Predilection to sepsis, acute tissue infections and delayed infected wound healing may depend on genetic polymorphisms

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Abstract

Most published studies on infections and patients' genetic polymorphisms are dealing with sepsis. Only few analyze the genetic predilection to less fulminant inflammatory processes as acute circumscribed organ or tissue infections and infections causing delayed healing of large wounds. There is only a quantitative difference between these three conditions in the host immune reaction regulated by the same mechanism, thus, the genetic basis should be common. We decided to study the polymorphisms of selected allele of cytokines and TLRs at 9 polymorphic sites in randomly selected groups of patients displaying symptoms of sepsis, acute tissue infections and prolonged wound suppuration. Our study provided the following observations: 1) in the entire group of patients presenting systemic and local infections, we found higher frequency of tumour necrosis factor α (TNF-α) G308A GG, TNF-β G525A mutated homozygote AA, and CCR2 G190A mutated homozygote AA than in controls (all $p < 0.0001$). At the TGF-β G25C site there was a low expression of GG compared with the controls ($p < 0.001$). 2) comparison of subgroups of sepsis, acute tissue infections and delayed infected wound revealed more of CD14 C-159T CT, TLR1.2 C2259A GA and C2029T CT in sepsis than other infections but the differences were not significant. There was lack of differences in the subgroups in expression of TNF-α G308A GG , TNF-β G525A heterozygote GA, CCR2 G190A AA, TLR4.1 A1036G AA and TLR4.2 C1336T CC. 3) TNF-α serum levels were higher in all patients compared to controls. The highest values of TNF-α were seen in G308A GA genotypes. They were high in cases with sepsis and acute infections. The TGF-β serum level was not higher in the whole group of patients with infection than in controls, however, in subgroups of sepsis and acute infections increased values were found in T941C CC genotype. Taken together, polymorphism of TNF-β and TNF-β, CD14, TLR2.1, CCR2 and TGF-β genes at certain mutation points may be predisposing to surgical type of infections. No significant differences in investigated polymorphisms were found between sepsis, acute local tissue infections and delayed infected wound healing.

Key words: *polymorphisms, TNF, TGF, CCR2, CD14, TLR, sepsis, infection, wound healing.*

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Introduction

Bacterial infections evoke host inflammatory response ranging from delayed infected wound healing, through abscesses and tissue necrosis to sepsis. The biological mechanism of host response is similar in all these cases and the differences in clinical symptoms are only quantitative. The host immune reaction is a polygenic and complex syndrome characterized by a local and more or less acute systemic inflammatory response. The clinical course and outcomes in the immune response to infection have been shown to be associated with genetic polymorphisms. Functional and association studies involving genetic polymorphisms in essential genes, including Toll-like

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receptors, cytokines, and coagulation factors, have provided important insights into the mechanisms involved in the pathogenesis of infection -induced organ dysfunction [1-3]. So far, more studies have been devoted to sepsis and less attention has been paid to the genetic predilection to acute and chronic local tissue and organ infections and surgical site infection in elective surgery. The advancement of high-throughput single nucleotide polymorphism (SNP) genotyping provides valuable information on the interaction of multiple allelic variants and clinical outcome not only of sepsis but also local infective processes. More precise categorization of patients based on genetic background might lead to individualized targeted treatment.

The usually reported studies of one or two genetic polymorphic sites give a limited insight into the correlation between the genotype and clinical symptoms. This prompted us to study a complex of nine mutation points in a randomly selected group of patients with present and at risk infections admitted to our surgical department. Here we present a genetic association study of:

- sepsis,
- acute local tissue or organ infections, and
- chronically infected wounds with delayed healing with focus on nine single nuclear polymorphisms linked to local and systemic septic conditions.

The genetic polymorphism of:

- TNF-α G-308A and TNF-β G252A,
- CCR2 G190A,
- CD14 C-159T,
- TLR2 G2259A and C2029T,
- TLR4 A1036G and C1336T and
- TGF-β G25C sites was studied.

Levels of TNF-α and TGF-β were measured and correlated with their gene polymorphism.

Materials and methods

Study population

One-hundred-thirty-four patients aged 21 to 78 (median 46) years were included into the study. These were the consecutive cases admitted to the Department of Surgery in the period 2004-2006 because of symptoms of sepsis, acute circumscribed tissue or organ infections and surgical-site infections after major gastrointestinal tract surgery. The study was approved by the Institute's ethics committee. Informed consent was obtained from patients, whenever it was possible, depending on the patient's clinical status.

Table. 1. Number of patients in the study and control groups

Study groups	Number of patients
TOTAL PATIENTS	134
1. Sepsis	36
2. Acute circumscribed infections	46
3. Surgical-site infections	
a. Gastric resection	8
b. Pancreatic resection	21
c. Colon resection	23
CONTROLS	125

Study groups

Patients were divided into three groups: 1) sepsis, 2) acute circumscribed organ or tissue infections, and 3) delayed infected surgical wound healing.

Inclusion criteria for the sepsis group were: 1) systemic infection (clinically suspected infected foci, positive blood cultures), 2) hyperthermia > 38°C, 3) tachycardia > 90/min, 4) tachypnoe > 20/min with need for respiration support, 5) indications for administration of vasopressors, 6) mental disturbances, 7) hypoxia PaO_2 < 280 mmHg at full oxygenation, 8) blood acidosis, 9) disseminated intravascular coagulation.

The group of patients with acute circumscribed tissue or organ infections comprised those with acute necrotizing pancreatitis, gangrenous appendicitis and soft tissue abscesses and necrosis with normal healing process.

The group with delayed wound healing was composed of patients who underwent acute or elective surgery of the gastrointestinal tract as colon perforation, gastric, pancreatic and colon/rectum cancer resection. The criterion of delayed wound healing was abdominal wound infection and partial dehiscence diagnosed on day 7 and hospital stay over 12 days (controls 4-6 days).

