitor SP600125. The quantity determination of TNF-α was performed by ELISA in culture supernatants on 24 h. Monocytes from patients produced significantly higher level of TNF-α in response to LPS stimulation in comparison with monocytes isolated from healthy donors (1926±690 pg/ml vs. 1465±771 pg/ml; p=0.034). Moreover, we observed that LPS-stimulated TNF-α production increased in stage-dependent manner; because monocytes from patients with advanced cancer secreted significantly more TNF-α than monocytes from patients in early stage of the disease (2298±127 pg/ml vs. 1687±780 pg/ml; p=0.041). Inhibition of JNK MAPK led to significant suppression of TNF-α production in both healthy individuals and patients with colorectal cancer. However, even after inhibition of JNK, monocytes from patients produced a greater quantity TNF-α, compared with monocytes from healthy donors. We conclude that monocytes from patients with colorectal cancer are prone to produce higher levels of TNF-α after stimulation and this production is disease stage dependent.

Keywords: colorectal cancer, JNK, TNF-α

Introduction

Colorectal cancer (CRC) represents a significant cause of morbidity and mortality worldwide (10). The anti-tumor immune response is regulated by numerous immune cells and proteins, including cytokines produced by the tumor-infiltrating lymphocytes and activated immune cells (macrophages, dendritic cells and lymphocytes). The mixture of cytokines that is produced both locally and systemically plays a pivotal role in the induction of host immune response, which can either block or facilitate tumor growth (4, 5).

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine secreted mainly by activated monocytes/macrophages. TNF-α gene is transcriptionally silent in unstimulated monocytes and is rapidly transcribed in response to variety of signals, such as bacterial endotoxin (LPS) and other stimuli. Transcriptional control of LPS-induced TNF-α gene expression is mediated by NF-kB and AP-1 binding sites present within the TNF-α gene promoter. Moreover, NF-κB and c-Jun acted independently, and no synergistic interaction were detected (7).

It is well known that TNF-α is critically involved in tumor promotion and progression. But, to date is still being discussed whether tumor-promoting or tumor-destructive role play TNF-α in tumorogenesis. TNF-α causes haemorrhagic necrosis *in vivo* with destruction of tumour vasculature and ischaemia. It also promotes tumour cells lysis by activating the anti-tumour immune response. TNF-α can act synergistically with other cytokines to induce tumor killing (12). Protumorogenic activities of TNF-α include further genetic alteration to malignant cells and enhancing malignant cell survival. TNF-α also induces increasing production of other cytokines and chemokines by the malignant cells. The combination of cytokines which is produced by myeloid cells in the tumor microenvironment may stimulate angiogenesis and local immuno-suppression. The end result is to enhance primary tumor growth and facilitation of metastatic spread (1). Unlike tumor associated macrophages the activity of peripheral blood monocytes still remains elusive.

In this study we examined the ability of monocytes of patients with colorectal cancer in different stages of the disease to produce TNF-α after stimulation and compared with TNF-α production from healthy volunteers. We also explored molecular regulatory mechanisms for the TNF-α production focusing on the signaling pathway of JNK activation.

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Monocytes expression of IL-12 related and IL-10 genes in association with development of colorectal cancer

Noyko S. Stanilov · Lyuba D. Miteva · Zlatka G. Dobreva · Jovcho P. Jovchev · Geo M. Cirovski · Spaska A. Stanilova

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Abstract The main regulator of anti-tumor immune response is the activity of monocytes, suggesting that the produced cytokines may have a prognostic role. This study investigates gene expression of interleukin (IL)-12-related cytokine and IL-10 in stimulated monocytes from colorectal cancer (CRC) patients. Relative quantification of IL-12A, IL-12B, IL-23A and IL-10 mRNA transcripts was performed on the third hours after stimulation by real-time qPCR. We also explored an inhibitor of JNK signaling pathway activation for the observed cytokine gene expression. A strong downregulation of IL-12B mRNA expression in CRC monocytes compared to healthy donors was observed. The rate of transcription of IL-12B in stimulated monocytes was associated with the stage of CRC. The expression of IL-12A gene in stimulated monocytes from patients with advanced was lower than early cancer. Moreover, we observed stage dependent JNK inhibition mediated reduction in IL-12A expression. The hyporesponsiveness was strongly expressed in monocytes from advanced then early stages of CRC. Expression of IL-10 mRNA was almost equally in CRC monocytes from early stages and healthy donors. We demonstrated that altered gene expression profiles of IL-12A, IL-12B, IL-23A at mRNA level in CRC monocytes was associated with tumor development and can be attributed to anticancer immune response.

Keywords Colorectal cancer · Gene expression · IL-10 · IL-12A · IL-12B · IL-23A

N. S. Stanilov · J. P. Jovchev · G. M. Cirovski
Department of Neurosurgery, Surgery and Urology, University Hospital, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

N. S. Stanilov
BMI Healthcare, The Harbour Hospital, St Mary's Road, Poole, Dorset, UK

L. D. Miteva · Z. G. Dobreva · S. A. Stanilova (✉)
Department of Molecular Biology, Immunology & Medical Genetics, Faculty of Medicine, Trakia University, Aremiska 11 Str., Stara Zagora, Bulgaria
e-mail: stanilova@mf.uni-sz.bg

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the Western countries [1]. A growing body of evidence established that cancer arises in chronically inflamed tissue and similarities in the regulatory mechanisms have been suggested [2, 3]. The same holds true in CRC. The main regulator of inflammation is the activity of immune system that is affected by pro- and anti-inflammatory cytokines. At the other side the tumor growth is under immune surveillance. The anti-tumor immune response is regulated by numerous immune cells and proteins, including cytokines produced by the tumor-infiltrating lymphocytes and activated immune cells (macrophages, dendritic cells and lymphocytes). The mixture of cytokines that is locally and systemically produced plays a pivotal role in the induction of host immune response, which can either block or facilitate tumor growth [4–6]. The proper balance between interleukin (IL)-12-related cytokines (IL-12p70, IL-12p40, IL-23) and IL-10 produced from antigen presenting cells controls the appearance of normal Th1-and Th2-mediated immune response.

Bioactive heterodimer IL-12p70, composed of p35 and p40 subunits, is a potent Th1-related cytokine clearly involved in the anti-tumor immune response [7, 8]. IL-12p70 and IL-23 are heterodimers, which share the

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EVENT ABSTRACT

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Association of -1082 A/G promoter polymorphism of IL-10 gene with colorectal cancer development

Noyko S. Stanilov[1*], Zlatka G. Dobreva[2], Jovcho Jovchev[1], Tashko Deliysky[3], Anelia Avramova[2], Lyuba Miteva[2] and Spaska A. Stanilova[2]

[1] Trakia University, Medical Faculty, Department of Neurosurgery, Surgery and Urology, Bulgaria

[2] Trakia University, Medical Faculty, Department of Molecular Biology, Immunology and Medical genetics, Bulgaria

[3] University School of Medicine, Oncology Center,, Bulgaria

The cancer-associated inflammation and anti-tumor immune response to colorectal carcinoma (CRC) is regulated by cytokines produced from activated immune cells. IL-10 is a Treg cytokine suppressing both Th1 immune response and anti-tumor immunity in hosts. The role of functional polymorphism of IL-10 gene in CRC development still remains elusive.

