TNF-α, TGF-β, IL-10, IL-6 and IFN-γ gene polymorphisms as risk factors for brucellosis

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SUMMARY

Polymorphisms in the regulatory regions of cytokine genes can affect the level of cytokine production, and may be associated with predisposition to infectious diseases as well as different clinical outcomes. The aim of this study was to investigate the association of the polymorphisms of IL-6 (-174), IL-10 (-1082, -819), IFNγ (+874), TGFβ (codon 10, codon 25) and TNFα (-308) genes with brucellosis in terms of susceptibility and resistance to the disease or occurrence of focal complications. A case control study was carried out in 85 patients with brucellosis and 85 healthy controls. We studied the polymorphisms of IL-6, IL-10, IFN-γ, TGF-β1 and TNFα genes, using the polymerase chain reaction with sequence-specific primers.

The IL-10 CT, TGF-β1 codon 10 CC and TGF-β1 codon 25 GG genotypes were significantly more frequent in the patients compared to the controls. The IL-10 CC genotype was higher in the controls than in the patients. In addition, the IL-6 (-174) GG genotype was more frequent in the patients without focal forms, while the GC genotype was more frequent in the patients with focal forms.

Our results showed that polymorphisms of IL-10 (-819) and TGFβ1 codons 10 and 25 were associated with susceptibility or resistance to brucellosis. The IL-6 (-174) GC genotype may be a risk factor for the development of focal complications of brucellosis, whereas the GG genotype may be a protective factor against brucellosis.

KEY WORDS: Cytokines, Gene polymorphism, Brucellosis, Risk factor

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INTRODUCTION

Brucella spp. are gram-negative, facultative and intracellular bacteria that can cause chronic zoonotic infections in humans (Young, 2005). The organism can be transmitted from animals to humans via direct contact with infected animals or with their secretions through cuts or abrasions in the skin. Moreover, it may be transmitted by means of infectious aerosols inhaled or inoculated into the conjunctiva, or by ingestion of unpasteurized dairy products (Young, 2005). The disease exists worldwide, but is especially prevalent in the Mediterranean countries, Middle East, Indian subcontinent and Central and South America (Young, 2005). In Turkey, brucellosis remains a major public health problem and the incidence of brucellosis was 25.65/100.000 population in 2004 (T.C. Sağlik Bakanliği, 2004). *Brucella spp*. survive within a variety of cells, including macrophages, and spread in mononuclear phagocytes to reticuloendothelial sites (Rasouli *et al*., 2007, Orozco *et al*., 2003). This infection activates cell mediated immune reactions (Serre *et al*., 1987, Araya *et al*., 1989). Cytokines help mediate many of the effector phases of the immune and inflammatory responses. Synthesis profiles of cytokines can be considered as either T-helper-cell

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type 1 (Th1) responses promoting cell mediated immunity, and interleukin-2 (IL-2) and interferon gamma (IFNγ) or T-helper-cell-type-2 (Th-2) responses promoting humoral immunity (IL-4, IL-5, IL-6, IL-10), and Th-3 subset characterized by TGFβ (Beutler *et al*., 1989, Bidwell *et al*., 1999). Polymorphisms in the regulatory regions of cytokine genes may not only increase susceptibility to some infectious diseases but also affect the course and prognosis of the disease (Bidwell *et al*., 1999, Bidwell *et al.,* 2001). A few studies report an association between cytokine gene polymorphism and development of brucellosis (Rasouli *et al*., 2007, Bravo *et al*., 2003, Rezazadeh *et al*., 2006, Caballero *et al*., 2000).

The aim of the present study was to investigate the association between cytokine gene polymorphisms and risk of development of brucellosis as well as to determine any possible influence of cytokine gene polymorphisms on the development of focal complications of brucellosis.

MATERIALS AND METHODS

Patients and controls

This study was performed in the Department of Infectious Diseases, Gaziantep University Medical School, between May 2007 and May 2008. The study was approved by the Ethical Committee of Gaziantep University School of Medicine. The study group included 85 patients with brucellosis and 85 healthy volunteers. Diagnosis of brucellosis was based on clinical findings (arthralgia, fever, sweating, malaise, hepatomegaly, splenomegaly, focal complication) and presence of high titres of specific antibodies. High titres were considered to be 1/160 for Wright's sero-agglutination test or 1/320 for Coombs' anti-brucella test. Involvement of a specific organ in the course of the disease was often referred to as focal complication (Young 2005). Sixty patients (71%) had acute uncomplicated disease and the other 25 (29%) had some type of focal complications. These included 15 (17.6%) spondylitis, 3 (3.5%) sacroiliitis, 2 (2.3%) lymphadenopathy and 4 (4.7%) neurobrucellosis cases. Blood samples were collected in ethylenediamine tetraacetate (EDTA) sterile tubes. Of 85 patients with brucellosis, 27 were males and 58 were females who aged from 10 to 64 years (mean age: 33). Of the healthy control volunteers, 42 were females and 43 were males who aged from 18 to 72 years (mean age: 41). The patients and controls were from the same geographical areas.

