SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2010; 27; 70-75 © Mattioli 1885

Cytokine gene polymorphisms in sarcoidosis

M. Vasakova[1], M. Sterclova[1], L. Kolesar[2], A. Slavcev[2], J. Skibova[3], I. Striz[2]

[1] Department of Respiratory Diseases 1st Medical School Charles University and University Thomayer Hospital Prague, Czech Republic; [2] Department of Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3] Medical Statistic Unit, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Abstract. *Background:* Sarcoidosis is a disease characterized by granuloma formation in many organs, but mostly in lung and lymph nodes. The immunopathogenic background of the disease is probably based on disregulation of immune response to different antigens. The imbalance of immune reactivity might be influenced by genetic background. In our study, we have investigated cytokine genetic polymorphisms in sarcoidosis group and compared the results with that of a group of healthy volunteers. *Methods:* Thirty one sarcoidosis patients were enrolled to our study. Basic demographic data were collected. Polymorphisms in the promoter regions of the IL-1alpha, IL-1beta, IL-1R, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, IFN-gamma and in the translated regions of the TGF-beta, IL-1 beta, IL-2, IL-4 and IL-4RA genes were characterized. *Results:* For IL-10, the (-819) and (-592) CC homozygosity was statistically more frequent in the sarcoidosis group compared to healthy controls. According to the haplotypes, the majority of sarcoidosis patients had IL-10 (-1082)(-819)(-592) ACC haplotype 2 compared to controls with ATA in most of the cases. *Conclusions:* The results of our study support the hypothesis of a genetically encoded immune regulation imbalance in sarcoidosis. The high-producer IL-10 (-819) and (-592) CC genotypes and intermediate- producer IL-10 (-1082) (-819) (-592) ACC haplotype 2 present in the majority of our sarcoidosis patients could support the role of genetically encoded disregulation of cell- mediated immune response to an unknown antigen *(Sarcoidosis Vasc Diffuse Lung Dis 2010; 27: 70-75)*

Key words: Sarcoidosis, IL-10, gene polymorphisms, immune regulation

Introduction

Sarcoidosis is a multisystem inflammatory disease of unknown ethiology characterized by epithelial granuloma formation with accumulation of CD4+T lymphocytes and macrophages. It is hypothesized that an imbalance of cytokine and chemokine production with a shift to a so-called T_H1 type immune reaction might play a role in its pathogenesis. The situation is not so clear in later phases of the disease characterized with transition to fibrosis, where the T_H-2 type and pro-fibrotic cytokines and chemokines probably play a dominant role (1, 2).

The cytokine and chemokine milieu could be influenced by genetic background, i.e. cytokine gene polymorphisms. Previous studies have pointed out a correlation between cytokine gene polymorphisms and sarcoidosis development. According to the tumor necrosis factor (TNF)-alpha (-857) C—>T

Received: 27 July 2009
Accepted after Revision: 19 March 2010
Correspondence: Martina Vasakova, Assoc. Prof., MD, Ph.D
Department of Respiratory Diseases,
1st Medical School Charles University
University Thomayer Hospital Prague
Videnska 800
140 59 Prague 4
Czech Republic
Fax +420 261082206
Tel. +420 261082372
E-mail: martina.vasakova@ftn.cz

The study is supported with MHCR grant No. 9131-3/2007

polymorphism, Seyhan et al revealed no differences in genotype and allele frequency between patients and control subjects, but described more relapses and more frequent involvement of three or more organs in Turkish sarcoidosis patients (3). For interleukin (IL)-6, Mayer et al demonstrated that the (-174) G—>C polymorphism may be a risk factor for sarcoidosis development in Slovenian patients (4). Grutters et al stated that although the investigated IL-6 polymorphisms are unlikely to contribute to sarcoidosis susceptibility, the IL-6(-174)C allele might have a role in the genetics underlying sarcoidosis severity or the progression towards pulmonary fibrosis in a particular subgroup (5). Kruit et al found no association of transforming growth factor (TGF)-beta1 gene polymorphisms with fibrosis in sarcoidosis patients in the Netherlands , but suggested the implication of genetic variation of TGF-beta3 in the predilection for pulmonary fibrosis development in sarcoidosis patients (6). The data of Mrazek et al suggest that the lymphotoxin alpha (LT)-alpha and HLA-DRB1 genes themselves or a gene located nearby contribute to the susceptibility for sarcoidosis and that TNF-alpha (-308)A, LT-alpha(+252)G and HLA-DRB1*03 alleles are associated (directly or via linkage with unknown causative locus) with Löfgren's syndrome as a specific manifestation of the disease (7). Interferon (IFN)- alpha (551)T—>G polymorphism was found to be associated with susceptibility for sarcoidosis but not to tuberculosis in Japanese (8). In the study of Takada et al, IL-18 gene polymorphisms, namely the C allele at position (-607) might be a genetic risk factor for sarcoidosis in the Japanese population (9). In the study of Hutyrova et al the IL-1alpha (-889) 1.1 genotype was significantly overrepresented in patients with sarcoidosis in comparison with control subjects in the Czech population (10). Yamaguchi et al found that the TNF-beta*1 allele is a marker for prolonged clinical course in patients with sarcoidosis in the Japanese population (11).