This study was designed to compare -1082 A/G IL10 genotype distribution in 119 CRC patients to a group of 154 matched healthy donors using ARMS-PCR assay. We also investigated serum IL-10 levels in an association to genotype by ELISA. In the study population the slightly enhanced frequency of homozygous genotype GG (18% vs 13%; OR=1.414; 95%CI=0.64÷3.11; p=0.347) was seen in cases versus controls. When CRC patient's group was divided into stages of disease by TNM classification we observed higher risk of advanced CRC (III-IV stages) for individuals with GG genotype: OR=2.229; 95%CI= 0.828÷6.018; p=0.077. G allele was also overrepresented in advanced vs early CRC (OR=1.581; 95% CI=0.912÷2.743; p=0.082). According to the IL-10 serum levels, CRC patients with GG genotype produced higher IL-10 than AA and AG patients in early stages, similar to healthy control. Moreover, advanced cancer patients with AA and AG genotype produced significantly higher IL-10 compared to patients in early stage (p=0.002 for AA; p= 0.007 for AG genotype), whereas for advanced cancer patients with GG genotype this difference does not reach statistical significance. In conclusion, this study demonstrated that -1082 A/G polymorphism of IL-10 gene has a role in CRC progression rather then CRC genetic predisposition.

Keywords: colorectal cancer, IL-10, promoter polymorphism, -1082A>GIL10, Cytokines

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* **Correspondence:** Dr. Noyko S Stanilov, Trakia University, Medical Faculty, Department of Neurosurgery, Surgery and Urology, Stara Zagora, 6000, Bulgaria, noyko.stanilov@gmail.com

EVENT ABSTRACT

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IL-12p40, IL-23 and IL-10 production from monocytes of patients with colorectal cancer: effect of inhibition of JNK signaling pathway

Noyko S. Stanilov[1*], Zlatka G. Dobreva[2] and Spaska A. Stanilova[2]

[1] Trakia University, Medical faculty, Department of Neurosurgery, Surgery and Urology, Bulgaria

[2] Trakia University, Medical Faculty, Department of Molecular Biology, Immunology and Medical genetics, Bulgaria

Cytokine production in patients with colorectal cancer (CRC) has an important impact on CRC development. We investigated changes of IL-12p40, IL-23 and IL-10 production from monocytes of CRC patients depending on cancer stages. Isolated monocytes were stimulated with LPS or C3bgp. We also explored molecular regulatory mechanisms focusing on the JNK activation by using selective inhibitor SP600125. The quantity determination of IL-12p40, IL-23 and IL-10 was performed by ELISA.

IL-12p40 production from patients' monocytes was diminished in early and advanced stages of the disease. Similarly, independent of disease stage, patients' monocytes secreted significantly lower IL-23 quantity compared to monocytes from healthy individuals. We observed significantly decreased IL-10 production from monocytes of CRC patients in early stage, but not in monocytes from patients with advanced CRC. Moreover, patients in early stages showed significant lower IL-10 in comparison with advanced stages of CRC. Inhibition of JNK is clearly upregulating IL-12p40 and IL-23 production from patients' monocytes, unlike healthy donors where inhibition of this kinase is slightly reduced IL-12p40 and IL-23 production is significantly downregulated. Regarding IL-10, the inhibition of JNK did not result in significant changes of its production, while the inhibition of JNK in monocytes from healthy donors led to downregulated IL-10 production.

In conclusion, this study showed altered functional activity of peripheral blood monocytes of CRC patients which are demonstrated through downregulation of IL-12p40 and IL-23, but not of IL-10. This different response of patients' monocytes is mediated partly by changes in involvement of JNK signaling pathway.

Keywords: colorectal cancer, Monocytes, IL-10, IL-12p40, IL-23 **Conference:** 15th International Congress of Immunology (ICI), Milan, Italy, 22 Aug - 27 Aug, 2013.

Presentation Type: Abstract **Topic:** Immune-mediated disease pathogenesis

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* **Correspondence:** Dr. Noyko S Stanilov, Trakia University, Medical faculty, Department of Neurosurgery, Surgery and Urology, Stara Zagora, Bulgaria, noyko.stanilov@gmail.com

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UPREGULATION OF Treg AND DOWNREGULATION OF TH17 RELATED CYTOKINE EXPRESSION IN PERIPHERAL BLOOD OF COLORECTAL CANCER PATIENTS

N. Stanilov[1,2], L. Miteva[3], G. Cirovski[1,4], S. Stanilova[3]

[1]Department of Surgery, Neurosurgery and Urology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Colorectal Surgery Unit, The Royal London Hospital, London, UK
[3]Department of Molecular Biology, Immunology and Medical Genetics, Trakia University, Medical Faculty, Stara Zagora, Bulgaria
[4]Hospital "St. Ivan Rilski", Stara Zagora, Bulgaria

ABSTRACT

The presence of distinct cell types of the innate and adaptive immune system in neoplastic lesion is a marker for local antitumoral responses and tumor elicited inflammation. The aim of the present study was to investigate the gene expression of interleukin (IL)-12A, IL-12B, IL-23A, IL-17, IL-10, IL-6 and TGF-β in peripheral blood cells from colorectal cancer (CRC) patients. The venous blood was collected preoperative and 10 days after surgery from 17 CRC patients and 22 healthy donors. After isolation of total RNA and synthesis of cDNA, the real-time polymerase chain reaction (qRT-PCR) for quantity determination of specific mRNA was performed on a 7500 Real - Time PCR System.

Our results demonstrated that among investigated cytokine genes IL-10 and TGF-b were significantly upregulated in patients with CRC compared to the control group, while the expression of IL-17 and IL23 mRNA was significantly decreased in CRC patients. Based on the results we could assume that peripheral blood gene expression programming in CRC patient's triggers local differentiation of Th cells towards Th17 and Treg instead Th1 anti-tumor subpopulation.

In conclusion we suppose that the established tumor elicited inflammation can contribute to multiple hallmark capabilities by supplying bioactive molecules not only benefit tumor grown, but also affecting epigenetic alternation in immune blood cells resulting in gene expression reprogramming.

