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High level of interleukin-10 in serum after therapy is characteristic of prostate carcinoma patients with high Gleason score, high tumor volume and present peritumoral infiltration

Visok nivo interleukina-10 u serumu nakon terapije karakterističan je za bolesnike sa karcinomom prostate koji imaju visok Gleason gradus, veliki volumen tumora i prisutnu peritumorsku infiltraciju

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Abstract

Background/Aim. Recent data imply the significance of certain cytokines in the appearance and development of prostate cancer (PC), as well as their association with pathohistological and/or clinical characteristics of PC. The aim of this study was to examine the relationship between the IL-10 concentration with histopathological and clinical characteristics of PC patients. **Methods.** IL-10 concentration was determined in serum, urine and prostate massage secret (pms) samples of 88 CP patients (initially and after therapy), 20 benign prostatic hyperplasia (BPH) patients and 15 healthy controls. **Results.** Compared to BPH and control groups, PC patients had the highest average serum IL-10 concentration. Interestingly, BPH patients demonstrated the highest concentration of IL-6 in urine and pms samples. Also, patients with G3 gradus and with the highest Gleason score (4 + 4) demonstrated the highest IL-10 serum level. PC patients without any histopathological sign of tumor invasion had a significantly increased serum IL-10, either before or after the therapy, compared to the patient group with evident invasion of tumor cells. The therapy induced different IL-10 profile in serum and urine samples. After the therapy, there was a clear significant IL-10 increase in serum of patients with unfavorable Gleason score (4 + 4), with present infiltration of tumor cells in peritumoral tissue (lymphatic, vascular and combined) and in patients with high tumor volume. **Conclusion.** PC patients without any histopathological signs of tumor invasion before the therapy have significantly increased serum IL-10 concentration compared to those with the signs of tumor invasion. There is a clear dissociation of IL-10 values between a serum sample and local, urine and pms samples from a particular patient. After the therapy, high IL-10 serum concentration is present only in patients with high Gleason score, present infiltration of peritumoral tissue and high tumor volume.

Key words:
interleukin-10; interleukins; neoplasm grading; prostate neoplasms; prostatic hyperplasia.

Apstrakt

Background/Aim. Skorašnji podaci ukazuju na značaj pojedinih citokina u pojavi i razvoju karcinoma prostate (KP), kao i na njihovu udruženost sa patohistološkim i/ili kliničkim karakteristikama KP. Cilj studije bio je da se ispita udruženost koncentracije interleukina-10 (IL-10) sa patohistološkim i kliničkim karakteristikama bolesti kod bolesnika sa KP. **Metode.** Koncentracija IL-10 određivana je u uzorcima seruma, urina i *prostate massage secret* (pms) kod 88 bolesnika sa KP (inicijalno i posle terapije), 20 bolesnika sa benignom hiperplazijom prostate (BHP) i 15 zdravih osoba (kontrolna grupa). **Rezultati.** U poređenju sa bolesnicima sa BPH i kontrolnom grupom, bolesnici sa KP imali su najveće serumske vrednosti IL-10. Interesantno, bolesnici sa BHP imali su najviše koncentracije IL-10 u urinu i uzorcima pms. Takođe, bolesnici sa G3 gradusom i najvećim Gleason skorom (4 + 4) imali su najveće serumske vrednosti IL-10. Bolesnici sa KP bez ikakvog patohistološkog znaka invazije tumora, u odnosu na bolesnike sa evidentnom invazijom tumorskih ćelija, imali su značajno povećane serumske vrednosti IL-10, kako pre, tako i posle terapije. Terapija je indukovala različite profile IL-10 u uzorcima seruma i urina. Nakon terapije detektovali smo značajno povišenje serumskih vrednosti

