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INTERLEUKIN 10 AND TGF-BETA GENE POLYMORPHİSMS CAN EFFECT GRANULOMATOUS FORMATIONIN CHRONIC GRANULOMATOUS DISEASE

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Introduction: Chronic granulomatous disease (CGD) is a rare and fatal inherited immunodeficiency syndrome. Recurrent bacterial and fungal infections, abnormal inflammatory responses and granuloma formation are common.

Purpose: Patients with CGD are susceptible to bacterial and fungal pathogens, with associated dysregulated inflammation and widespread granuloma formation. The objective of this study was to evaluate the clinical presentation, granuloma formation and association with cytokine gene polymorphisms.

Patients and Methods: Four patients with CGD and 60 healthy controls were enrolled in this study. All genotyping (TNF-α, TGF-β, IL-10, IL-6, and IFN-γ) studies were performed using sequence-specific primers (PCR-SSP).

Results: Frequencies of IL-10 (-1082, -819, -592) ACC/ATA polymorphism were significantly greater in the patients with CGD.

Conclusion: The results suggest that the IL-10 ACC/ATA polymorphism is associated with granuloma formation.

Key Words: Cytokine, gene polymorphism, chronic granulomatous disease.

İNTERLÖKİN 10 VE TGF-BETA GEN POLİMORFİZMLERİ KRONİK GRANÜLOMATÖZ HASTALIKTA GRANÜLOM FORMASYONU OLUŞUMUNDA ETKİLİ OLABİLİR Mİ?

Giriş: Kronik granülomatöz hastalık, nadir görülen fatal seyirli ve kalıtsal geçiş gösteren bir immün yetmezlik hastalığıdır. Hastalarda sıklıkla tekrarlayan bakteriyel ve fungal enfeksiyonlar, anormal inflamatuvar cevap gelişimi ve granülom oluşumları gözlenmektedir. Bu hastalarda fonksiyonel bozukluklara da neden olabilen granülom oluşumlarının nedeni tam olarak açıklanamamıştır

Amaç: Bu çalışma kronik granülomatöz hastalık tanısı almış olan hastalarda bakteriyal ve fungal patojenlerin neden olduğu inflamasyon ve granülom formasyonu oluşumu ile sitokin gen polimorfizmleri arasında bir ilişki olup olmadığını saptamak amacıyla yapılmıştır.

Hasta ve Metod: Kronik granülomatöz hastalık tanısı almış 4 hastanın ve 60 sağlıklı gönüllü donörün kanlarından elde edilen DNA örneklerinden, önceden seçilmiş olan sitokin gen polimorfizmleri (TNF-α, TGF-β, IL-10, IL-6, and IFN-γ) PCR SSP yöntemi ile çalışılmıştır.

Sonuç: Hasta grubunda interlökin-10 -1082 ACC/ATA polimorfizm varlığında anlamlı fark saptanmıştır.

Yorum: IL-10 ACC/ATA polimorfizm varlığının granulom formasyonu ile ilişkili olabileceğini göstermektedir.

Anahtar Kelimeler: Sitokin, gen polimorfizm, kronik granülomatöz hastalık.

INTRODUCTION

Chronic granulomatous disease (CGD) is a rare (one per billion), fatal, inherited immunodeficiency syndrome caused by a profound defect in the oxygen metabolic burst machinery. Phagocytes fail to produce antimicrobial superoxide because of NADPH oxidase deficiency. Abnormal function of polymorphonuclear leukocytes in patients with CGD was first reported in 1967 (1). Recurrent life-threatening bacterial and fungal infections, as well as abnormally exuberant inflammatory responses, are common (2, 3). Patients with CGD exhibit deficient generation of reactive oxygen intermediates (ROI) and hydrogen peroxide and susceptibility to bacterial and fungal pathogens, with associated dysregulated inflammation and widespread granuloma formation. Granulomatous complications usually affect the gastrointestinal and genitourinary tracts (4).

The diagnosis of CGD should be considered in patients with unusually severe infections with a common pathogen or in patients with infections caused by a rare pathogen in the general population that is observed with a higher frequency in patients with CGD. Aspergillosis is the most important cause of death in patients with CGD (5). The mechanisms underlying abnormal inflammatory responses in GCD are undefined. In some cases, granulomatous complications arise from unresolved infection. Cytokines participate in the induction and effector phases of immune and inflammatory response and levels of their production are associated with polymorphisms of cytokine genes (6). Many researchers have reported associations between cytokine gene polymorphism and the development of certain infectious diseases, allergies, autoimmune disorders, and cancers (7-10). The balance among the Th1 (pro-inflammatory cytokines such as TNF-alpha and interleukin (IL)-1), Th2 (anti-inflammatory cytokines such as IL-10 and IL-4) and T reg (regulatory cytokines such as TGF-β) cells is extremely important for the development of immune response (11).

PATIENTS AND METHODS

The study included 4 patients (male) and 60 (34 female, 26 male) healthy controls.

CGD was confirmed by abnormal neutrophil nitroblue tetrazolium reduction test.