Key words: CRC, Th17, mRNA, TGF-betta, IL-17, IL-10, RT-PCR

INTRODUCTION

Colorectal cancer follows definitive genetic changes including at least the adenomatous polyposis coli (APC) tumour suppressor gene and mutations in K-Ras oncogene, providing the reproduction of tumor cells. Genome instability and epigenetic alteration ensure on certain mutant genotypes selectively advantage for tumor growth and present a first enabling hallmark of cancer (1, 2). The second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions that is driven by cells of the immune system, some of which serve to promote tumor progression through various means (3-5).

Inflammation is likely to be involved with both forms of sporadic as well as heritable colon cancer. The "Evading immune destruction" as characteristic of tumor illustrated the remarkable progress made in the last decade to describe the role of inflammation in promoting cancer development. The inflammation promote cancer progression and accomplishes the full malignant phenotype, such as tumor tissue remodeling, angiogenesis, metastasis and the suppression of the innate anticancer immune response (6, 7).

The presence of distinct cell types of the innate and adaptive immune system in neoplastic lesion is a marker for local antitumoral responses and tumor elicited inflammation. Additionally,

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The prognostic value of preoperative serum levels of IL-12p40 and IL-23 for survival of patients with colorectal cancer

NOYKO STANILOV,[1,2] LYUBA MITEVA,[3] JOVCHO JOVCHEV,[1] GEO CIROVSKI[1,4] and SPASKA STANILOVA[3]

[1]Department of Surgery, Neurosurgery and Urology, Faculty of Medicine, Trakia University, Stara Zagora; [2]Colorectal surgery unit, The Royal London Hospital, London, UK; [3]Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, Stara Zagora; and [4]Hospital 'St. Ivan Rilski', Stara Zagora, Bulgaria

Stanilov N, Miteva L, Jovchev J, Cirovski G, Stanilova S. The prognostic value of preoperative serum levels of IL-12p40 and IL-23 for survival of patients with colorectal cancer. APMIS 2014.

Colorectal cancer (CRC) patients were previously shown to express a signature of cytokines that contribute to cancer pathogenesis and are detectable in serum. The aim of this study was to evaluate the potential clinical use of circulating cytokine measurements in CRC patients preoperatively as markers for disease outcome. The levels of cytokines IL-12p40 and IL-23 were assessed by ELISA in the sera of 91 patients with previously untreated CRC and then 5-year survival was determined using Kaplan–Meier analyses. The levels of circulating interleukin IL-12p40 significantly decreased with the progression of CRC, whereas the levels of IL-23 remained with no significant differences between disease stages. None of the cytokine levels were influenced by age, gender and colon vs rectum localization. We found that preoperative serum concentration of IL-12p40 cytokine is a good prognostic marker for survival; as for IL-23 levels, we found no outcome prognostic value. In addition, 5-year survival confirmed that tumor grade, bowel wall invasion, lymph node and metastatic status have an impact on overall survival. In conclusion, we believe that our findings show clinical significance of the preoperative serum concentration for IL-12p40 and provide an additional prognostic biomarker for CRC survival.

Key words: CRC; survival; cytokines; IL-12p40; IL-23.

Spaska Stanilova, Department of Molecular Biology, Immunology & Medical Genetics; Faculty of Medicine, Trakia University, Aremiska 11 str., Stara Zagora, Bulgaria. e-mail: stanilova@mf.uni-sz.bg

Colorectal cancer is a common and very aggressive malignant disease in the contemporary industrialized society (1). It has been previously shown that cytokines are a major part of the local and systemic microenvironment and can inhibit or promote tumor growth (2, 3). A growing number of cytokines and related transcription factors have been reported to be associated with CRC development (4–6). Identifying new prognostic markers can contribute to tailoring adequate treatment models and lower mortality. Serum levels for a number of cytokines and transcription factors in cancer patients have shown potential to possess such properties (7, 8).

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IL-12 was discovered by two separate groups as a cytokine-inducing differentiation of cytotoxic lymphocytes (CTL) or stimulating proliferation of natural killer cells (9, 10). IL-12 is a proinflammatory, Th1-inducing and Th1-maintaining cytokine, which bridges innate and acquired immunity (11). It is a heterodimeric cytokine formed of two subunits, IL-12p35 and IL-12p40, each encoded by a different gene, IL12A for the light chain p35 and IL12B for the heavy chain p40. IL-12 belongs to a family of cytokines, which includes IL-23, IL-35 and IL-27 (12). The subunit IL-12p40, which is present in both IL-12 and IL-23, has not been widely recognized as having intrinsic functional activity. The independent role of IL-12p40 is a recent perception supported by data from a growing number of

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Association of insulin-like growth factor-I receptor polymorphism with colorectal cancer development

Noyko S. Stanilov · Iliya A. Karakolev · Tashko S. Deliysky · Jovcho P. Jovchev · Spaska A. Stanilova

Received: 18 January 2013 / Accepted: 26 August 2014

Abstract The insulin-like growth factor (IGF) system plays a prominent role in the cancer development. The IGF-1 receptor (IGF-1R) and its associated signalling pathway is an important growth regulatory pathway that has been implicated in colorectal carcinogenesis. This study was designed to compare +3179G/A *IGF1R* (rs 2229765) genotype distribution in 110 colorectal cancer (CRC) patients to a group of 143 healthy controls (HCs). We also investigated serum IGF-1 levels in CRC patients and HCs in an association to genotype. IGF-1 serum levels were measured by enzyme-linked immunosorbent assay and genotyping for the +3179G/A polymorphism was performed by restriction fragment length polymorphisms–polymerase chain reaction assay. Although the genotype frequencies were comparable in both groups, higher frequency of dominant genotypes [AA/AG; 71 vs. 62 %; odds ratio (OR) = 1.52] and lower frequency of GG genotype (29 vs. 38 %) was seen in cases versus controls. When CRC patient's group was divided into stages of disease by tumor–node–metastasis classification we observed the significantly highest frequency of AA genotype in III stage compared to controls: 22.5 versus 15 %; OR = 3.37, $p = 0.026$. There was a significant association between IGF-1R rs2229765 polymorphism and advanced CRC (AA/AG vs. GG: OR = 3.06, $p = 0.004$). The frequency of A-allele in advanced CRC was significantly higher then early CRC (52 vs. 37.7, OR = 1.78). According to genotype serum IGF-1 levels was significantly decreased in patients with GG genotype then patients with dominant genotypes. Our results showed a relationship between the +3179G>A polymorphism of the IGF-1R and serum IGF-1 with the progression of colorectal carcinoma. A dominant genetic model was established for IGF-1R rs2229765 polymorphism and CRC progression.