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IL-10 kod bolesnika sa nepovoljnim Gleason skorom (4 + 4), prisutnom infitracijom tumorskih ćelija u peritumorskom tkivu (limfnom, vaskularnom ili kombinovano) i u grupi bolesnika sa visokim volumenom tumora. **Zaključak.** Bolesnici sa KP bez patohistoloških znakova invazije tumora pre terapije imaju značajno povišene vrednosti serumskog IL-10 u odnosu na grupu bolesnika sa dokazanom invazijom. U našem istraživanju postoji jasna disocijacija vrednosti IL-10 u uzorcima seruma, urina i pms pojedinačnog bolesnika. Posle terapije, visoka koncentracija IL-10 u serumu prisutna je samo kod bolesnika sa visokim Gleason skorom, prisutnom infiltracijom peritumorskog tkiva i visokim volumenom tumora.

Ključne reči:
interleukin-10; interleukini; neoplazme, određivanje stadijuma; prostata, neoplazme; prostata, hipertrofija.

Introduction

Prostate cancer (PC) is one of the most common malignant tumors in male population, and the most frequent among the population above the age of 50 [1]. Prostate tissue samples of the PC patients often demonstrate signs of chronic inflammation, with abundant inflammatory cellular infiltrate composed of T lymphocytes, B lymphocytes, macrophages, neutrophils and mastocytes. Epithelial prostate cells (both androgen-dependent and independent) influence the growth and differentiation of both normal and cancer cells. It has been shown that the epithelial prostate cells produce proinflammatory cytokines [2], which suggests that cytokines are associated with the pathophysiology of the prostate cancer [3]. In healthy people, immune system components specifically recognize and eliminate tumor cells. Tumor infiltration with T lymphocytes, natural killer (NK) cells and/or NKT cells should represent favourable response, i.e. a positive outcome of the treatment of a malignant disease [4]. However, chronic, alternative and uncontrollable activation of immune cells (macrophages, neutrophils and mastocytes) has a protumoral role rather than a defensive one, thus enabling the tumor to avoid the protective immune response, to further grow and to metastasize [5, 6]. Cytokines produced from tumorous cells and infiltrating immune cells are important factors which enable the survival of tumorous cells and their growth in spite of the existence of tumor specific response. So far, several studies focused on cytokines determined in the blood samples, prostate exprimate and urine of the patients with PC [3, 7–11]. The results of these studies pointed to the possible association of cytokines interleukin (IL)-4, IL-1α, IL-1β, IL-6, tumor necrosis factor alpha (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN-γ), IL-2, IL-8, IL-10, IL-12, IL-1RA, IL-4, IL-12, MIC1, IL-5, hepatocyte growth factor (HGF), IL18BP, intercellular adhesion molecule 1 (ICAM-1), IL-17, NT-3, GITR and epithelial neutrophil activating peptide-78 (ENA-78) with the appearance, growth, histopathological and/or clinical characteristics of the PC. The aim of our study was to analyze the IL-10 concentration in the PC patient samples [serum, urine, prostate massage secret (pms)] and to estimate their association with clinical and histopathological parameters of the disease.

Methods

The research was conducted at the Clinic for Urology, Institute of Radiology, and the Institute for Medical Research at the Military Medically Academy in Belgrade, after it had been approved by the Military Medical Academy Ethical Committee. Eighty eight men above 18 years of age were included in this prospective clinical observational case-control study. All patients were confirmed as PC after the initial screening of infiltrative change in prostate [digit rectal examination of prostate (DRP), elevated prostate-specific antigen (PSA)] and positive histopathological confirmation of tissue sample taken with diagnostic biopsy. Control groups consisted of 20 men with benign prostatic hyperplasia (BPH) and 15 healthy men (regular physical examination) that had never suffered from active/chronic genitourinary disease or any malignant disease. Tumorous tissue samples for further histopathological analysis were obtained during the surgical procedure of the PC patients who had undergone the surgery. Blood and urine and samples were obtained at the control examinations of these patients (before and two months after the surgery) Pms samples were obtained after the prostate massage during DRP. The existence of other malignant diseases, autoimmune diseases, kidney diseases, bladder diseases and the consumption of medications which affect hematopoiesis were the criteria for the exclusion from the research. All the subjects included in the study signed an informed consent form, approved by the Military Medical Academy Ethical Committee. According to the National Guidelines for Good Clinical Practice for PC, beyond DRP, all patients were submitted to bone scintigraphy. Histopathological analysis of the tumor tissue included determination of histological type, tumor volume, Gleason score [12], Gleason differentiation degree, histological grade, existence of lymphovascular and/or perineural tumor invasion and pathological tumor, nodus, metastasis (TNM) stage. The clinical stage of the disease was determined according to the 8th edition TNM classification [13].