DNA Extraction

Genomic DNA was extracted from mononuclear cells obtained from EDTA-treated peripheral venous blood using the salting out method techniques (Miller *et al*., 1988).

Cytokine Gene Polymorphism

Cytokine genotyping was performed by the polymerase chain reaction sequence-specific primer method, using the Cytokine Genotyping Tray kit according to the manufacturer's instructions. Single nucleotide polymorphism for five cytokines (IL-6, IL-10, IFN-γ, TGF-β1, TNFα) was analyzed. The presence of a G or A nucleotide in position -308 of the promoter region was analyzed for TNFα. The AA and AG genotypes represent the potential to produce high levels of TNFα whereas the GG genotype represents the potential to produce low levels of TNFα (Wilson *et al.,* 1997).

The presence of T or A nucleotide in position +874 of IFN-γ was analyzed. Three genotypes are possible: TT, TA and AA. The genotype AA is associated with low production, TA with intermediate production and TT with high production of the cytokine (Pravica *et al*., 1999).

The presence of a single nucleotide modification in position -174 was examined for the IL-6 promoter. Both the GG and GC genotypes are associated with increased levels of IL-6 while CC genotype leads to decreased expression of the cytokine (Fishman *et al*., 1988).

Two different polymorphisms were analyzed for the IL-10 promotor region: position -1082 and position -819. Two different polymorphisms in coding region were surveyed for TGF-β1: codon +10 can be either T or C, +25, either C or G.

Statistical analysis

All data were analyzed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL; USA). Categorical data were analyzed using Pearson's chi-square analysis. Logistic regression models. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. OR (95% CI) was adjusted by age and sex. The data were analyzed for appropriateness between the observed and expected genotypes as well as for Hardy-Weinberg equilibrium. All

analyses were two-tailed, and differences were interpreted as statistically significant when p <0.05.

RESULTS

There was no significant difference between the patients and controls regarding TNFα (-308), IL-6 (-174) and IL-10 (-1082) polymorphisms. A statistically significant difference was found between the patients and controls regarding the polymorphisms, IL-10 (-819), INFγ (+874) and TGFβ1 codons 10 and 25. The frequency of a high INFγ (+874) producing TT genotype was significantly more common in the patients compared to the controls (p=0.013). The frequencies of intermediate and low INFγ (+874) producing AT and AA genotypes were significantly higher in the controls when compared with the patients (p=0.030, p=0.027, respectively). The genotypes for TGFβ1 codon 10 were significantly different between the patients and the controls. Regarding TGFβ1 codon 10 gene polymorphism, the frequency of the CC genotype was more common in the patients when compared with the controls (p= 0.048). For TGFβ1 codon 25 polymorphism, the GG genotype was more common in the patients in comparison to the controls (p=0.033). The CT genotype of IL-10 (-819) was significantly more common in the patients than in the controls (p=0.042), while the CC genotype was higher in the controls than in the patients (p=0.016). The results of the genotype analyses are shown in Table 1.

No differences were found between the patients with focal complications and the patients without focal complications regarding IL-10, IFN-γ, TGF-β1 and TNFα gene polymorphisms except for IL-6 (-174). For the IL-6 (-174) polymorphism, the GC genotype was more common in the patients with focal complication (p=0.035), while the GG

TABLE 1 - *Comparison of frequencies of TNF-α, TGF-β, IL-10, IL-6 and IFN-γ gene polymorphisms between patients with brucellosis and healthy controls.*