Keywords Insulin-like growth factor · IGF-1R · rs 2229765 · Colorectal cancer · Polymorphism

Introduction

The insulin-like growth factor (IGF) system is comprised of ligands (IGF-I and IGF-II), receptors (IGF-1R and IGF-2R), and a family of six binding proteins. The IGF pathway plays an important role in growth and development and in the maintenance of tissue homeostasis through regulation of cell cycle progression [1]. Both IGF-1 and IGF-2 exert their biological effects through activation of IGF type 1 receptor (IGF-1R). IGFs binding activate intrinsic tyrosine kinase activity, resulting in receptor autophosphorylation and stimulation of signaling cascades that include the RAS/RAF/MEK/ERK and PI3K/AKT/TOR pathways. Activation of the receptor and transduction of the intracellular signaling kinase cascades culminates in cell proliferation, differentiation and anti-apoptotic effects [2].

N. S. Stanilov · J. P. Jovchev
Department of Neurosurgery, Surgery and Urology, 2nd Surgery Clinic, University Hospital, Stara Zagora, Bulgaria

N. S. Stanilov
BMI Healthcare, Winterbourne Hospital, Dorchester, Dorset, UK

I. A. Karakolev · S. A. Stanilova (✉)
Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Aremiska 11 street, Stara Zagora, Bulgaria
e-mail: stanilova@mf.uni-sz.bg; spaska.stanilova@abv.bg

T. S. Deliysky
Oncology Center, University School of Medicine, Pleven, Bulgaria

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RESEARCH ARTICLE

Significance of −1082A/G polymorphism of *IL10* gene for progression of colorectal cancer and IL-10 expression

Lyuba D. Miteva · Noyko S. Stanilov · Tashko S. Deliysky · Spaska A. Stanilova

Received: 17 July 2014 / Accepted: 3 September 2014

Abstract The role of functional polymorphism within *IL10* (rs1800896) in colorectal cancer (CRC) still remains elusive. The aim of present study was to investigate the significance of −1082A/G polymorphism in *IL10* on CRC risk, progression, and overall survival in a cohort of Bulgarian patients. Also, a functional role of this polymorphism on systemic and local level of *IL10* mRNA quantity and serum IL-10 level was explored. A group of 119 patients with sporadic CRC and 154 age-sex-matched controls were genotyped by allele-specific PCR. The quantification of mRNA and serum IL-10 levels was performed by real-time PCR and ELISA assays, respectively. The genotype and allelic frequency among cases and controls was similar. However, we observed significant elevation of G-allele and GG-genotype frequencies among advanced CRC. G-allele was overrepresented in advanced CRC patients (49 %) compared to early CRC (35 %) with OR=1.77; 95%CI 1.018÷3.083; P=0.031. A significant up-regulated expression of *IL10* mRNA was observed among AG/GG-genotypes in tumor tissue compared to homozygous AA-genotype (RQ value 68.3 vs. 6.68; P=0.0062). Also, GG-genotype of −1082A/G polymorphism in *IL10* was positively associated with higher serum IL-10 among early CRC patients and controls, in contrast to advanced cases. Although, investigated polymorphism in *IL10* has no significant impact of overall survival among Bulgarian CRC patients, we found a significant relationship of high pre-operative serum level of IL-10 with poor survival of CRC (P=0.023). Our findings indicate a significant impact of −1082A/G polymorphism of *IL10* on CRC progression, rather than genetic predisposition and prognosis of CRC.

Keywords rs1800896 · Tumor · mRNA · Serum

L. D. Miteva · S. A. Stanilova (✉)
Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University,
Armeiska 11 St., 6000 Stara Zagora, Bulgaria
e-mail: stanilova@mf.uni-sz.bg

N. S. Stanilov
Department of Neurosurgery, Surgery and Urology; Medical Faculty, Trakia University, Stara Zagora, Bulgaria

N. S. Stanilov
Colorectal Surgery Unit, The Royal London Hospital, London, UK

T. S. Deliysky
Oncology Center, University School of Medicine, Pleven, Bulgaria

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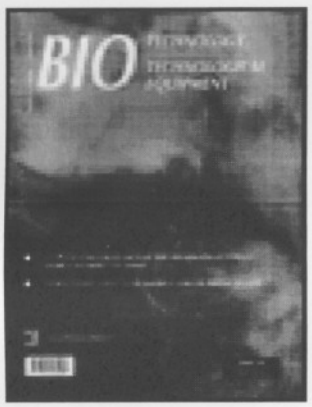
Introduction

Colorectal cancer (CRC) is one of the most common cancers associated with high morbidity and mortality worldwide. An inflammatory process plays the key role in neoplasia of CRC in both sporadic and hereditary forms [1]. Given that numbers of cytokines are involved in tumor development and progression, functional polymorphisms in their genes are pointed as potential candidate genes associated with carcinogenesis.

Interleukin 10 (IL-10) is an important immunoregulatory cytokine, crucial for maintaining intestinal homeostasis with central role in controlling inflammation [2, 3]. The relationship between IL-10 and cancer has been studied extensively. Several investigators consider IL-10 as immunosuppressive cytokine that blunts the immune response against cancer [4, 5]. Respectively, the decreased IL-10 production could improve cancer-specific immune responses in some preclinical tumor models [2]. The source of IL-10 can be variety of immune cells, including inducible regulatory T cells (iTregs), especially those in tumor-infiltrating lymphocytes, mediate strong inhibition of anti-tumor immunity and immune tolerance favoring tumor growth [6]. In addition, it was suggested that IL-10 is secreted by tumors to allow tumor cells to escape from immune surveillance [7]. However, the role of IL-10 in



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Colorectal cancer severity and survival in correlation with tumour necrosis factor-alpha

Noyko Stanilov[ab], Lyuba Miteva[c], Zlatka Dobreva[c] & Spaska Stanilova[c]

[a] Department of Neurosurgery, Surgery and Urology, University Hospital, Trakia University, Stara Zagora, Bulgaria

[b] Department of Colorectal Surgery, Milton Keynes Hospital, Milton Keynes, UK

[c] Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

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sensitive. Both, AT and PQ, which stimulate the intracellular formation of H2O2 or superoxide anions, respectively, trigger cell death in loh2 but do not lead to visible damage in atr7. The gene responsive for atr7 phenotype has been mapped and the gene product is a highly conservative hydroxyproline-rich glycoprotein. The transfection of both Arabidopsis mesophyll protoplast and entire plants with chimeric 35S:ATR7/GFP construct reveal subcellular localization of HRGP. Additionally it has been shown that HRGP is strongly induced by oxidative stress and acts as a positive regulator of the process of programmed cell death. Finally, to study gene expression during oxidative stress and ROS-induced programmed cell death, two platforms for RT-qPCR analysis of 217 antioxidant and 180 ROS marker genes were employed. The analyses revealed ROS-induced expression of many ROS-responsive genes mainly in loh2, confirming that an oxidative burst plays a role in the activation of the cell death in this mutant. Some of the genes were specifically regulated by either AT or PQ, serving as markers for particular types of ROS. Many of these genes were upregulated in atr7 compared to loh2 under non-stress conditions at the first time point, indicating that higher basal levels of ROS and higher antioxidant capacity in atr7 are responsible for the enhanced tolerance to oxidative stress and suggesting a possible tolerance against multiple stresses.