PSA serum values were determined with a commercial test, ABBOTT ARCHITECT *i*System analyzer PSA assay. IL-10 cytokine concentration in all investigated samples was determined using a commercial kit for cytokine concentration determination (YSL flow multiplex cytokine test kit) with the flow cytometer (Beckman Coulter FC500).

All statistical analyses were done using GraphPad Prism 5 version 5.01 software. Unmatched comparisons between groups were done using Student's *t*-test and Mann-Whitney test. For unmatched comparison of more groups One-way ANOVA test with additional Bonferroni test was used. To estimate statistical significance of relationships between different parameters Pearson's and Spearman's correlation tests were used. The results were presented as mean ± standard deviation (SD).

Results

PC patients have the highest average serum IL-10 concentration

We have investigated IL-10 concentration in various samples of all groups, healthy control men (C), and the BPH and PC patients (Table 1). According to our investigation design, since there were no serial samples of the C and BPH groups, only initial values of the PC group (0th day) were taken into this analysis. Comparison of serum samples demonstrated the highest average IL-10 in the PC group (PC > BPH > C), but without significant difference compared to other participants.

Table 1

Concentration of interleukin-10 (pg/mL, mean ± SD) in various samples of the investigated groups at day 0

Group	serum	urine	pms
C	55 ± 14	36 ± 17 [a]	35 ± 12
BPH	72 ± 22	56 ± 15 [a,b]	49 ± 6
CP	77 ± 69	37 ± 17 [b]	40 ± 23

C – control; BPH – benign prostatic hyperplasia;
PC – prostate cancer;
pms – prostate massage secret;
SD – standard deviation.
[a]BPH/C, $p < 0.05$; [b]BPH/CP, $p < 0.01$.

Interestingly, IL-10 in urine samples demonstrated the highest average value in BPH patients, significantly increased compared to other two groups (BPH > C, $p < 0.05$; (BPH > PC, $p < 0.01$), with no difference between the C and PC groups.

Similarly, local IL-10 concentration found in pms had the highest value in the BPH group, but without significant differences compared to other two groups (BPH > PC > C).

Patients with G3 gradus demonstrated the highest average serum and urine IL-10 concentration

Our patients were divided in four groups according to pathological findings of tumor gradus. Interestingly, patients with gradus 4 (G4) demonstrated the lowest average IL-10 values in serum samples at both time intervals (Table 2). Patients from the G3 group had the highest average IL-10 values, significantly increased compared to the G2 and G4 groups (G3 > G2, 0th day, 6th day; (G3 > G4, 0th day, 60th day) (Table 3). After the therapy (60th day), the G3 group IL-10 serum concentrations were still significantly increased compared to those of the G2 and G4 patient groups (Table 3).

Urine IL-10 values reflected serum concentrations, again with G3 group patients having the highest average IL-10 concentration, with only significant difference between the G3 and G4 groups in the initial time interval (G3 > G4, 0d).

Table 2

Concentration of interleukin-10 (pg/mL, mean ± SD) in prostate cancer samples according to the investigated parameters.