In our study, we compared cytokine genetic polymorphisms frequency in sarcoidosis group with group of healthy volunteers. We tried to find the polymorphisms which could prime the immune system to granuloma formation in response to environmental antigens. We are aware that the nature of antigen triggering the granuloma formation could itself be the answer, but we suppose that also genetic mechanisms might modify the immunopathogenetic response.

Material and Methods

Study subjects

Thirty one sarcoidosis patients, all Czech Caucasians, were enrolled to our study.

The sarcoidosis patients (mean age 47,5 years, 14 men and 17 women) were diagnosed according to the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Granulomatous Disorders statement on sarcoidosis, based on history, clinical symptoms, standard chest radiography, high resolution computed tomography (HRCT), bronchoscopy with bronchoalveolar lavage (BAL) and laboratory tests (serum angiotensin converting enzyme, calcemia and calciuria). Seven patients had radiological stage I, 21 patients stage II and 3 patients stage III. All patients underwent transbronchial biopsy or transbronchial lymph node puncture or videothoracoscopic lung biopsy with histopathologic evidence supporting the diagnosis of sarcoidosis.

Basic demographic data such as age and sex were collected (Table 1).

The control population of 145 unrelated individuals (24 males, 121 females) were all Caucasians from the Czech Republic with no previous history of fibrosing lung disease. These patients were potential bone marrow donors in generally good health status, without known current lung disease. The normal controls had a mean age of 43.1(standard deviation (SD)=16.17) years (range 19-80 years).

The study design and informed consent form was approved by the Central Ethical Comittee of the

Table 1. Demographic and basic clinical data of sarcoidosis patients

	Sarcoidosis n=31
Mean age	47.52; SD=13.49
Men:women ratio	14:17
FVC (l / %)	3.85; SD=1.42/95.52%; SD=17.32
TLCO %	72 (SD=23.35)
BAL LY %	26.3 (SD=25.57)
BAL PMN%	12.83 (SD=22.27)
BAL EOS%	0.8 (SD=1.99)

SD: standard deviation

University Thomayer Hospital and the Institute for Clinical and Experimental Medicine. The patients all signed the informed consent form before submitting a blood sample for genotyping.

Methods

Polymorphisms in the promoter regions of the IL-1alpha, IL-1beta, interleukin-1 receptor (IL-1R), IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, IFN-gamma as well as polymorphisms in the translated regions of the transforming growth factor (TGF)-beta, IL-1 beta, IL-2, IL-4 and IL-4RA genes were characterized (Table 2).

DNA samples were extracted from 350 µl of EDTA whole blood using the commercial EZ1 DNA Blood 350 µl Kit in combination with automated platform Biorobot EZ1 according to the manufacturer's protocol. The quality of DNA was determined spectrophotometricaly.

Cytokine genotyping: We evaluated polymorphisms of thirteen different cytokine genes utilizing CYTOKINE GENOTYPING KIT (Dynal, Biotech, Norway). The test is designed as a polymerase chain reaction (PCR) with sequence- specific primers. In detail,, each well of a 48 well tray contains specific primer pair for amplifying desired unique sequence. The whole procedure was performed according to the manufacture's manual. The obtained pattern of positive and negative PCR was documented and interpreted according to the manufacture's worksheet.