UPREGULATION OF T-HELPER-17 RELATED CYTOKINE EXPRESSION AND INDUCIBLE NITRIC OXIDE SYNTHASE IN COLORECTAL CANCER IN ASSOCIATION WITH HISTONE DEACETYLASE-3

Spaska Stanilova[1], Noyko Stanilov[1], Geo Cirovski[2], Totina Cirovska[1], Lyuba Miteva[1]
[1]Medical Faculty, Trakia University, [2]Trakia Hospital, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Key words: *Th17, cytokine, qPCR, CRC*

The aim of the present study was to investigate the gene expression of cytokines which are relevant to the differentiation and function of Th17 cells (TGF-β, IL-6 and IL-17) as well as inducible NO synthase (iNOS) and histone deacetylase-3 (HDAC3) in colorectal cancer (CRC) tissues. Total RNA was isolated from tumor and normal mucosa samples from 16 CRC patients. After cDNA synthesis quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 7500 Real-Time PCR System with validated PCR primers and TaqMan MGB probes for TGF-b, IL-6, IL-17, iNOS and HDAC3 mRNA. Relative quantitative evaluation of mRNAs was performed by the comparative ΔΔCt method. Results are presented as n-fold mean difference relative to calibrator (RQ = 2-ΔΔCt). We found significantly increased mRNA in tumor tissue compared to normal mucosa with RQ around 10 times that of TGF-β, IL-17, iNOS and HDAC3 (RQ = 10.01; p = 0.034; RQ = 11.3; p = 0.047; RQ = 9.7; p = 0.028; RQ = 9.7; p = 0.013). The highest increased mRNA in tumor tissue compared to normal mucosa was detected for IL-6 (RQ = 107.3; p = 0.00021). A significantly positive correlation of IL-17 expression with iNOS in colorectal cancer specimens was established (r = 0.6757; p = 0.046). In addition, iNOS gene expression was also strongly correlated with HDAC3 expression (r = 0.8290; p = 0.006). Another positive correlation was found between expression of IL-6 and HDAC3 genes (r = 0.7501; p = 0.02). Conclusion: The results demonstrated gene expression profile which favors Th17 differentiation. Based on these results we suppose that the established Th17 type inflammation can contribute to tumor promotion, including enhancing epigenetic alteration.

Original paper

Increased transforming growth factor β and interleukin 10 transcripts in peripheral blood mononuclear cells of colorectal cancer patients

Noyko S. Stanilov[1], Lyuba Miteva[1], Geo Cirovski[2], Spaska A. Stanilova[1]

[1]Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Hospital "Trakia", Stara Zagora, Bulgaria

Aim of the study: The ability of immune cells in peripheral blood to produce certain cytokines affects tumour-elicited inflammation. The aim of this study was to investigate the gene expression of interleukin 12A (IL-12A), IL-12B, IL-23A, IL-10, IL-6, transforming growth factor β (TGF-β), HDAC3, and iNOS in peripheral blood mononuclear cells (PBMC) from colorectal cancer (CRC) patients.

Material and methods: The venous blood for PBMC isolation was collected preoperatively and 10 days after surgery, from CRC patients. After isolation of total RNA and synthesis of cDNA, quantitative real-time PCR assays were performed.

Results: Our results demonstrated that among investigated cytokine genes IL-10 and TGF-β were significantly upregulated in patients with CRC compared to the control group, while the expression of IL-23 mRNA was significantly decreased in CRC patients. We observed significantly increased mRNA levels in CRC patients' PBMC before surgery for IL-10 and TGF-β compared to both postoperative and control groups. We also found a significant upregulation of iNOS in early compared to advanced CRC.

Conclusions: Based on the results we can assume that PBMC gene expression programming in CRC patients drives local differentiation of Th cells towards Treg instead of the Th1 anti-tumour subpopulation.

Key words: mRNA, cytokine, qPCR, HDAC3, iNOS, Treg, CRC.

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Introduction

Colorectal cancer follows definitive genetic changes including, at least, the adenomatous polyposis coli (APC) tumour suppressor gene and mutations in K-Ras oncogene, providing the reproduction of tumour cells. Genome instability and epigenetic alteration ensure on certain mutant genotypes a selective advantage for tumour growth and present the first enabling hallmark of cancer [1, 2]. The second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions, which is driven by cells of the immune system, some of which serve to promote tumour progression through various means [3–5]. Inflammation is likely to be involved with both forms of sporadic as well as heritable colon cancer. The "cancer-related inflammation" as characteristic of tumour illustrates the remarkable progress made in the last decade to describe the role of inflammation in promoting cancer development. The inflammation promotes cancer progression and accomplishes the full malignant phenotype, such as tumour tissue remodelling, angiogenesis, metastasis, and the suppression of the innate anticancer immune response [6, 7]. A specific cause-effect relationship between colorectal cancer angiogenesis and the upregulation of the inducible NOS (iNOS) gene in the tumour specimens has been recently demonstrated [8]. Moreover, the iNOS upregulation requires induction in response to proinflammatory cytokines. Several studies support the concept that histone deacetylase (HDAC) is responsible for transcriptional induction of proinflammatory cytokines and modulation of antigen-presenting cell activity [9, 10].

The presence of distinct cell types of the innate and adaptive immune system in neoplastic lesion is a marker for local antitumoral responses and tumour-elicited inflammation. Additionally, innate and specific immune cells can release different cytokines, responsible for pro- and anti-tumoural effect [11]. Tumour-infiltrating immune cells and cytokine-related signalling pathways are critical components in colorectal cancer initiation and progression. The mixture of cytokines that is locally and systemically produced can either block or facilitate tumour growth [7, 11, 12]. Most intratumoural immune cells are recruited from peripheral blood. Whether they are already programmed or can change their functions after falling into tumour microenvironment remains elusive. The ability of immune cells in peripheral blood to produce certain cytokines and possibility for their reprograming after entering into a tumour is a key feature for tumour growth or immune destruction. Previously, altered gene expression profiles of cytokines at mRNA level in CRC monocytes after *in vitro* stimulation were demonstrated, and this was associated with tumour development [13].