Parameter	Group	Serum		Urine		Prostate massage secret	
		0th day	60th day	0th day	60th day	0th day	60th day
Gradus	G1	72 ± 47	89 ± 40	30 ± 7	37 ± 17	23 ± 9	22 ± 7
	G2	54 ± 37	55 ± 37	34 ± 16	33 ± 15	34 ± 19	30 ± 16
	G3	77 ± 34	89 ± 37	44 ± 19	42 ± 10	54 ± 24	42 ± 22
	G4	29 ± 11	50 ± 31	27 ± 7	30 ± 10	26 ± 5	24 ± 10
Gleason score	3 + 3	62 ± 46	62 ± 40	31 ± 13	37 ± 16	34 ± 22	32 ± 15
	3 + 4	50 ± 21	40 ± 17	56 ± 30	31 ± 11	31 ± 14	27 ± 13
	4 + 3	75 ± 43	71 ± 35	45 ± 21	41 ± 13	56 ± 26	41 ± 25
	4 + 4	42 ± 14	81 ± 32	33 ± 6	39 ± 9	29 ± 7	34 ± 11
Lymphatic invasion	no	72 ± 55	68 ± 46	35 ± 17	36 ± 13	35 ± 22	31 ± 15
	yes	66 ± 28	93 ± 43	43 ± 17	40 ± 13	51 ± 21	40 ± 22
Vascular invasion	no	72 ± 50	70 ± 38	37 ± 16	38 ± 12	39 ± 22	32 ± 15
	yes	53 ± 30	93 ± 75	38 ± 22	32 ± 18	43 ± 27	35 ± 27
Neural invasion	no	95 ± 62	89 ± 55	38 ± 17	39 ± 16	43 ± 27	34 ± 16
	yes	52 ± 24	54 ± 26	36 ± 18	35 ± 14	38 ± 22	32 ± 20
Invasion score	0	90 ± 67	93 ± 58	35 ± 16	37 ± 16	39 ± 26	34 ± 17
	1	51 ± 22	53 ± 26	39 ± 20	37 ± 9	36 ± 22	29 ± 13
	2	70 ± 31	78 ± 23	35 ± 3	42 ± 7	41 ± 13	36 ± 11
	3	53 ± 30	59 ± 38	38 ± 22	32 ± 18	43 ± 27	35 ± 27
Tumor volume (%)	> 15 (high)	48 ± 20	52 ± 10	34 ± 13	33 ± 17	40 ± 20	32 ± 18
	< 15 (low)	76 ± 46	81 ± 52	40 ± 19	36 ± 13	42 ± 25	34 ± 20
PSA (ng/mL)	> 10 (high)	64 ± 42	59 ± 31	38 ± 20	35 ± 14	35 ± 21	29 ± 15
	< 10 (low)	69 ± 46	87 ± 64	39 ± 15	38 ± 14	48 ± 16	33 ± 15
Therapy	RRP	45 ± 35	48 ± 20	33 ± 20	33 ± 15	34 ± 32	34 ± 24
	RTh	61 ± 22	70 ± 36	39 ± 15	38 ± 13	43 ± 15	33 ± 15

PSA – prostate specific antigen;RRP – retropubic radical prostatectomy; RTh – radiation therapy;
SD – standard deviation.

Table 3

Statistical analysis of the differences in interleukin-10 concentrations among investigated PC groups

Parameter	Group	Serum		Urine		Prostate massage secret	
		0th day	60th day	0th day	60th day	0th day	60th day
Gradus	G1/G2						
	G1/G3					***	**
	G1/G4	*					
	G2/G3	*	*			*	
	G2/G4						
	G3/G4	***	**	*		*	*
Gleason score	3 + 3 / 3 + 4			*		*	
	3 + 3 / 4 + 3						
	3 + 3 / 4 + 4						
	3 + 4 / 4 + 3		*			*	
	3 + 4 / 4 + 4		*	*			
	4 + 3 / 4 + 4					*	
Lymphatic invasion	no / yes		**			*	
Vascular invasion	no / yes						
Neural invasion	no / yes	***	**				
Invasion score	0/1	*	*				
	0/2						
	0/3						
	1/2						
	1/3						
	2/3						
Tumor volume (%)	< 15 / > 15	*	*				
PSA (ng/mL)	< 10 / > 10		*			*	
Therapy	RRP / RTh		**			*	

PC – prostate cancer; PSA – prostate specific antigen; RRP – retropubic radical prostatectomy; RTh – radiation therapy.