The common denominator for all studied groups was documented systemic or local bacterial infection . Some few patients met the criteria of two groups. Five patients displayed sepsis and local infections and the other eight had major surgical site infections and delayed wound healing. The end-point of observations was death or recuperation from sepsis and healing of infected tissue. A control group of 125 blood donors, ethnically matched to the study group, was randomly selected for comparing the studied group data. Numerical data of the studied groups have been shown in Table 1.

Study design

Detection of genetic polymorphisms of 1) TNF-α G-308A and TNF-β G252A, 2) CCR2 G190A, 3) CD14 C-159T, 4) TLR2 G2259A and C2029T, 4) TLR4 A1036G and T1336C, and 5) TGF-β G25C was performed using the PCR technique with appropriate primers. Levels of TNF-α and TGF-β were measured using commercially available Quantikine Human TGF-β-1 kit and Quantikine Human TNF-α kit according to the manufacturer's protocol (R&D Systems, Minneapolis, USA) with ELISA method.

Genotyping

Isolation of DNA

Genomic DNA was isolated from whole blood using NucleoSpin Kit according to the manufacturer's protocol (Macherey – Nagel Dueren, Germany). Quantification of DNA was performed spectrophotometrically on ND-1000 spectrophotometer (Nanodrop Wilmington, USA). Quality of isolating DNA was checked electrophoretically on 1% agarose gel with ethidium bromide.

RFLP-PCR analysis of TNF-α G-308A and TNF-β G252A polymorphism

Polymerase chain reaction (PCR) was performed employing RedTaq polymerase (Sigma-Aldrich St.Louis, USA). An approximately 40 ng of sample DNA was added to a reaction volume of 25 µl containing 2.5 µl 10 × buffer with $MgCl_2$, 0.5 µl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich St. Louis, USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in the Table 2. The PCR amplification was carried out in a Thermal Cycler (MJ Research Watertown, USA) with following conditions: 95˚C for 5 min, followed by 30 cycles of 94˚C for 30 s, 64˚C for 30 s, and 72˚C for 45 s followed by one elongation step at 72˚C for 10 min. 10 µl of the PCR products were incubated for 2 h with 1 U Nco I (Roche Mannheim, Germany) in a total volume of 20 µl at 37˚C.

RFLP-PCR analysis of CCR2 G190A and CD14 C-159T polymorphism

Polymerase chain reaction was performed employing Expand Long Templete polymerase (Roche Mannhaim, Germany). An approximately 40 ng of sample DNA was added to a reaction volume of 25 µl containing 2.5 µl 10 × buffer with $MgCl_2$, 0.5 µl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich, St.Louis ,USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in the table. The PCR amplification was carried out in a Thermal Cycler (MJ Research Watertown, USA) with following conditions: 95˚C for 5 min, followed by 30 cycles of 94˚C for 30 s, 58˚C for 30 s, and 72˚C for 30 s followed by one elongation step at 72˚C for 10 min. 10 µl of the CCR2 G190A PCR products were incubated for 3 h with 0.25U Fok I (Roche, Mannheim, Germany) in a total volume of 20 µl at 37˚C. 10 µl of the CD14 C-159T PCR products were incubated for 2 h with 0.5U Mae III (Roche, Mannheim, Germany) in a total volume of 20 µl at 37˚C.

RFLP-PCR analysis of TLR2 G2259A and C2029T polymorphism

Polymerase chain reaction was performed employing FastStart Taq DNA polymerase (Roche Mannheim, Germany). An approximately 40 ng of sample DNA was added to a reaction volume of 25 µl containing 2.5 µl 10 ×

Table 2. Primers used for polymerase chain reactions

No.	Gene polymorphism	Primer sequence
1.	TNF-α G-308A	F 5'AGGCAATAGGTTTTGAGGGCCAT3' R 5'TCCTCCCTGCTCCGATTCCG 3'
2.	TNF-β G252A	F 5'GGTTTCCTTCTCTGTCTCTGACTCTCC 3' R 5'GAGAGAGATCGACAGAGAAGGGGAC 3'
3.	CCR2 G190A	F 5' GAAAGTGGATTGAACAAGGAC 3' R 5' CAGGTTGAGCAGGTAAATGT 3'
4.	CD14 C-159T	f 5' GTGCCAACAGATGAGGTTCA 3' r 5' CGCAGCGGAAATCTTCATC 3'
5.	TGF-β G25C	F 5'-TTC AAG ACC ACC CAC CTT CT 3' R 5'-TCG CGG GTG CTG TTG TAC A
6.	TLR2.1 G2259A	F 5' GCCTACTGGGTGGAGAACCT 3' R 5' GGCCACTCCAGGTAGGTCTT 3'
7.	TLR2.2 C2029T	F 5' GCCTACTGGGTGGAGAACCT 3' R 5' GGCCACTCCAGGTAGGTCTT 3'
8.	TLR4.1 A1036G	F5'GATTAGCATACTTAGACTACTACCTCCATG3' R 5'GATCAACTTCTGAAAAAGCATTCCCAC 3'
9.	TLR4.2 C1336T	F5'GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA3' R5'CCTGAAGACTGGAGAGTGAGTTAAATGCT 3'

buffer with $MgCl_2$, 0.5 μl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich St.Louis, USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in Table 1. The PCR amplification was carried out in a Thermal Cycler (MJ Research Watertown, USA) with following conditions: 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s followed by one elongation step at 72°C for 10 min. 10 μl of the PCR products were incubated for 2 h with 1U Aci I (Fermentas, Burlington, Canada) in a total volume of 20 μl at 37°C.