Upregulation of Treg-related genes in addition with IL6 showed the significant role for the distant metastasis in colorectal cancer

Upregulation of Treg-related genes in addition with IL6 showed the significant role for the distant metastasis in colorectal cancer

Miteva LD[1], Stanilov NS[2], Cirovski GM[3], Stanilova SA[1*]

[1]Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Breast Oncoplastic Unit, University College London Hospital , London, UK
[3] Trakia Hospital, Stara Zagora, Bulgaria

***corresponding author:**
Prof Spaska Angelova Stanilova, DSc, PhD
Head of Department of Molecular Biology, Immunology and Genetics,
Faculty of Medicine, Trakia University
Armeiska 11 St, 6000 Stara Zagora,
Bulgaria
Tel: +359-42-600-675; fax: +359-42-600-705
E-mail address: stanilova@mf.uni-sz.bg
ORCID ID: 0000-0003-1368-9081

Abstract:
T helper 17 (Th17) and T regulatory (Treg) cytokines appear to be contributing greatly to colorectal cancer (CRC) development and progression. The aim of the current study was to investigate the expression of *Foxp3*; *IL10*; *TGFB1*; *IL17A*; *IL6* and *NOS2* genes in tumor tissue, regional positive lymph nodes and distant metastasis obtained from 26 patients with advanced CRC. Quantitative real-time polymerase chain reaction (qPCR) was performed for mRNA detection by TaqMan gene expression assay.
In distant metastasis, *IL6* was strongly expressed, over 7.5 fold, followed by Treg-related genes *Foxp3*; *IL10* and *TGFB1* in contrast to *IL17A* and *NOS2*. The similar pattern of expression was observed in positive regional lymph node in addition to significant down-regulation of *NOS2* (RQ=0.287; p=0.011) and a trend for the elevation of *IL17A*. In tumor tissue, *Fopx3* was significantly upregulated and *Foxp3* mRNA positively correlated with *TGFB1* in all investigated tissue types. In tumor tissue, expression of *IL17A* was correlated with *NOS2* (r=0.68; p=0.005), while in distant metastasis *IL10* was in strong relation with *TGFB1* and *IL6*. In addition, a reverse correlation between *IL6* and *NOS2* (r=-0.66; p=0.009), was observed in distant metastasis.
The simultaneous expression of given Treg and Th17-related genes found both in the primary tumor and in the regional lymph nodes appears to provide suitable microenvironment sufficient for promoting metastatic growth. The upregulation of *Foxp3*; *IL10*, *TGFB1* and *IL6* might be a transcriptional profile hallmark for colorectal metastases.

Key words: mRNA; qPCR; colorectal cancer; Th17; Treg

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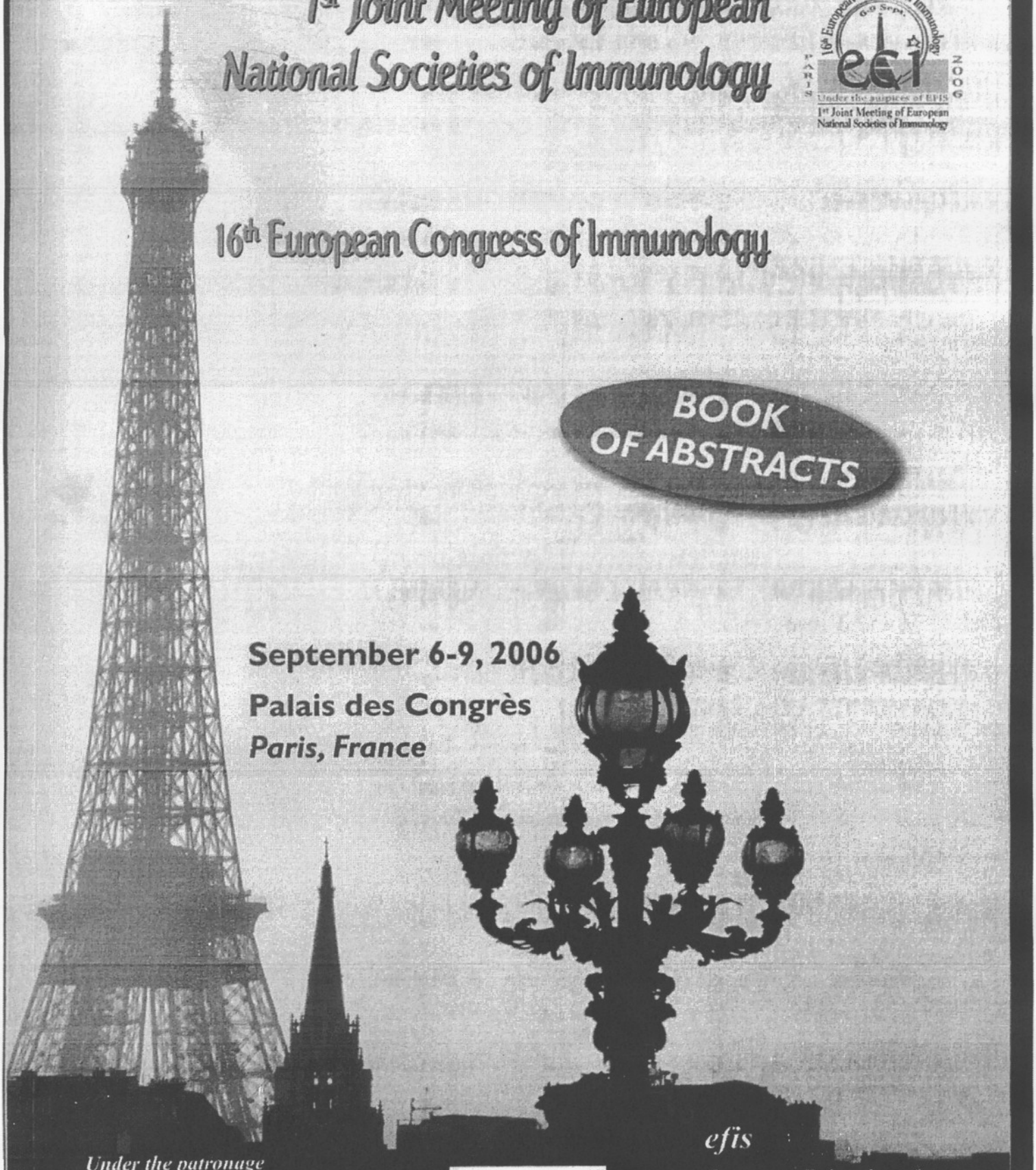

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ZAP-70 KINASE: A NEWLY IDENTIFIED JUNCTION OF THE T-CELL RECEPTOR AND GLUCOCORTICOID RECEPTOR SIGNAL TRANSDUCTION PATHWAYS

D BARTIS[1], F BOLDIZSÁR[1], K KVELL[1], P NÉMETH[1], T BERKI[1] - (1) Department of Immunology and Biotechnology, Faculty of Medicine, University of Pécs, Pécs, Hungary.