***p* < 0.05; ****p* < 0.01; *****p* < 0.001 (Mann-Whitney test).**

In urine samples taken before the therapy, prostate massage induced the highest IL-10 values in the G3 group, significantly increased compared to all others groups (G1, G2, G4) (Table 3). After the therapy (60th day), in voided urine there was no significant difference among the investigated groups, while prostate massage again induced a significant IL-10 concentration increase in patients of the G3 group compared to the G1 and G4 patients.

Different association of Gleason score with IL-10 concentration in urine and serum samples

Our patients were stratified in groups according to the updated Gleason score scale [12]. Interestingly, the analysis of serum samples before the therapy (0th day) demonstrated the highest IL-10 values in groups with the lowest Gleason score (3 + 3), together with 4 + 3 group. There were no significant differences between the groups. After the therapy, the highest IL-10 sera level was noticed in the group with the highest Gleason score (4 + 4), significantly higher compared to group 3 + 4.

Analysis of urine samples demonstrated different association of Gleason score and IL-10 concentration. Initially, the highest IL-10 values were detected in groups with intermediate Gleason score (3 + 4, 4 + 3) (Table 2).

At the same time interval, prostate massage induced a significant increase only in 4 + 3 group, at the level significantly elevated compared to all other groups (Tables 2 and 3).

The therapy induced no significant differences between the investigated groups either in urine or pms.

Tumor infiltration of lymphatic, vascular and neural structures and IL-10 concentration in urine and serum samples

Absence of perilymphatic infiltration was associated with insignificant serum IL-10 increase before the therapy (0th day). To the contrary, after the therapy (60th day), the invasion of lymph vessels was associated with a significant average IL-10 value increase compared to noninvasive finding (Tables 2 and 3). Urine IL-10 value was constantly higher in patients with verified tumor perilymphatic infiltration. Patients with positive perilymphatic infiltration had significantly more IL-10 in pms samples initially.

Serum IL-10 according to perivascular infiltration showed same pattern as in perilymphatic infiltration, with higher IL-10 initially in patients without infiltration and lower IL-10 value after the therapy in the same patient group. Again, urine IL-10 was increased in the samples of patients with perivascular infiltration, without statistical significance. IL-10 in pms samples demonstrated insignificant differences.

Perineural infiltration demonstrated completely different association to IL-10 levels. Namely, patients without per-

ineural tumor infiltration demonstrated higher IL-10 average concentration, both in serum and urine samples, and either before or after therapy (Table 2). This difference was significant for serum samples taken initially (0th day) and after the therapy (60th day). IL-10 value in urine and pms samples showed no differences in either group.

Finally, we analyzed our patients according to the sum of all investigated infiltration types (invasion score – Table 2). According to this organization, patients without any sign of tumor invasion had significantly increased serum IL-10, either before or after the therapy (Table 3). The investigation of urine and pms IL-10 concentrations did not demonstrate any significant differences.

Patients that had more than 15% of tumor in prostate tissue (high volume) demonstrated significantly increased average IL-10 concentration in serum samples, both initially (0th day) or at the second time interval (60th day) (Tables 2 and 3). The analysis of IL-10 concentrations in urine and pms samples showed insignificant differences between these two groups of patients.

Initial serum IL-10 concentration (0th day) demonstrated similar values in both groups of patients (Table 2), while 60 days after therapy significant IL-10 increment was noted in the high PSA group (Table 3). Both groups demonstrated similar IL-10 values in urine samples. The group with high serum PSA level showed increased average IL-10 in pms samples before the therapy.