Genomic DNA was extracted from whole ethylenediamine tetraacetate (EDTA)-treated blood with the Machery Nagel DNA isolation kit (Duren, Germany) according to the manufacturer's instructions.

Single nucleotide polymorphisms were analyzed in five cytokines for genotype assignment. The presence of a G or A nucleotide in position -308 of the promoter region was analyzed for

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TNF-α (TNF-alpha). Two single nucleotide modification in the coding region were surveyed for TGF-β1: codon 10 can be either T or C, and codon 25 either C or G. Three different polymorphisms were analyzed for the IL-10 promoter region: position -1082 (G vs. A), position -819 (C vs. T), and position -592 (A vs. C). The presence of a single nucleotide modification at position -174 was examined for IL-6 promoter. An additional coding sequence mutation (T vs. A) at position +874 was analyzed for IFN-γ.

Cytokine genotypes were determined using PCR-sequence-specific primers (SSP) with a commercially available kit (One lambda, Inc., Canoga Park, CA, USA) in accordance with the manufacturer's instructions. The DNA extractions and PCR amplifications were performed by a technician blinded to the study groups.

Statistical analysis:

Statistical analysis was performed with Epi Info Version 3.2.2 (Centers for Disease Control and Prevention, USA). The distributions of cytokine genes polymorphisms were compared between patients with CGD and healthy controls by the χ^2 or Fisher's exact test. P values smaller than 0.05 were considered significant.

RESULTS

Frequencies of IL-10 (-1082, -819, -592) ACC/ATA polymorphism, which was associated with low production, were significantly greater (patients: 100%, control: 31.6%, p: 0.014) in the patients with CGD. TGF-beta TT/GG and TC/GG haplotypes, which were associated with high production, were higher in patients with CGD (100%) than in the control group (31.6%) but no significant difference was found. No significant difference in cytokine genes was found between patients with CGD and the healthy controls (Figure 1).

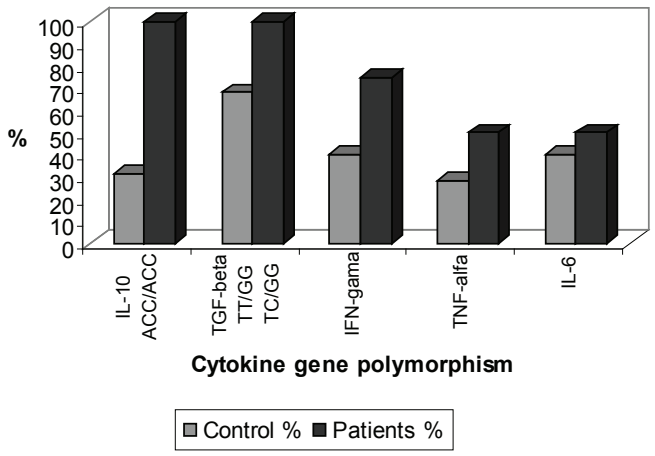

Figure 1: Cytokine gene polymorphism frequencies in patients and controls.

DISCUSSION

In addition to recurrent infections, patients with CGD frequently have exuberant and persistent tissue granuloma formation. Inflammatory manifestations, especially granulomatous involvement of the gut, have been recognized as central to CGD (12, 13).

If the immune system fails to eradicate the infection, activated macrophages continue to produce cytokines and growth factors, which progressively modify the local tissue environment. Tissue injury is followed by replacement with connective tissue. Fibrosis is a hallmark of chronic DTH reactions. Clusters of activated macrophages produce nodules of inflammatory tissue called granulomas. Granulomatous inflammation, representing a form of chronic DTH, is a characteristic response to persistent microbes and frequently associated with tissue fibrosis. DTH reactions appear due to Th1 type response (14).

IL-10 is an inhibitor of activated macrophages and is thus involved in the homeostatic control of innate immune reactions and cell-mediated immunity. Because it inhibits macrophage functions, it is an excellent example of a negative feedback regulator. Increased serum IL-10 is expected to decrease serum levels of IFN-gamma and TNF-alpha, and elevated serum IL-10 also suppresses DTH (15).

TGF-beta is another inhibitory cytokine. However, it has many diverse actions apart from those concerning the immune system. It causes synthesis of extracellular matrix proteins such as collagens and of cellular receptors such as integrins (11).

Our study showed that frequencies of IL-10 (-1082, -819, -592) ACC/ATA polymorphism, which is associated with low production, were significantly greater in the patients with CGD. Low levels of IL-10, failure to inhibit macrophage activation and persistent Th1 type response cause the DTH reactions.

Although we found no significant difference, TGF-beta TT/GG and TC/GG haplotypes, which are associated with high production, were higher in patients with CGD than in the control group. High levels of TGF-beta may promote tissue repair and fibrosis after local immune and inflammatory reactions.

CONCLUSION

IL-10 (-1082, -819, -592) ACC/ATA and TGF-beta codon 10, codon 25 TT/GG and TC/GG haplotypes can cause granuloma formation in patients with CGD. However, this conclusion requires the study of larger series. Knowledge of these haplotypes may be of predictive value in the treatment of granulomatous lesions. Our study is the first preliminary study to show an association between cytokine gene polymorphism and development of granuloma formation.

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