RFLP-PCR analysis of TLR4 polymorphism A1036G

Polymerase chain reaction was performed employing FastStart Taq DNA polymerase (Roche, Mannheim, Germany). An approximately 40 ng of sample DNA was added to a reaction volume of 25 μl containing 2.5 μl 10 × buffer with $MgCl_2$, 0.5 μl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich St.Louis, USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in the table. The PCR amplification was carried out in a Thermal Cycler (MJ Research Watertown, USA) with following conditions: 95°C for 4 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by one elongation step at 72°C for 10 min. 10 μl of the PCR products were incubated for 1.5 h with 1 U NcoI (Fermentas, Burlington, Canada) in a total volume of 20 μl at 37°C.

RFLP-PCR analysis of TLR4 polymorphism C1336T

Polymerase chain reaction was performed employing FastStart Taq DNA polymerase (Roche, Mannheim, Germany). An approximately 40 ng of sample DNA was added to a reaction volume of 25 μl containing 2.5 μl 10 × buffer with $MgCl_2$, 0.5 μl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich St. Louis, USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in the table. The PCR amplification was carried out in a Thermal Cycler (MJ Research, Watertown, USA) with following conditions: 95°C for 4 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by one elongation step at 72°C for 10 min. 5 μl of the PCR products were incubated for 3 h with 2U HinfI (Fermentas, Burlington, Canada) in a total volume of 10 μl at 37°C.

RFLP-PCR analysis of TGF-β polymorphism G25C

Polymerase chain reaction was performed employing FastStart Taq DNA polymerase (Roche, Mannheim, Germany). An approximately 40 ng of sample DNA was added to a reaction volume of 25 μl containing 2.5 μl 10 × buffer with $MgCl_2$, 0.5 μl deoxyribonucleoside trisphosphate mix (Sigma-Aldrich, St. Louis, USA) and 25 pmol of each primer (Oligo, Warsaw, Poland). Primers' sequences are shown in the Table. The PCR amplification was carried out in a Thermal Cycler (MJ Research Watertown, USA) with following conditions: 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 35 s followed by one elongation step at 72°C for 10 min. 10 μl of the PCR products were incubated for 1 h with 1 U MspAI (Promega, Madison, USA) in a total volume of 20 μl at 37°C.

Electrophoretic detection of restriction fragments

Samples was electrophoresed through ultrathin 12.5% polyacrylamid gel (PAGE) (Muliphore II System Amersham Pharmacia Biotech, Uppsala, Sweden) and silver stained (Silver Staining Kit, Amersham Pharmacia Biotech, Uppsala, Sweden). The gels were scanned and analysed by OneDscan Software (Scanlytics).

Statistical analysis

Deviation from Hardy-Weinberg equilibrium was tested using χ^2 goodness fit. Comparison of allele and genotype frequencies for each polymorphism was by Fisher's exact test or χ^2 analysis, and significance was set at $p < 0.05$. For cytokine levels the median values were used. Correlation between detected allell and cytokine levels was carried out.

Results

Genotypes in the entire studied (sepsis, acute circumscribed organ or tissue infections and delayed infected wound healing) and control groups.

The obtained data have been presented in Fig. 1-3. There were differences in genotypes of TNF-α G308A of patients and control subjects. Patients showed higher frequency of GG (46%) and lower of GA (53%) than controls (24 and 74%, respectively) ($p < 0.0002$) (Fig. 1A). Tumour necrosis factor β G525A GG was 3.5%in patients and 0% in controls, GA was less (45%) and mutated AA was more (52%) represented in patients than in controls (81 and 20%, respectively) ($p < 0.0001$) (Fig. 1B). CD14 C159T CC genotype was represented in 20-50% and CT in 50-60% at a similar level to controls (Fig. 1C). There was less of CCR2 GG patients (57%) than healthy subjects (76%) ($p < 0.0001$) (Fig. 2A) TLR2.1 G2259A GG was detected in 30%, GA in 30% and AA in 40-60% (Fig. 2B). TLR2.2 C2029T CC reached 70% whereas CT 30% (Fig. 2C). TLR4.1 A1060G AA was close to 100% (Fig. 3A) as was TLR4.2 C1363T CC (Fig. 3B). No statistically significant differences of the last four genes of patients compared with controls were found . CCR2 G190A GG remained at 60%, GA at 25% and AA at 15%). Neither the TGF-β G25C TT or TC genotypes were detected in patients, whereas the mutated homozygote CC genotype was found in 20% of patients (Fig. 3C). Interestingly,