The comparison of serial samples of the investigated PC patients after/before the therapy demonstrated increased average IL-10 values in serum according to all investigated parameters (60th/0th day, Table 4). There was a clear significant increase in sera of patients of unfavorable Gleason score (4 + 4), with present infiltration of tumor cells in peritumoral tissue (lymphatic, vascular and combined) and in patients with high tumor volume. Interestingly, local urine IL-10 demonstrated different profile. Among the PC patients, after the therapy, IL-10 was significantly increased only in the Gleason score 4 + 4 group and significantly decreased in the PC vascular and/or combined peritumor invasion, as well as in patients with high tumor volume. Similarly, pms samples demonstrated significant increase after the therapy only in the Gleason score 4 + 4 group and significant decrease in the G3 patients, those with present peritumor invasion (any kind), high tumor volume, high serum PSA and in patients with RTh.

Discussion

Role of IL-10 in benign prostatic inflammation is far from clear. Vignozzi et al. [14] investigated cytokine profile produced from primary human prostatic smooth muscle cell lines derived from six patients out of 42 investigated with BPH. Although all BPH patients demonstrated intraprostatic inflammation of some grade, they found clear indirect correlation between inflammation and testosterone level, with the most intensive inflammation in severe hypogonadal patients. *In vitro*, prostatic stromal cell lines produced spontaneously significant amount of IFN-γ, IL-12, IL-6, IL-8, CXCL-10,

Table 4

Comparison of interleukin-10 concentrations in serial samples of prostate cancer patients, 60th/0th day, according to the investigated parameters

Parameter	Group	Samples		
		serum	urine	pms
Gradus	G2	=	▼	▼▼
	G3	▲	▼	▼▼▼
Gleason score	3 + 3	=	▲	▼
	3 + 4	▲	▼	▼
	4 + 3	▼	▼	▼▼▼
	4 + 4	▲▲▲	▲▲	▲▲
Lymphatic invasion	absent	▼	▲	▼
	present	▲	▼	▼▼
Vascular invasion	absent	▼	▲	▼
	present	▲	▼▼▼	▼▼
Neural invasion	absent	▼	=	▼
	present	▲	▼	▼
Invasion score	absent	▼	▲	▼
	1	▲	▼	▼
	2	▲	▲	▼
	3	▲	▼▼▼	▼▼
Tumor	< 15% (Hi)	▼	▼	▼▼▼
Volume	> 15% (Low)	▲	▼	▼▼▼
PSA	< 10 ng/mL	▼	▼	▼
	> 10 ng/mL	▲	=	▼▼▼
Therapy	RRP	▲	=	=
	RTh	▲	▼	▼▼▼

PSA – prostate specific antigen; RRP – retropubic radical prostatectomy; RTh – radiation therapy.
▲ increased; ▼decreased; ▲$p < 0.05$; ▲▲$p < 0.01$; ▲▲▲$p < 0.001$; = not significant (Wilcoxon test).

monocyte chemoattractomil protein-1 (MCP-1) and basic fibroblast growth factor (bFGF). Simulation of infection or further inflammation, with exogenously added lipopolysaccharide (LPS) or TNF-α, dose dependently increased this production several times. Additionally, prostatic stromal cell lines exhibited an antigen presenting function, because their co-culture with $CD4^+$T cell clones induced significant increase of inflammatory mediators (TNF-α , IL-1β, MIP-1α, and MCP-1) and importantly, inhibited production of IL-10. Dihydrotestosterone treatment of prostatic stromal cell lines significantly reduced response to TNF-α or LPS stimulation, significantly reduced $CD4^+$T cell clones proliferation and production of proinflammatory mediators, but increased IL-10 production. Authors concluded that activation of prostate cell lines *via* androgen receptors attenuates inflammation and increase IL-10 production, diminishing autoimmune and inflammatory processes. Our BPH patients demonstrated high IL-10 values in all samples, serum, urine and prostate massage secret, with average urine IL-10 concentrations significantly higher than those in the C and PC groups. Since all our BPH patients had physiological testosterone level, it is attractive to speculate that locally produced high IL-10 found in urine and pms could be physiological, testosterone driven response aimed to control underlying prostatic inflammation.