Table 2. List of investigated cytokine gene polymorphisms

Polymorphism	Genotype
IL-1alpha –889	C/C C/T T/T
IL-1beta –511	C/C C/T T/T
IL-1beta +3962	C/C C/T T/T
IL-1R pst 1970	C/C C/T T/T
IL-1 RA mspa 11100	C/C C/T T/T
IL-4 RA +1902	A/A A/G G/G
IL-12 –1188	A/A A/C C/C
INF-gamma UTR 5644	A/A A/T T/T
TGF beta1 codon 10	C/C C/T T/T
TGF beta1 codon 25	C/C C/G G/G
TNF alpha –308	A/A A/G G/G
TNF alfa –238	A/A A/G G/G
IL-2 –330	G/G G/T T/T
IL-2 +166	G/G G/T T/T
IL-4 –1098	G/G G/T T/T
IL-4 –590	C/C C/T T/T
IL-4 –33	C/C C/T T/T
IL-6 –174	C/C C/G G/G
IL-6 +565	A/A A/G G/G
IL-10 –1082	A/A A/G G/G
IL-10 –819	C/C C/T T/T
IL-10 –592	A/A A/C C/C

Statistical analysis

The genotype frequencies and allele carriage frequencies in IPF were determined by direct counting and they were compared with those in the control population and between each other group using Fisher's exact test or chi-squared test. The basic statistical characteristics, i.e. mean values and standard deviation were calculated for continous variables. Statistical analysis was performed using MedCalc statistical software. A p value less than 0.05 was considered significant. Correction of level of significance was assessed using Holm's sequentially multiple test procedure (modification of Bonferroni test) for free hypotheses concerning IL-10 (12).

Results

For IL-10 at the position (-819) the CC genotype was statistically more frequent in the sarcoidosis group compared to healthy controls and, on the contrary, T allele was present at this position in half of controls (48.9%) but only in 19.4% of sarcoidosis patients (p= 0,009; 0.009<0.0167) (Table 3). Also, for the IL-10 at the position (-592), CC homozygosity were more frequently seen in the sarcoidosis group compared to controls where allele A was significantly more frequently seen (p=0.022; 0.022<0.025) (Table 4) .

Table 3. IL-10 (-819) gene polymorphisms in sarcoidosis patients compared to controls (p=0,009; 0.009<0.0167)

IL-10 (-819) genotype	CC	CT	TT
Control group	74 (51.0%)	64 (44.1%)	7 (4.8%)
Sarcoidosis group	25 (80.6%)	6 (19.4%)	0 (0.0%)

Table 4. IL-10 (-592) gene polymorphisms in sarcoidosis patients compared to controls (p=0.022; 0.022<0.025)

IL-10 (-819) genotype	AA	AC	CC
Control group	7 (4.8%)	64 (44.1%)	74 (51.0%)
Sarcoidosis group	0 (0.0%)	7 (22.6%)	24 (77.4%)

Table 5. IL-10 (-1082)(-819)(-592) haplotype 2 frequencies in sarcoidosis and control groups (p=0.0037).

	ACA	ACC	ATA	GCC
Control group	0 (0.0%)	54 (37.2%)	71 (49.0%)	20 (13.8%)
Sarcoidosis group	1 (3.2%)	19 (61.3%)	6 (19.4%)	5 (16.1%)

According to the haplotypes of TGF-1- beta, TNF- alpha, IL-2, IL-4, IL-6 and IL-10 genes at investigated positions, we found different frequencies of haplotypes 2 in the promoter region of the IL-10 (-1082)(-819)(-592) compared to healthy controls (p=0.0037) (Table 5). The majority of the patients with sarcoidosis had haplotype ACC compared to healthy subjects who, in most of the cases, had ATA.

Discussion

IL-10 genetic polymorphisms were investigated with relationship to sarcoidosis before, but the authors tested only IL-10 (-1082) polymorphisms and did not report any differences between sarcoidosis and controls and consensually neither did we (13). However, we found significant differences in polymorphisms frequency between sarcoidosis patients and healthy population for IL-10 polymorphisms at (-592) and (-819) positions and also for IL-10 haplotypes 2. Both these polymorphisms are known to influence the IL-10 secretion. Furthermore, the -1082, -819 and -592 polymorphisms are shown to be in close linkage disequilibrium and construct only three haplotypes in the white population (GCC, ACC and ATA in an order of -1089/-819/-592). These haplotypes are associated with high (GCC), intermediate (ACC), and low (ATA) IL-10 production (14, 15). In our study, we have observed ACC haplotype, i.e. which are associated with intermediate production of IL-10, more frequently in sarcoidosis group compared with control group where the low- producer haplotype ATA was seen more frequently.

In the work of Thye et al, the influence of IL-10 haplotypes on IL-10 production on the response to mycobacterial antigens and tuberculosis development was studied in the Ghanaian population. The authors have found that the IL-10 intermediate-producer haplotype (-2849)A(-1082)A(-819)C (-592)C, compared to the high-producer haplotype (-2849)G(-1082)G(-819)C(-592)C, occurred less frequently among tuberculin skin test- protein purified derivate (PPD)-negative controls than among tuberculosis cases and PPD-positive controls (16). This could be rather confusing when we realize that our patients are mostly genetical intermediate- producers. But in this case, we must consider the differences in the study populations and the interaction of Mycobacterium tuberculosis with the immune system, which itself could influence the cytokine secretion and cell- mediated immune reaction.