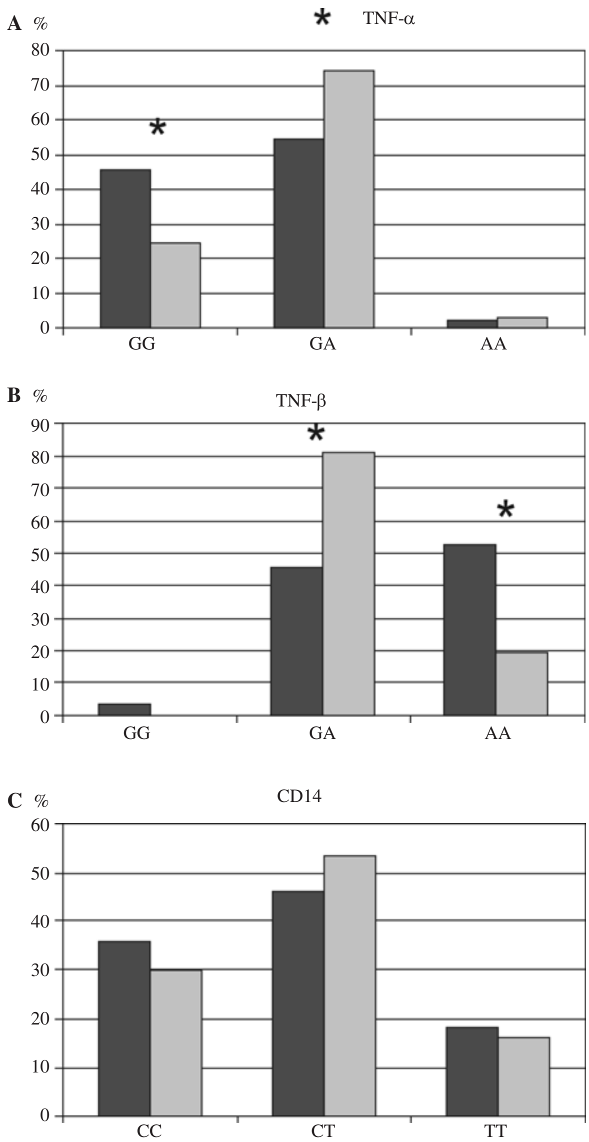

Fig. 1. RFLP-PCR analysis of A – TNF-α G-308A, B – TNF-β G252A and C – CD14 C-159T polymorphism in the whole investigated group. Note that patients with TNF-α G308A showed higher frequency of GG and lower of GA than controls (*$p < 0.0002$). The TNF-β G525A mutated AA was more frequent in patients than in controls (*$p < 0.0001$). CD14 C159T genotypes were represented at similar levels to controls

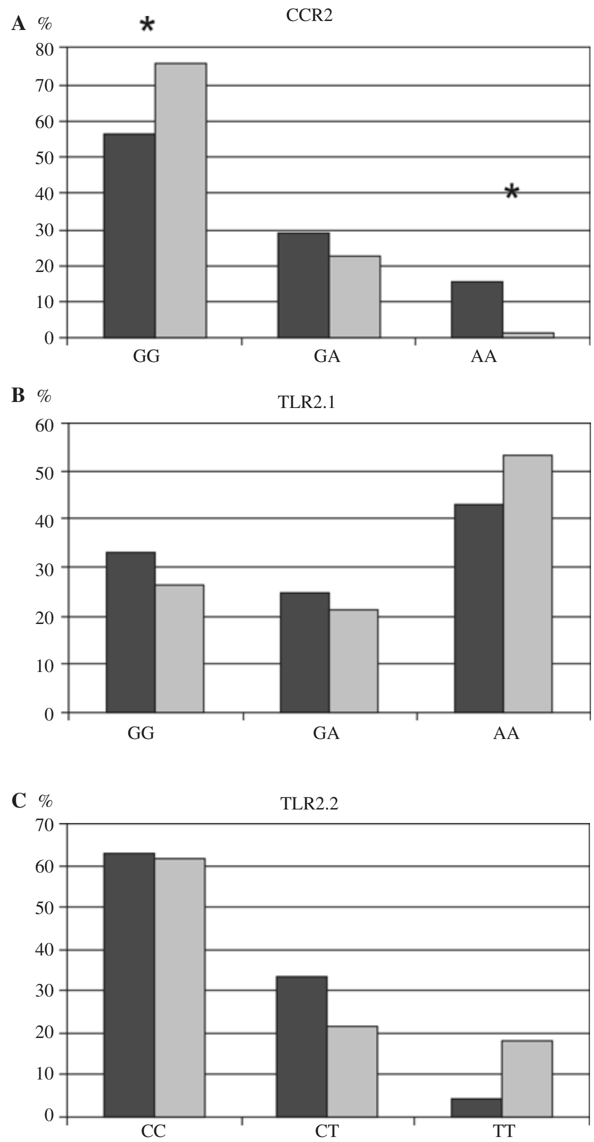

Fig. 2. RFLP-PCR analysis of A – CCR2 G190A, B – TLR2 G2259A and C – C2029T polymorphism in the whole investigated group. There was less of CCR2 GG patients than healthy subjects and more of mutated AA (*$p < 0.0001$). TLR2.1 G2259A GG was detected in 30%, GA in 30% and AA in 40-60%. TLR2.2 C2029T CC reached 70%, whereas CT 30%

another genotype was additionally detected using primer for TGF-β G25C. Around 80% of patients carried this genotype (Fig. 3C).

Genotypes in subgroups of sepsis, acute circumscribed organ or tissue infections and delayed infected wound healing

The data have been presented in Fig. 4-6. The TNF-α G308A GG was represented in all subgroups at similar level, with higher prevalence (39 to 42%) than in controls (23%) ($p < 0.05$) (Fig. 4A). GA genotype was found at similar level in all groups being lower than in controls ($p < 0.05$) (Fig. 4A). The TNF-β G525A GG genotype