Cytokine gene	*Genotype*	*Brucellosis n[a] (%)*	*Healthy Control n[a] (%)*	*OR*	*95% CI*	*p*
TNFα (-308)	GG[&]	75 (88.2)	71 (83.5)	0.676	0.282-1.620	0.378
	AG[#]	9 (10.6)	12 (14.1)	1.588*	0.538-4.691*	0.402*
	AA[#]	1 (1.2)	2 (2.4)	1.567*	0.105-23.428	0.745*
IL-6 (-174)	GG[#]	49 (57.6)	49 (57.6)	1.000	0.544-1.838	1.000
	GC[#]	27 (31.7)	31 (36.5)	1.193*	0.547-2.602*	0.657*
	CC[&]	9 (10.6)	5 (5.9)	0.322*	0.082-1.271*	0.106*
IL-10 (-1082)	AA	30 (35.3)	32 (37.6)	1.004*	0.302-3.342*	0.995*
	AG	45 (52.9)	36 (42.4)	0.402*	0.137-1.178*	0.097*
	GG	10 (11.8)	17 (20)	0.533	0.229-1.244	0.142
IL-10 (-819)	TT	9 (10.5)	4 (4.7)	2.398	0.709-8.112	0.149
	CT	36 (42.4)	32 (37.6)	5.206*	1.061-25.554*	0.042*
	CC	40 (47.1)	49 (57.6)	7.433*	1.449-38.131*	0.016*
IFNγ (+874)	TT[#]	20 (23.5)	8 (9.4)	0.338	0.140-0.817	0.013
	AT[β]	38 (44.7)	45 (53)	3.237*	1.121-9.347*	0.030*
	AA[&]	27 (31.8)	32 (37.6)	3.446*	1.152-10.312*	0.027*
TGFß1 (T10/C10)	TT	17 (20.0)	24 (28.2)	0.635	0.312-1.294	0.209
	TC	41 (48.2)	49 (57.6)	0.847*	0.361-1.991*	0.704*
	CC	27 (31.8)	12 (14.2)	0.338*	0.115-0.992*	0.048*
TGFß1 (T25/G25)	GG	74 (87.0)	63 (74.1)	0.426	0.192-0.945	0.033
	GC	10 (11.8)	20 (23.5)	2.588*	0.991-6.758*	0.052*
	CC	1 (1.2)	2 (2.4)	2.371*	0.151-37.300*	0.539*

[a]n=85, [#]high expression, [&]low expression, [β]intermediate expression, *: OR (95% CI) was adjusted by age and sex.

genotype was more frequent in the patients without focal complication (p=0.014) (Table 2).
All single nucleotide polymorphisms were in Hardy Weinberg equilibrium (p>0.05) in patients with brucellosis and controls by chi-square test.

DISCUSSION

Cytokines play a key role in the regulation of the immune response. The maximal capacity of cytokine production varies among individuals and correlates with the polymorphism in the cytokine gene promoters (Ben Ari Z *et al.*, 2003). Polymorphic gene sequences of certain cytokines could be the potential markers of susceptibility and clinical outcome of different infectious diseases in humans (Hajilooi *et al.*, 2006). The aim of this study was to determine potential associations between cytokine gene polymorphisms and brucellosis. We found no significant differences between allele frequency and genotype distribution of TNFα (-308), IL-6 (-174) and IL-10 (-1082) polymorphisms between the patients and controls. Our data suggest that genetic polymorphisms of TNFα (-308), IL-6 (-174) and IL-10 (-1082) do not influence the susceptibility to brucellosis in theTurkish population.
IFNγ, which is a product mainly of natural killer and activated T-cells, has an important role in host defense. IFNγ modulates the activation of macrophages, increases class II molecules expression and antigen presentation, and increases differentiation of the lymphocyte population (Casanova *et al.*, 2002, Schroder *et al.*, 2004). IFNγ is essential for an efficient response to brucella infection. The AA genotype of IFNγ +874, which is associated with decreased production, is significantly associated with infectious diseases such as brucellosis, tuberculosis, coronavirus and hepatitis B

TABLE 2 - *Cytokine gene polymorphism distribution in brucellosis with and without focal forms.*

Cytokine gene	*Genotype*	*Without focal forms* n^a *(%)*	*With focal forms* n^b *(%)*	*OR*	*95% CI*	*p*
TNFα (-308)	GG$^{\&}$	52 (86.6)	23 (94)	1.769	0.348-8.988	0.487
	AG$^{\#}$	7 (11.7)	2 (8)	2.405*	0.343-16.840*	0.377*
	AA$^{\#}$	1 (1.7)	0 (0)	1.017	0.984-1.051	0.516
IL-6 (-174)	GG$^{\#}$	39 (65)	9 (36)	0.303	0.114-0.802	0.014
	GC$^{\#}$	14 (23.3)	13 (52)	0.237*	0.062-0.906*	0.035*
	CC$^{\&}$	7 (11.7)	3 (12)	0.299*	0.048-1.851*	0.194*
IL-10 (-1082)	AA	23 (38.3)	7 (28)	0.913*	0.107-7.805*	0.934*
	AG	29 (48.3)	16 (64)	0.814*	0.114-5.801*	0.837*
	GG	8 (13.4)	2 (8)	1.769	0.348-8.988	0.487
IL-10 (-819)	TT	8 (13.4)	1 (4)	3.692	0.437-31.207	0.203
	CT	23 (38.3)	13 (52)	0.514*	0.100-2.640*	0.425*
	CC	29 (48.3)	11 (44)	0.307*	0.049-1.938*	0.209*
IFNγ (+874)	TT$^{\#}$	13 (21.7)	7 (28)	0.711	0.245-2.068	0.531
	AT$^{\beta}$	27 (45)	11 (44)	1.020*	0.221-4.715*	0.980*
	AA$^{\&}$	20 (33.3)	7 (28)	2.058*	0.450-9.417*	0.352*
TGFβ1 (T10/C10)	TT	13 (21.7)	4 (16)	1.452	0.423-4.983	0.552
	TC	30 (50)	11 (44)	0.514*	0.100-2.640*	0.425*
	CC	17 (28.3)	10 (40)	0.307*	0.049-1.938*	0.209*
TGFβ1 (T25/G25)	GG	51 (85)	23 (92)	2.029	0.406-10.146	0.381
	GC	8 (13.3)	2 (8)	2.350*	0.343-16.074*	0.384*
	CC	1 (1.7)	0 (0)	0.230	0.013-4.224	0.322