Data concerning IL-10 in PC is controversial, indicating both pro- and antitumor activity. Considering its anti-inflammatory function, IL-10 is potent local and systemic negative regulator of T lymphocyte mediated response, enabling tumor cells with opportunity to escape from host immune surveillance. On the other hand, there are i*n vitro* and *ex vivo* data reporting that IL-10 exerts anti tumor activity by inhibiting tumor vessel formation and metastasis. Majority of data concerning IL-10 and PC came from genetic studies, based on the notion of significant genetic influence in PC and hypothesis that variations in genes that regulate inflammation could differentially affect risk inherited for this type of tumor. Practical significance of IL-10 gene polymorphism is represented by argument that particular genotype is associated with potential to produce less or more IL-10. Turner et al. [15] were the first who demonstrated that healthy persons that carry GG alleles for IL-10 at 1,082 position have the capacity to produce significantly more IL-10 than those who are carriers of AA allele. Namely, *in vitro* lymphocyte cultures from GG-1,082 persons stimulated with concanavalin A produced more IL-10 compared to AA-1,082 cultures. In different model (whole blood sample stimulated with LPS) IL10R2 / IL10G14 haplotype produced significantly more IL-10 than IL10R3 / IL10G7 haplotype [16].

Generally, the results of IL-10 polymorphism studies are still confusing, ranging from no significant association [17–24] to association of particular alleles in PC patients [15, 25–36]. Several of these studies that confirmed association of particular IL-10 polymorphism with PC reported also a significant correlation of GG-1082 gene alleles with low grade tumors [18, 26, 28, 32, 34, 37], absence of bone metastasis [30] or low recurrence rate [31]. Taken together, these data indicate that genetically determined low IL-10 producers among PC patients more frequently demonstrate high grade tumors, higher Gleason score or more aggressive disease course. In our patients, the G3 group demonstrated the highest average IL-10 value, either in serum, urine or pms sample, both before and after therapy. Surprisingly, patients with G4 grade showed the lowest average IL-10 concentration.

First data that associate Gleason score and IL-10 were from study conducted twenty years ago, from Stearns et al. [38] who investigated effects of IL-10 on carcinoma prostate cell lines on microvasculature formation. They established cell lines from PC patients either with high or low Gleason stage. Cell lines originating from more advanced PC (higher Gleason score) induced formation of significantly more microvessels from human bone marrow endothelial cells (HBMCE-1 cells) in three dimensional gel model. All cell lines produced significant amounts of MMP2 and MMP9, especially those originating from high Gleason tumors. They demonstrated that IL-10 treatment of cell lines significantly decreased MMP secretion and microvasculature formation, underlying the significance of locally produced IL-10 in PC. Generally, there are few studies that investigated Gleason score and IL-10 association in PC patients. In the study of 120 PC patients, Horvat et al. [37] demonstrated significant association of PC patients with IL-10 -1,082 AA haplotype and high Gleason score, more than 7. This haplotype is generally considered as low IL-10 producer. Similar findings came for study of Liu et al. [39] in Chinese population, that demonstrated significant association of gene alleles that are distinguished for high IL-10 production (G -1082, C -819, C -592) with low Gleason score. Immunohistochemical study of Cardillo and Ippoliti [40] demonstrated no association between Gleason score and IL-10 presence and positivity (in tumor stroma and epithelium), although they found correlation of IL-10 positivity with tumor grade and TNM scoring. Hu et al. [41] investigated presence of tumor associated macrophages (TAM) and alternatively activated macrophages (AAM) in PC tissue with pathological and clinical parameters. They found that $CD68^+$ TAM was significantly more present in tissue of PC patients with metastasis, that AAM were significantly more present in tumors of patients with higher grade, higher Gleason score, and serum PSA level. According to this group, these macrophages were the dominant cellular source of IL-10, indicating that patients with the highest Gleason score will have the highest IL10 value. In our PC patients stratified according to Gleason score, the lowest average IL-10 was in groups with the smallest (3 + 3) or the highest (4 + 4) Gleason score, in all kind of samples before therapy. Interestingly, when we analyzed samples from every particular patient after/before therapy (Table 4), only patients with the highest Gleason score (4 + 4) demonstrated significant increase, either in serum, urine or pms samples. In second sample of our patients, since tumor tissue was reduced by therapy, local IL-10 production in urine and pms samples probably originate from other cell type, most probably TAM, AAM and lymphocytes, as considered in study of Hu et al. [41].