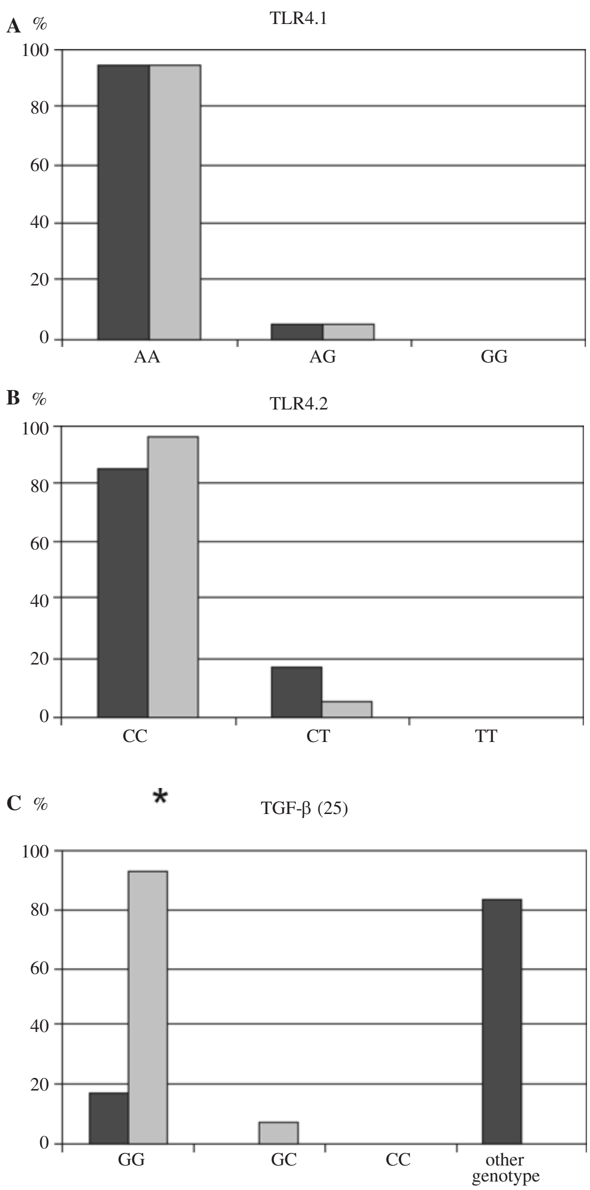

Fig. 3. RFLP-PCR analysis of A – TLR4 A1036G and B – C1336T, and C – TGF-β G25C polymorphism in the whole investigated group. TLR4.1 A1060G AA was close to 100% as was TLR4.2 C1363T CC. Neither the TGF-β G25C GG or GC genotypes were detected, whereas the mutated homozygote CC genotype was found in 20% of patients. Another genotype was additionally detected in Polish population using primer for TGF-β G25C. Around 80% of patients carried this genotype, whereas controls 57%

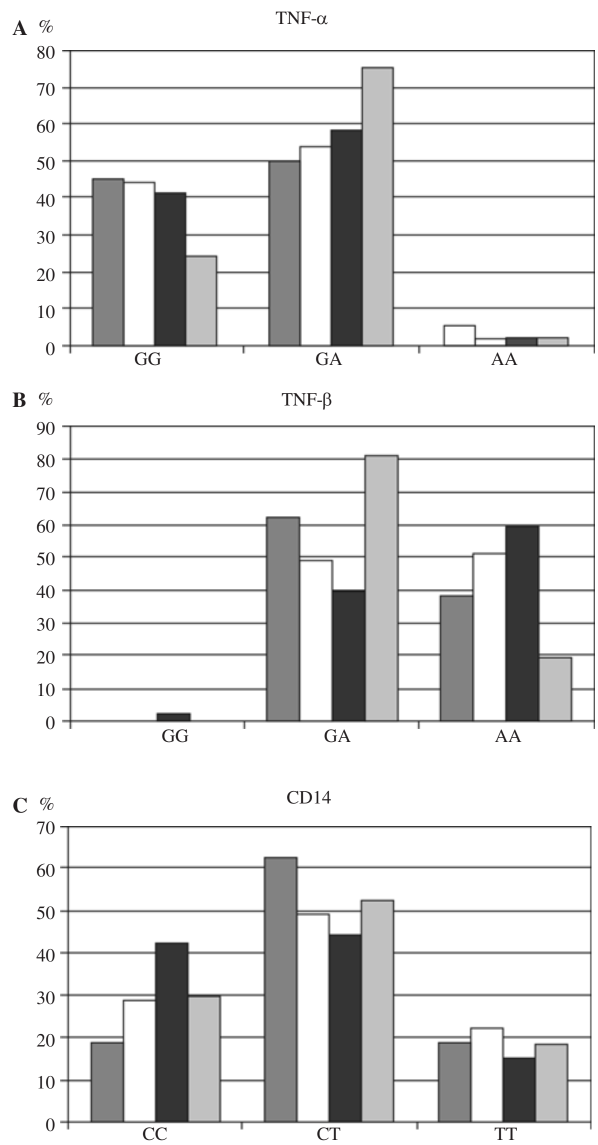

Fig. 4. RFLP-PCR analysis of A – TNF-α G-308A, B – TNF-β G252A and C – CD14 C-159T polymorphism in subgroups. The TNF-α G308A, and TNF-β G525A genotypes were represented in all subgroups at similar level. Differences between sepsis, acute infection and delayed infected healing at CD14 C159T CC and CT were not significant

was low both in patients and controls. The GA genotype was represented at highest values in sepsis, lower in acute and chronic infections (NS). All these genotypes were lower than in controls ($p < 0.05$) (Fig. 4B). The mutated homozygote AA was lower in sepsis than acute and chronic wounds patients but higher than in controls (Fig. 4B). There were differences between sepsis, acute infection and delayed infected healing at CD14 C159T CC (Fig. 4C) but statistically non-significant (NS). There were no differences between patients subgroups at CCR2