Besides the classical genomic glucocorticoid hormone (GC) actions mediated by the cytoplasmic glucocorticoid receptor (GR), GCs are known to mediate rapid non-genomic effects occurring within minutes. The GC hormone has significant role in the regulation of T-cell maturation and activation; but the cross talk between the GC and T-cell receptor (TcR) signal transduction pathways need still to be elucidated.
We examined the rapid effects of GC exposure on *in vitro* cultured Jurkat T-cells. Our results showed that Dexamethasone (DX), a GC analogue, when applied at high dose (10 μM), induced rapid (within 5 minutes) tyrosine-phosphorylation events. Short DX pre-treatment inhibited the tyrosine-phosphorylation stimulated by CD3 cross-linking. ZAP-70 kinase plays a key role in transmitting TcR-derived signals. We demonstrate that DX induced rapidly the phosphorylation of ZAP-70 on tyrosine in Jurkat cells but not in in the absence of active p56-lck as examined in the p56-lck kinase-deficient Jurkat cell line JCaM1.6. The DX-induced ZAP-70 phosphorylation could be inhibited by RU486 (GR antagonist), suggesting that this process was GR mediated. Investigating the relation of GR and ZAP-70, we found that ligand bound GR associates with ZAP-70 kinase as shown by co-immunoprecipitation experiments sampling Jurkat cell lysates. We confirmed the DX induced co-localisation of the two molecules with confocal microscopy in Jurkat cells. The association of liganded GR and ZAP-70 was observed in HeLa cells stably transfected with ZAP-70, suggesting that the phenomenon is independent of T-cell specific factors.
Our data about the GC-caused alterations in the tyrosine phosphorylation of ZAP-70 and the physical association of the GR with the ZAP-70 kinase suggest that ZAP-70 takes part in transmitting non-genomic GC action in T-cells.

FETAL LTI CELLS CAN BE DIFFERENTIATED INTO ADULT TYPE OF CD4+CD3- CELLS WHICH MAINTAIN SECONDARY LYMPHOID STRUCTURE

MY KIM[1], F MCCONNELL[1], KM TOELLNER[1], F GASPAL[1], A WHITE[1], E JENKINSON[1], G ANDERSON[1], P LANE[1] - (1) Birmingham Medical School, Birmingham, United-Kingdom.

Here we demonstrate that fetal lymphoid tissue inducer (LTi) cells can be differentiated into adult type of $CD4^+CD3^-$ cells after transfer into an adult host. Adult $CD4^+CD3^-$ cells are responsible for providing survival signals to the T cells that help B cells via their constitutive expression of T cell survival molecules, OX40-ligand and CD30-ligand. Transferred LTi cells, which do not express OX40-ligand and CD30-ligand, upregulate these molecules in vivo, and LTi cells cultured with IL-7 and TL1A (TNFSF15) upregulate them in vitro. The adult $CD4^+CD3^-$ cells share similar genetic fingerprints with LTi cells (including IL-7Rα, lymphotoxin (LT) α and β, TNFα, TRANCE and RANK expression) but not with other cell types; dendritic cells, plasmacytoid dendritic cells, T cells, B cells and NK cells. Fetal LTi cells are critically involved in the development of lymphoid tissues during organogenesis via their expression of $LT\alpha1\beta2$. We show that the adoptive transfer of adult $CD4^+CD3^-$ cells or LTi cells into LTα-deficient mice, which lack segregation of their B and T cell areas in the spleen, allows them to acquire normal lymphoid organization, indicating that these $CD4^+CD3^-$ cells are critical in maintaining the segregation of B and T cell areas. Consistent with this idea we found that adult $CD4^+CD3^-$ cells are associated with VCAM-1-expressing stromal cells in B and T cell areas where stromal cells upregulate homeostatic chemokines, CXCL13, CCL19 and CCL21. We suggest that both of these functions of adult $CD4^+CD3^-$ cells; (1) providing survival signals to the T cells and (2) maintaining secondary lymphoid structure are integral to the development and maintenance of high affinity antibody responses.

ARAP1 (ARF GAP AND RHO GAP WITH ANKIRIN REPEAT AND PH DOMAIN),A NOVEL BINDING PARTNER FOR CRKII

A GIVONI[1], S GELKOP[1], N ISAKOV[1] - (1) Department of Microbiology and Immunology and the Cancer Research Center, Ben Gurion University of the Negev, Beer Sheva, Israel.

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The Crk adaptor proteins are involved in multiple signaling pathways in different cell types, including lymphocytes. Because of their lack of catalytic activit, many studies on Crk were aimed at the identification of their binding partners and determination of the physiological meaning of these interactions. Crk proteins were found to be involved in the early steps of lymphocyte activation. They constitutively associate with effector molecules that mediate cell adhesion,and thereby regulate lymphocyte extravasation and cell recruitment to sites of inflammation. To identify additional Crk binding proteins in human T cells we used bead-immobilized GST-CrkII and pulled down proteins from a lysate of resting and activated Jurkat T cells. Bound proteins were released by 5 min of boiling and protein mixtures were separated by gel electrophoresis, stained with colloidal Coomassie blue dye, excised, and digested with trypsin. The resulting peptides were analyzed by LC-tandem mass spectrometry (MS/MS) and assigned to specific proteins with the Mascot search engine. A prominent Crk-binding partner was identified by LC-MS/MS as a product of the ARAP1(Art GAP and Rho GAP with ankyrin repeat and PH domain) protein. This protein serves as a potential effector in multiple signaling pathways and as a potential regulator of several GTP-binding proteins that are involved in cell adhesion processes. Pull-down assays and coimmunoprecipitation studies confirmed the association between CrkII and ARAP1 in lysates of Jurkat T cells and COS-7 cells. Preliminary studies suggest that the SH3N and SH2 domains of CrkII are involved in the interaction with ARAP1 and that CrkII-ARAP1 interaction occurs in resting cells but is amplified following cell activation.

POLYMORPHISMS IN THE IL12B GENE IN ASSOCIATION WITH SEPSIS SUCSEPTIBILITY

S STANILOVA[1], L MITEVA[1], Z KARAKOLEV[2], C STEFANOV[3], N STANILOV[4] - (1) Trakia University, Faculty of Medicine, Department of Molecular Biology, Immunology & Medical Genetics, Stara Zagora, Bulgaria. (2) Trakia University, Faculty of Medicine, Department of Intensive Medicine and ICU, Stara Zagora, Bulgaria. (3) Medical University, Department of Anesthesiology and Intensive Care, Plovdiv, Bulgaria. (4) Trakia University, Faculty of Medicine, Department of Surgery, Stara Zagora, Bulgaria.