When considering noninfectious diseases, IL-10 (-1082) (-819)(-592) haplotype with high IL-10 production was protective against paediatric heart transplant rejection (17). This is also the case for adult lung transplant recipients, where the increased IL-10 production genotype was protective against acute persistant rejection when compared with the intermediate or decreased IL-10 production genotypes (18). As for systemic diseases, the IL-10 haplotype and genotype associated with high IL-10 production may alter the susceptibility to systemic sclerosis and/or its expression and also showed susceptibility for primary Sjogren's syndrome development (19,20). In the study of Chung et al, IL-10 promoter (-1082) (-819)(-592) GCC homozygous haplotype is strongly associated also with the pathogenesis of systemic lupus erythematodes (21).

According to IL-10 role in the pathogenesis of sarcoidosis, there were a few studies dealing with this topic. In the majority of them, an increased secretion of IL-10 by alveolar macrophages spontaneously and/or after a stimulation was demonstrated when compared to healthy controls (22-25). A couple years before this study, Bansal et al observed the elevated levels of IL-10 in serum of sarcoidosis patients when compared to controls (25). On the contrary, Kawaguchi et al proved in their study lower IL-10 production by peripheral blood mononuclear cells (PBMC) from patients with active sarcoidosis compared to healthy controls. They also found a negative

correlation of CD86 expression with IL-10 production (26). Hauber et al failed to prove a difference in IL-10 mRNA expression in BALF cells or PBMC in sarcoidosis patients when compared to healthy controls as well (27). In the context of these studies, the role of IL-10 in sarcoidosis seems unclear. In our group of patients, we have found the prevalence of patients with alleles C on the positions IL-10 (-592) and (-819), i.e., the alleles connected with higher IL-10 production, and haplotype IL-10 (-1082) (-819) (-592) ACC which is ascribed to intermediate production of IL-10. Our sample of healthy population from the Czech Republic tends to have mostly the ATA haplotype 2 which is known to be a low- producer one. Thus, a hypothesis about the role of these polymorphisms in sarcoidosis pathogenesis could be formulated. We are aware that the sarcoidosis group is not so numerous and our findings cannot be generalised. Nevertheless, our conclusions could support the suspicion of the genetically encoded disregulation of immune response in sarcoidosis pathogenesis. We will continue in our research trying to compare the IL-10 polymorphisms with IL-10 production by PBMC and alveolar macrophages.

Conclusion

The results of our study could support the hypothesis of immune regulation imbalance in sarcoidosis pathogenesis. The IL-10 (-819) and (-592) high- producing CC genotypes and IL-10 (-1082) (-819)(-592) ACC haplotype 2 known for intermediate production of IL-10, seen in most of our sarcoidosis patients, could support the hypothesis of a genetically encoded disregulation of immune system in sarcoidosis leading to granuloma formation and impaired cell-mediated immune response.

Further study concerning the correlationof IL-10 genotypes and haplotypes and IL-10 production in lung and peripheral blood in sarcoidosis patients shall be our next task.