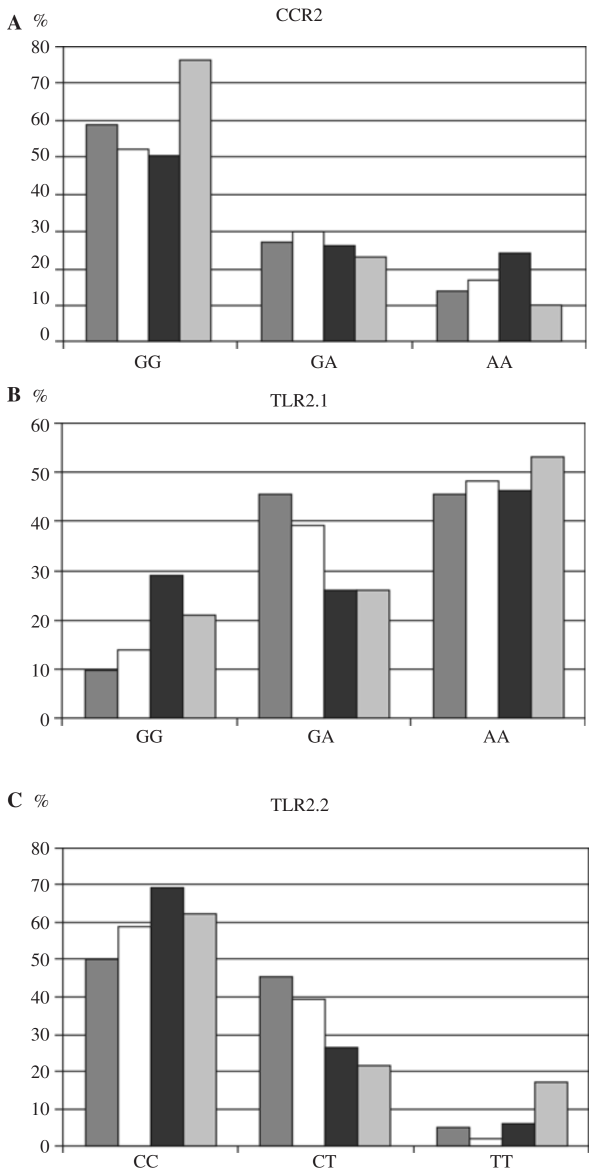

Fig. 5. RFLP-PCR analysis of A – CCR2 G190A, B – TLR2 G2259A and C – C2029T polymorphism in subgroups. There were no differences between patients subgroups at CCR2 G190A. TLR2.1 G2259A GA and TLR2.2 C2029T CT genotypes were higher in sepsis and acute infections than in controls but differences were statistically non-significant

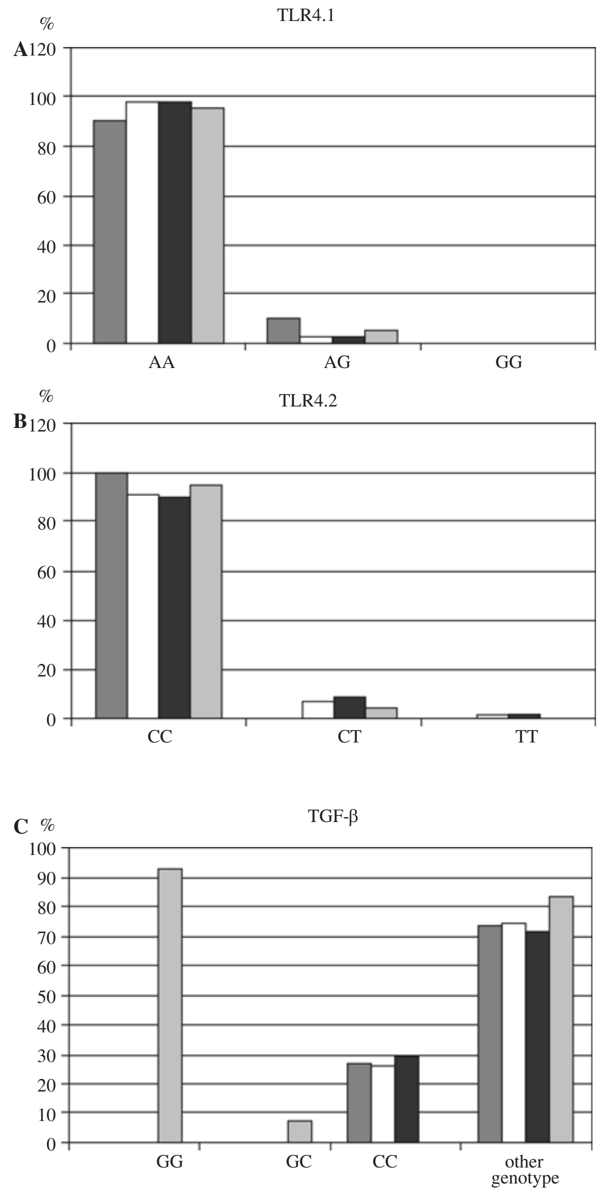

Fig. 6. RFLP-PCR analysis of A – TLR4 A1036G and B – C1336T, and C – TGF-β G25C polymorphism in subgroups. No significant differences were found at TLR4.1 A1060G and TLR4.2 C1363T. In the TGF-β group there were no difference between subgroups. Another genotype was also detected using primer for TGF-β G25C but again without differences in subgroups

G190A, however, data of these subgroups were lower than in controls ($p < 0.05$) (Fig. 5A). TLR2.1 G2259A GA and TLR2.2 C2029T CT genotypes were higher in sepsis and acute infections than in controls (NS) (Fig. 5B and 5C). No significant differences were found at TLR4.1 A1060G (Fig. 6A) and TLR4.2 C1363T (Fig. 6B) in subgroups group of patients and controls. In the TGF-β group, patients expressed the CC genotype (22%) and there were no difference between groups (Fig. 6C). This genotype was not detected in controls. Interestingly, another genotype was also detected using primer for TGF-β G25C. Around 80% of patients in each subgroup carried this genotype without differences in subgroups (Fig. 6C).

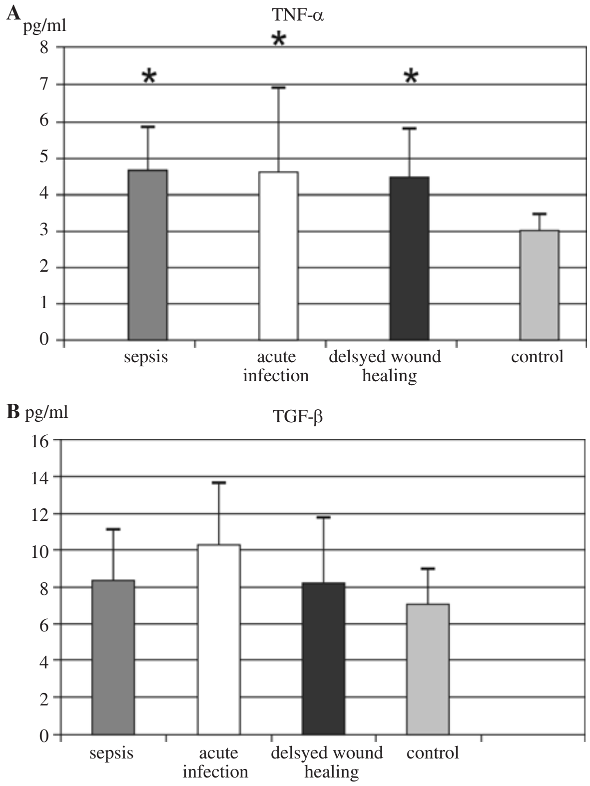

Fig. 7. All investigated patients had TNF-α serum levels higher than controls (*$p < 0.05$)