The pathology of humans faces the challenge of diversity addressed in genetic terms as polymorphism. Interleukin-12 has an important immunoregulatory function, since it promotes Th1 differentiation and cell-mediated immunity, which appears to contribute to the development of sepsis. This study investigates the allele and genotype distribution of the A/C SNP located at position 1188 *TaqI* in the 3'UTR of the IL-12p40 gene (IL12B) and the AGAG microinsertion together with the GC/TT transition (allele 1) within the promoter region of this gene (IL12B*pro*) in association with susceptibility of sepsis. A total of 43 patients in ICU meeting the criteria for severe sepsis and 114 healthy volunteers were included. The polymorphism in 3'UTR of the IL12B gene was typed by PCR-RFLP analysis. The ARMS-PCR system was used for IL12B*pro* polymorphism detection. Secreted IL-12p40 quantities from PBMC stimulated with C3bgp, LPS, PHA and PWM were measured in culture supernatants by ELISA. The results showed that healthy controls had significant elevation of IL12B*pro*-1 allele frequencies, compared to patients with sepsis (60% vs 44%; OR=1.895; 95%CI 1.123÷3.196;p=0.016). Homozygous genotype of this allele "11" was also more frequent in healthy than in sepsis group (34% vs 16%; OR=0.375; 95% CI, 0.152÷0.931; p=0.034). Thus, the data provided evidence that IL12B*pro*-1 allele has a protective effect on the risk of sepsis. Individuals homozygous for IL12B*pro*-2 were over-represented among the sepsis group (28% vs 14%; OR=2.355; 95% CI, 0.969÷5.730; p=0.059), which suggests that IL12B*pro*-"22" genotype could have a role in the predisposition to human sepsis. No significant association was identified between IL-12B 3'UTR alleles and genotypes and susceptibility to severe sepsis. Our results also indicated that the presence of "A" allele in IL12B3'UTR correlated with increased production of IL-12p40 of stimulated PBMC from sepsis patients. When the sepsis patients were stratified according to IL12B3'UTR AA-genotype, heterozygous IL12B*pro*-"12" subjects were found to have lower IL-12p40 induced production, however the significance was reached for cultures stimulated with C3bgp and PHA. In conclusion our results suggest that IL12B*pro* genotypes have a relevant role in the susceptibility to sepsis in contrast to IL12B 3'UTR polymorphism.

CLONING AND CHARACTERISATION OF A NOVEL KINASE DEFICIENT ZAP-70 VARIANT IN JURKAT CELLS

F BOLDIZSÁR[1], K KVELL[1], T CZÖMPÖLY[1], D BARTIS[1], L PÁLINKÁS[1], P NÉMETH[1], T BERKI[1] - (1) University of Pécs, Faculty of Medicine, Department of Immunology and Biotechnology, Pécs, Hungary.

We have cloned a new ZAP-70 variant molecule from cDNA generated from wild type (wt) Jurkat cell total RNA with RT-PCR. Although the PCR product was similar in size to cDNA coding for the normal ZAP-70 molecule (~1900 bp), sequencing revealed, that only the first 837 bases of the cloned cDNA is identical to the normal ZAP-70 cDNA. After analysing the whole ZAP-70 gene, we found that this sequence might represent a new ZAP-70 splice variant that is 1956 bp long, contains exons 1-5, intron 5, exon 6 and most of intron 6, respectively. Among the 3 stop codons found in the variant sequence, the first is in intron 5, that gives an intelligible open reading frame. Based on this we predicted that the novel variant protein contains only the N-terminal part of the normal ZAP-70 molecule: the SH2 domains, interdomain A and part of interdomain B (1-278 amino acid residues, respectively), but lacks the kinase domain. Predicted residues 278-339 are unique to this molecule. Parallely we have also cloned the normal ZAP-70 molecule from human peripheral T cell total RNA.
We have transfected both the variant and normal ZAP-70 into wt and ZAP-70 deficient (P116) Jurkat cells with lentiviral vector. Expression of the variant and normal ZAP-70 molecules in the transfected cells was verified by both RT-PCR and flow cytometry. The variant ZAP-70 expressing P116 cells could only be stained with antibodies recognising the N terminal part or the SH2 domains, but not the kinase domain of the ZAP-70 molecule, while the normal ZAP-70 expressing P116 cells could be stained with several available anti-ZAP-70 antibodies. Transfection of the normal but not the variant ZAP-70 molecule normalized the Ca-signaling of P116 cells upon anti-CD3 treatment. In the variant ZAP-70 transfected wt Jurkat cells the amplitude of the Ca-signal decreased significantly after anti-CD3 treatment compared to untransfected controls.
Based on these data we conclude that the newly cloned molecule is a truncated, kinase deficient ZAP-70 variant, that is not capable of restoring the Ca-signalling in ZAP-70 deficient P116 cells, but its overexpression in wt Jurkat cells negatively influences the function of the normal ZAP-70, possibly because of a competition for docking sites.

ALTERED T CELL SIGNAL TRANSDUCTION IN HISTIDINE DECARBOXYLASE KNOCKOUT MICE

A KÖNCZ[2], G NAGY[2], M MAZAN[1], E BUZAS[1], A FALUS[1] - (1) Semmelweis University, Department of Genetics, Cell- and Immunobiology, Budapest, Hungary. (2) Heim Pal Children's Hospital, Budapest, Hungary. (3) Polyclinic, Hospitaller Brothers of St. John of God, Budapest, Hungary.

Histamine is a key regulator of the immune system, increased histamine production is a well known feature of many allergic diseases. Several lines of evidence suggest the role of histamine in T cell activation: T cells express both type 1 and type 2 histamine receptors, histamine inhibits the production of Th1 cytokines such as IL-2 and interferon-ó (IFN) and enhances the secretion of Th2 cytokines. IFN-ó was previously shown to regulate nitric oxide (NO) production. NO is a multifunctional intracellular and intercellular messenger, NO mediates lymphocyte proliferation differentiation and apoptosis. Our previous work indicates that NO is necessary for effective T cell activation.
To study the role of histamine in T cell activation, we investigated T cell signal transduction in histidine decarboxilase knockout (HDC-KO) and wild type mice.
Splenocytes from wild type and HDC-KO mice were isolated following *in vivo* stimulation with complete Freund's adjuvant. NO, cytoplasmic Ca^{2+} concentrations and reactive oxygen intermedier (ROI) levels were determined by flow cytomerty. IFN-ó mRNA level was measured by RT PCR.
Increased INF-ó mRNA level of HDC-KO splenocytes was associated with a markedly increased (2.5 fold, p=0,00086) NO production, compared to splenocytes of wild type animals. Histamine treatment decreased NO production of both wild type and KO splenocytes (p=0,01, p=0,045 respectively). Activation of T cells through the TCR initiates a biphasic elevation in cytosolic Ca^{2+} concentration, a rapid initial peak observed within seconds and a plateau phase lasting up to 48 h. In response to Con-A stimulation, rapid Ca^{2+} fluxing was diminished ($p<0.05$) while the plateau phase was increased (p=0.0024) in HDC-KO T cells. T cell activation-induced NO and superoxide signals were similar in HDC-KO and wild type mice (p=0.45; p=0.23 respectively).
Our present data support the effect of histamine on T cell signal transduction.