References

1. Costabel U, Ohshimo S, Guzman J. Diagnosis of sarcoidosis. Curr Opin Pulm Med 2008; 14: 455-61.
2. Grunewald J. Genetics of sarcoidosis. Curr Opin Pulm Med 2008; 14: 434-9.
3. Seyhan EC, Cetinkaya E, Altin S, et al. Tumor necrosis factor gene polymorphisms in Turkish patients with sarcoidosis. Intern Med 2008; 47: 833-8.
4. Mayer A, Medica I, Salobir B, Sabovic M, Tercelj M, Peterlin B. Polymorphisms in genes coding for mediators in the interleukin cascade and their effect on susceptibility to sarcoidosis in the Slovenian population. Int J Mol Med 2007; 20: 385-90.
5. Grutters JC, Sato H, Pantelidis P, et al. Analysis of IL6 and IL1A gene polymorphisms in UK and Dutch patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2003; 20: 20-7.
6. Kruit A, Grutters JC, Ruven HJ, et al. Transforming growth factor-beta gene polymorphisms in sarcoidosis patients with and without fibrosis. Chest 2006; 129: 1584-91.
7. Mrazek F, Holla LI, Hutyrova B, et al. Association of tumour necrosis factor-alpha, lymphotoxin-alpha and HLA-DRB1 gene polymorphisms with Löfgren's syndrome in Czech patients with sarcoidosis. Tissue Antigens 2005; 65: 163-71.
8. Akahoshi M, Ishihara M, Remus N, et al. Association between IFNA genotype and the risk of sarcoidosis. Hum Genet 2004; 114: 503-9.
9. Takada T, Suzuki E, Morohashi K, Gejyo F. Association of single nucleotide polymorphisms in the IL-18 gene with sarcoidosis in a Japanese population. Tissue Antigens 2002; 60: 36-42.
10. Hutyrová B, Pantelidis P, Drábek J, et al. Interleukin-1 gene cluster polymorphisms in sarcoidosis and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2002; 165: 148-51.
11. Yamaguchi E, Itoh A, Hizawa N, Kawakami Y. The gene polymorphism of tumor necrosis factor-beta, but not that of tumor necrosis factor-alpha, is associated with the prognosis of sarcoidosis Chest 2001; 119: 678-9.
12. Holm S. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 1979; 6: 65-70.
13. Muraközy G, Gaede KI, Zissel G, Schlaak M, Müller-Quernheim J. Analysis of gene polymorphisms in interleukin-10 and transforming growth factor-beta 1 in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2001; 18: 165-9.
14. Stewart D, Fulton WB, Wilson C, Monitto CL, Paidas CN, Reeves RH, De Maio A: Genetic contribution to the septic response in a mouse model. Shock 2002; 18: 342-347.
15. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1-8.
16. Thye T, Browne EN, Chinbuah MA, et al. IL10 haplotype associated with tuberculin skin test response but not with pulmonary TB. PLoS One 2009; 4: e5420.
17. Awad MR, Webber S, Boyle G, et al.The effect of cytokine gene polymorphisms on pediatric heart allograft outcome. J Heart Lung Transplant 2001; 20: 625-30.
18. Zheng HX, Burckart GJ, McCurry K, et al. Interleukin-10 production genotype protects against acute persistent rejection after lung transplantation. J Heart Lung Transplant 2004; 23: 541-6.
19. Beretta L, Cappiello F, Barili M, Scorza R. Proximal interleukin-10 gene polymorphisms in Italian patients with systemic sclerosis. Tissue Antigens 2007; 69: 305-12.
20. Hulkkonen J, Pertovaara M, Antonen J, Lahdenpohja N, Pasternack A, Hurme M. Genetic association between interleukin-10 promoter region polymorphisms and primary Sjogren's syndrome. Arthritis Rheum 2001; 44: 176-9.
21. Chung EY, Liu J, Zhang Y, Ma X. Differential expression in lupus-associated IL-10 promoter single-nucleotide polymorphisms is mediated by poly(ADP-ribose) polymerase-1. Genes Immun 2007; 8: 577-89.
22. Bingisser R, Speich R, Zollinger A, Russi E, Frei K. Interleukin- 10 secretion by alveolar macrophages and monocytes in sarcoidosis. Thorax 2003; 58 (6): 519-24.

23. Oltmanns U, Schmidt B, Hoernig S, Witt C, Matthias J. Increased spontaneous interleukin-10 release from alveolar macrophages in active pulmonary sarcoidosis. Experimental Lung Research 2003; 29 (5): 315-28.
24. Minshall EM, Tsicopoulos A, Yasruel Z, et al. Cytokine mRNA gene expression in active and nonactive pulmonary sarcoidosis. Eur Respir J 1997; 10: 2034-9.
25. Barbarin V, Petrek M, Kolek V, Van Snick J, Huaux F, Lison D. Characterization of p40 and IL-10 in the BALF of patients with pulmonary sarcoidosis. Journal of Interferon and Cytokine Research 2003; 23 (3): 92-97.
26. Bansal AS, Bruce J, Hogan PG, Allen RK. An assessment of peripheral imunity in patients with sarcoidosis using measurements of serum vitamin D3, cytokines and soluble CD23. Clin Exp Immunol 1997; 110 (1): 92-97.
27. Kawaguchi H, Sato S, Sugiura Y, et al. IL-12 and IL-10 released from peripheral blood mononuclear cells in sarcoidosis is associated with differential modulation of B7-1(CD80) and B7-2(CD86) expression on monocytes. Nagoya Med J 2001; 45 (1): 17-29.
28. Hauber HP, Gholami D, Meyer A, Pforte A. Increased interleukin-13 expression in patients with sarcoidosis. Thorax 2003; 58: 519-24.