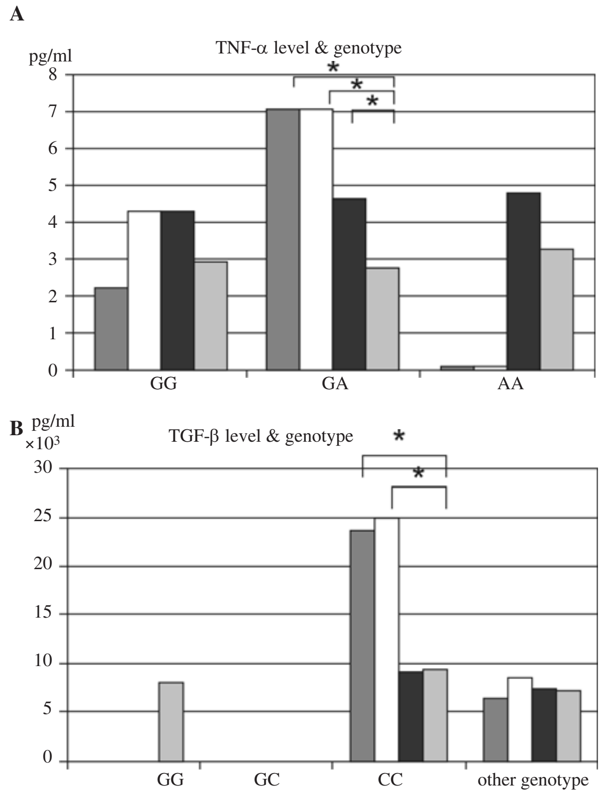

Fig. 8. TNF-α and TGF-β polymorphism and protein levels. A. Patients with sepsis, acute infections and delayed infected wound healing of TNF-α G308A GG genotype had TNF-α levels similar to controls, whereas those with GA had levels significantly higher than controls ($p < 0.05$). Moreover, there was less TNF-α protein in serum in sepsis than acute infection in individuals with GG phenotype. B. The TGF-β level in patients with sepsis and acute infections with mutated homozygote CC was significantly higher than in controls ($p < 0.05$). Patients with sepsis and acute infections with genotype other than the TGF-β G25C had serum levels lower than these of the CC genotype individuals

Genotypes of TNF-α and TGF-β and their cytokine serum levels

All investigated patients had TNF-α serum levels higher than controls ($p < 0.05$) (Fig. 7A). Patients with sepsis, acute infections and delayed infected wound healing of TNF-α G308A GG genotype had TNF-α levels similar to controls, whereas those with GA had levels significantly higher than controls ($p < 0.05$) (Fig. 8A). Moreover, there was less TNF-α protein in serum in sepsis than acute and chronic infection in individuals with GG phenotype (Fig. 8A). The TGF-β level in patients with sepsis and acute infections with mutated homozygote CC was significantly higher than in controls ($p < 0.05$) (Fig. 8B). There was more TGF-β in sepsis and acute than chronic infections group. Patients with sepsis and acute infections with genotype other than the TGF-β G25C had serum levels lower than these of the CC genotype individuals (Fig. 8B).

Discussion

Most of published studies on infections and genetic polymorphisms are dealing with sepsis. Only few analyze the correlation between less fulminant inflammatory processes as circumscribed organ or tissue infections and infections causing delayed healing of large wounds. However, there seems to be only a quantitative difference in the immune reaction between these three conditions whereas the basic mechanism should remain the same. This being so, we decided to study the polymorphisms of selected allele of cytokines and TLRs in a large groups of patients displaying symptoms of systemic and local response to infection irrespective of the advancement of the process. Our study provided the following observations: 1) in the whole randomly recruited group of infected patients there was higher frequency of TNF-α G308A GG and lower of GA, and lower of TNF-β G525A GA and higher of AA than in controls. There was less of CCR2

G190A GG genotype in patients than healthy subjects. The GA genotype was around 20%. CD14 C159C CT was at the level of 50% similar to controls. TLR2.1 G2259A GA and AA were found at 30% and 50%, respectively. TLR2.2 C2029T CT reached 25%. There was a low level of polymorphism in TLR4.1 and TLR4.2. In the TGF-β G25C investigated group patients expressed only the mutated homozygote CC genotype. Interestingly, additional genotype was detected using TGF-β G25C primer expressed in patients, 2) in subgroups of patients with sepsis, acute tissue infections and delayed infected wound healing the TNF-α G308A GG genotype was represented at the same level in all groups. Patients' TNF-β G525A heterozygote GA genotype was higher in sepsis than acute infections and chronic infected wounds. The CD14 C159T CC and TLR1.2 C2259A and C2029T genotypes were higher in sepsis than acute and chronic infections. 3) TNF-α and TGF-β serum levels were higher in all patients compared to controls. The highest values of TNF-α were seen in G308A GA genotypes. They were higher in sepsis and acute than chronic infections. The TGF-β was found higher in T941C CC group of sepsis and acute infections than chronic infected wounds.