Generally, increased IL-10 is associated with the higher capacity of tumor cells for migration and invasion, at least in

melanoma[42], neuroblastoma[43], hepatocellular carcinoma [44] and non-small-cell lung cancer [45]. Newer data suggest that increased expression of IL-10 could have even anti tumor effects, as documented in breast cancer patients, showing significant correlation with favorable clinical parameters as well as disease free interval [46]. Twenty years ago, Stearns and Wang [47] demonstrated anti tumor properties of IL-10 at prostate carcinoma, both experimentally and *in vitro*. They showed that IL-10 transfection of human PC cell line given to severe combined immunodeficient (SCID) mice suppressed tumor growth and formation of metastasis, and prolonged animal survival. In the next study, they demonstrated that IL-10 treatment significantly suppressed angiogenesis induced by of PC cell lines [38]. In recent study, Yu et al. [48] investigated pro- and antitumor effects of IL-3, IL-6, IL-11, IL-10 and IL-24 on testosterone sensitive and insensitive PC cell lines. Contrary to other tested cytokines, IL-10 and IL-24 suppressed proliferation, growth, migration and invasion, inhibited expression of CD44, SOX2 and ABCG2, and increased sensitivity to docetaxel treatment. Author concluded that IL-10 exert significant antitumor effects on PC cell lines. Analysis of IL-10 in our study demonstrated significantly increased average value only in those PC patients without any infiltration (lymphatic, vascular or neural), with the lowest infiltration score (Table 2) and only in serum samples both before and after therapy. In other words, high serum IL-10 value was significantly associated with absence of invasion in our PC patients. The simplest interpretation could be in line with antitumor effects presented by Yu et al. [48]. But, when we analyzed IL-10 in serial samples of particular patients (after/before therapy, Table 4), we found significantly increased serum concentrations of IL-10 after therapy in patients with lymphatic, vascular or combined infiltration. We can only speculate that this invasion associated IL-10 increment could be either a consequence of T lymphocytic activity as a attempt of tumor spread control, or reflection of TAM/AAM activity, as a pro tumor setting of further disease recurrence.

Among rare studies that associated PSA level to cytokines, Dwivedi et al. [49] investigated IL-18 and IL-10 serum concentrations together with PSA values in almost 150 PC patients, BPH and control subjects. They found that serum IL-10 concentration directly followed PSA value, being the highest in PC patients with T3 and T4 stage, and also in patients with evident clinical progression. Tazaki et al. [3] studied values of Th1 (IL-12, IFN-γ, IL-2), Th2 (IL-4, IL-5, IL-6, IL-10), inflammatory (IL-1β, TNF-α) cytokines and IL-8 in serum samples of PC patients, with limited, advanced or metastatic form of disease [51]. They found significant increase of IL-10 in patients with disseminated disease and with cachexia compared to patients with limited disease and controls. Although informative, these results must be taken with reserve because of small number of investigated patients.

Conclusion

We consider as our key result that PC patients without any signs of tumor invasion (lymphatic, vascular, neural) before therapy have significantly high serum IL-10 compared to those with signs of tumor invasion. There is a clear dissociation of IL-10 value between a serum sample and local, urine and pms samples from a particular patient. This is especially noted after therapy in patients with high grade and high Gleason score, present infiltration and high tumor volume, with increase of serum IL-10 and decrease of urine/pms IL-10. Average IL-10 concentration does not have needed specificity and sensitivity (ROC curves not shown), but evaluation of its value in serial serum samples (after/before therapy) demonstrated significant association of increased IL-10 with advanced disease.

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