^{a}n=60, ^{b}n=25, $^{\#}$high expression, $^{\&}$low expression, $^{\beta}$intermediate expression, *: OR(95% CI) was adjusted by age and sex.

virus (Rasouli *et al*., 2007, Bravo *et al*., 2003, Ben Ari Z *et al*., 2003, Amim *et al*., 2007, Chong *et al*., 2006). Bravo *et al.* reported that the IFNγ AA genotype was significantly high in patients with brucellosis when compared to the controls. Another study from Turkey (Budak *et al*., 2007) reported that no association was found between IFNγ +874 polymorphism and susceptibility to brucellosis. Our results of IFNγ polymorphism are not in agreement with the previous studies. In our study, the TT variant, which leads to IFNγ high producer genotype, was significantly higher in the patients when compared with controls whereas IFNγ low producer genotype was more common in the controls. Thus, high IFNγ producer genotype would be expected to show resistance to brucellosis. Our results did not show a possible association between IFNγ polymorphism +874 and brucellosis in the Turkish population. IL-10 was known as cytokine synthesis inhibitory factor because of its ability to inhibit cytokines such as IL-2 and IFNγ. On the other hand, IL-10 also has a direct comitogenic effect on T cells and B cells and promotes B cell antibody production (Oppenheim *et al*., 1997). Fernandes *et al*. showed in the murine model that IL-10 may down-regulate the immune response to *B. abortus* by affecting both macrophage effector function and the production of IFNγ. Our data suggest that IL-10 (-819) gene polymorphisms may affect susceptibility to brucellosis. The frequency of CT genotype of IL-10 (-819) polymorphisms is more common in patients compared with controls. Accordingly, the CT genotype may be considered a genetic factor leading to susceptibility to brucellosis. Moreover, CC genotype at position -819 of IL-10 gene was more prevalent in controls than patients, suggesting that the CC genotype may serve as a protective factor against brucellosis. Bravo *et al*. reported no association between the IL-10 polymorphisms and brucellosis susceptibility. Budak F *et al*. from Turkey reported that IL-10 gene polymorphism may affect susceptibility to brucellosis. TGFβ1 is a multifunctional cytokine that regulates several events in growth and differentiation of many cell types (Toosi *et al*., 1998). TGFβ1 is suppressive for the cellular immune response at multiple levels including proliferation, and inhibits lymphocyte proliferation and function (Toosi *et al*., 1998). The increased frequency of the TGFβ1 high producer genotype in brucellosis suggests a suppression of immune status by TGFβ1 (Rafiei *et al.,* 2006). In the present study, TGFβ1 (codon 10, codon 25) genotypes influenced susceptibility to brucellosis. The CC genotype of TGFβ1 codon 10 polymorphism contributes to susceptibility to brucellosis. Also, the GG genotype of TGFβ1 codon 25 polymorphism contributes to susceptibility to brucellosis. In a similar study from Iran (Rafiei *et al.,* 2006), it was reported that the CC and GG genotypes of TGFβ1 gene could be potential risk factors for brucellosis. These results are compatible with our results.

The function of IL-6 in Brucellosis is not known well. A study from Turkey (Budak *et al*., 2007) found an association between IL-6 (-174) polymorphism and Bucellosis. Our study failed to find a difference in the distribution of IL-6 variants between the patients and controls while we found a difference in the frequency of IL-6 (-174) between the patients with and without focal complications. Our study showed that IL-6 (-174) GG genotype was more common in patients without focal complications than in patients with focal complications. The IL-6 (-174) GC genotype was more common in patients with focal complications than in patients without focal complications. The presence of GC genotype may be a predisposing factor for the development of focal complications whereas the presence of GG genotype may be protective against development of focal complications in brucellosis. This is the first report that IL-6 (-174) polymorphism may influence susceptibility to development of focal complications in brucellosis.

In conclusion, our data suggest that IL 10 (-819) and TGFβ1 codon 10 and codon 25 polymorphisms may be associated with an increased risk of brucellosis and IL-6 (-174) polymorphism may be associated with susceptibility to or protection against development of brucellosis complications.

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