The TNF-α promoter gene is in linkage disequilibrium with several HLA alleles that may be involved with the control of TNF-α secretion, or that may be independent risk factor for the development of various forms of sepsis. We found increased frequency of TNF-α G308A GG genotype in 45% of patients, significantly higher than in controls. In the pertinent literature the elispot analysis demonstrated that existence of A allele was associated with higher TNF production compared with G allele [4]. This was not observed in our studies as high TNF-α levels were found both in patients with A and G allele. Stuber and coworkers reported an association study of the TNF-α 308 allele and outcome of 80 postoperative patients with severe sepsis [5]. Mira and coworkers reported an association study of the TNF-α-308 promoter region SNP and septic shock [6]. Others did not find any association between TNF-α, IL-1β, PAI-1, CD14 and TLR4 polymorphisms and outcome of Gram negative sepsis. Waterer also analyzed the TNF-α – 308A: TNF-β-252A haplotype (high secretors of TNF) and found no association between the risk of septic shock and this haplotype [7]. Altogether conflicting results have been reported for TNF-α (G – 308A), which was associated to disease severity and outcome in some but not other studies [6-11]. We found a high percentage of TNF-α GG in all patients but TNF-α protein remained at the control level. It was high in patients with GA genotype. Patients' TNF-β G525A heterozygote GA genotype was higher in sepsis than acute infections and chronic infected wounds. However, the number of patients with sepsis in our study was too low for statistical evaluation and comparison with the reported data.

The TNF-α (lymphotoxin A) is expressed and released by lymphocytes. A TNF-β polymorphism exists at position 252 within the first intron of the TNF-β gene, consisting of a G (TNF-β-252G) on one allele and an A (TNF-β-252A) on the alternate allele. Stuber and co-workers compared non survivors of postoperative severe sepsis with survivors and found that 65% of non survivors were homozygous for the variant (252A) allele compared to 12% of survivors [10]. Another study examined the association between outcome from blunt trauma and the TNF-β-252A polymorphism. The authors stress that the polymorphism may not be directly linked to sepsis susceptibility but may be a marker for another gene in the MHC region [6]. We studied the polymorphism at TNF-β G525A. The GA genotype was less (45%) and AA was more (52%) represented in patients than in controls (81 and 20%, respectively). The GA was found highest in sepsis.

Recently a C to T polymorphism in the promoter region at base-pair – 159 from the major transcription start site (CD-14-159) has been identified that is important in modulating sCD-14 levels [9]. The CD-14-159 T homozygotes had greater circulating sCD-14 levels in the blood. Stimulation of peripheral blood mononuclear cells from the variant homozygotes was associated with increased interferon γ production. Polymorphisms within the CD-14 gene (chromosome 5) and TLR4 gene (chromosome 9) may alter the inflammatory response. A number of SNPs have been identified in the promoter region of the CD14 gene. One polymorphism at – 159 in the promoter has been associated with an increased susceptibility to inflammatory diseases. It has been shown that homozygous carriers of the T allele at – 159 have elevated levels of sCD14. No significant differences were found in genotypes CD14 C159T in our studied experimental and control groups. However, GA was most represented in sepsis. Jensen *et al.* did not find any association between TNF-α, IL-1β, PAI-1, CD14 and TLR4 polymorphisms and outcome of Gram negative sepsis [8-13]. Other studies suggests that the CD14 – 260 polymorphism is not associated with an increased risk of severe sepsis in trauma patients [12]. The CD14 – 260 polymorphism does not affect the CD14 expression of unstimulated circulating monocytes or soluble CD14 plasma levels [13].

Toll-like receptor 2 (TLR2) is a member of the TLR family, which plays a central role in the innate immune response to a wide variety of microorganisms. TLR2 is a signaling receptor that also responds to endotoxin and activates NF-κB [15] TLR2 binds to CD-14 to serve as an endotoxin receptor complex. Interlekin1 receptor-associated kinase is recruited to the TLR2 complex. Intracellular deletion variants of TLR2 fail to recruit IL-1 receptor-associated kinase, impairing endotoxin signaling [16]. The CD-14, TLR4, and TLR2 polymorphisms could be important in determining an individual's response to sepsis [14-19]. TLR2 has a special place among the 10 members of the human TLR family. TLR4 is a member of the TLR superfamily and is a transmembrane receptor with a leucine-rich extracellular domain and an intra-cellular domain with high similarity to the interleukin-1 (IL-1) receptor [20]. TLR-ligand complexes activate signal transduction pathways in both the innate and adaptive immune systems, leading to the release of inflammatory mediators. TLR4 operates in synergy with CD14, a leucine-rich 55 kDa glycoprotein.

No significant differences were found in our studies in the percentage of genotypes TLR2.1 G2259A, TLR2.2 C2029T, TLR4.1 A1060G and TLR4.2 C1363T of patients and controls. There was, however, more GA and CT heterozygotes in sepsis in TLR2.1 G2259A, TLR2.2 C2029T and acute infections than in chronic wounds.

The TGF-β1 is a multifunctional cytokine that plays an important role In modulating cell growth. Although TGF-β1 inhibits the growth of normal epithelial cells, it promotes the proliferation of malignant cancer cells and loss of the growth inhibitory effects of TGF-β1 accompanies the transformation of colorectal adenoma to cancer. The mechanism by which the loss of growth inhibition occurs is unclear. It was found that TGF-β1-509TT and 10Pro/Pro genotypes were associated with an increased risk of advanced colorectal adenoma. It is reasonable to hypothesize that TGF-β1 polymorphisms that increase TGF-β1 serum levels may have a great effect on advanced adenocarcinoma [21-26]. Our group of patients comprised a large number of gastric, pancreatic and colon cancer cases. We found that the TGF-β CC mutated homozygote genotype was represented only in patients but not in controls. Interestingly, another genotype was additionally detected in our ethnic population using primer for TGF-β T941C. Around 80% of patients carried this genotype. Whether these data may be attributed to the presence of cancer cell genotype remains to be elucidated.

The TNF-α serum levels were higher in all patients compared to controls. The highest values of TNF-α were seen in G308A GA genotypes. It was higher in sepsis and acute than chronic infections. The TGF-β was also found higher in G25C CC group of sepsis and acute infections than chronic infected wounds.

Taken together, polymorphism of TNF-α and TNF-β, CD14, TLR2.1 and TGF-β genes at the investigated mutation points may be responsible for the predilection to inflammatory processes manifested by sepsis, acute local tissue infections and delayed infected wound healing. Sepsis may be more dependent on the gene polymorphisms than other studied less fulminant infective processes.

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