Biochem Genet (2013) 51:967–975
DOI 10.1007/s10528-013-9621-x

Pathological Implications of Th1/Th2 Cytokine Genetic Variants in Behçet's Disease: Data from a Pilot Study in a Sicilian Population

L. Vaccarino · G. Triolo · A. Accardo-Palombo · L. Scola · M. Palmeri · M. Bova · G. Candore · D. Lio · C. R. Balistreri

Received: 29 August 2012 / Accepted: 11 March 2013 / Published online: 16 July 2013

Abstract Cytokines act as pleiotropic polypeptides able to regulate inflammatory/immune responses and to provide important signals in physiological and pathological processes. Several cytokines (Th1, Th2, and Th17) seem to be involved in the pathophysiology of Behçet's disease, a chronic immune-mediated disease characterized by oral and genital lesions and ocular inflammation. Its individual susceptibility seems to be modulated by genetic variants in genes codifying these cytokines. Th1 and Th17 seem to be involved in the disease's active phases, and Th2 seems to affect the development or severity of the disease; however, contrasting data are reported. In this study, some genetic variants of the Th1/Th2 cytokine genes were investigated in Sicilian patients and age- and gender-matched controls. Three very significant associations with Behçet's disease were detected, and combined genotypes associated with increased disease risk were identified. Results obtained point to the key role of Th1/Th2 cytokine genetic variants in disease susceptibility.

Keywords Th1 and Th2 cytokines · Immune imbalance · Behçet's disease · Polymorphisms · Susceptibility

Introduction

Currently, data that demonstrate the fundamental impact of genetics in human autoimmune diseases are rapidly accumulating. In particular, genetic variants of multiple inflammatory/immune genes may play roles as risk or predisposing factors.

L. Vaccarino · L. Scola · M. Palmeri · M. Bova · G. Candore · D. Lio · C. R. Balistreri (✉)
Department of Pathobiology and Medical and Forensic Biotechnologies, University of Palermo, Corso Tukory 211, 90134 Palermo, Italy
e-mail: carmelarita.balistreri@unipa.it

G. Triolo · A. Accardo-Palombo
Rheumatologic Unit of Palermo University Hospital, University of Palermo, Palermo, Italy

This also confirms the involvement of different inflammatory/immune pathways in the complex pathophysiology of these diseases. In this scenario, the Th1 and Th2 cytokines and their receptors seem to be crucial (Gregersen and Olsson 2009; Cho and Gregersen 2011).

The Th1 (i.e., IL-12, IL-18) and Th2 (IL-4, IL-13, IL-10) cytokines, involved in host protection from pathogen invasions and maintenance of immune balance, are finely tuned, and their imbalance seems to be the cause of various immune disorders, including autoimmune diseases such as Behçet's disease (Zygmunt and Veldhoen 2011; Bluestone et al. 2009; Kuchroo et al. 2012). Emerging evidence is also demonstrating the promotion of Th17 responses and the suppression of T regulatory cells (Tregs) in the pathophysiology of these diseases (Hamzaoui et al. 2011, 2012; Pineton de Chambrun et al. 2012; Zhou et al. 2012).

Behçet's disease is a systemic vasculitis characterized by oral and genital lesions and ocular inflammation. Its pathogenesis is unknown; however, an increased and deregulated immune response triggered by pathogenic agents characterizes its pathophysiology (Kapsimali et al. 2010). An increased infiltration of both T cells and innate immune cells into the affected organs and tissues has been identified. In particular, excessive Nucleotide-binding oligomerization domain-containing 2 (NOD2) and Toll-like receptor (TLR) expression, responsible for the induction of pro-inflammatory cytokines and Th1 response, has been observed in cells from broncho-alveolar lavage of Behçet's patients with pulmonary manifestations (Hamzaoui et al. 2012). Expansion of Th17 and Th1 cells has also been found in cerebrospinal fluid, neuro-Behçet's disease, and skin lesions. Furthermore, the analysis of transcription factor ratios revealed an increase in the RORC/FOXP3 and TBX21/GATA3 ratios in Behçet's patients with neurological manifestations, indicating plasticity between Th1, Th17, and Treg cells during inflammation (Hamzaoui et al. 2011; Pineton de Chambrun et al. 2012; Shimizu et al. 2012). A high proportion of cytotoxic CD8+CD56+NKT, CD8+CD56+T, and γδT cells has been reported in intraocular infiltration and skin lesions of Behçet's patients (Hamzaoui et al. 2012; Shimizu et al. 2012). An increased level of Th2 cytokines has been assessed in patients with systemic complications (Kuchroo et al. 2012; Pineton de Chambrun et al. 2012).

In addition, susceptibility seems to be associated with HLA-B51, particularly in close family members (Pirim et al. 2004; Remmers et al. 2010; Kapsimali et al. 2010). Association of HLA-B51 has been recognized as the strongest genetic susceptibility factor discovered so far, even though the pathogenic role of HLA-B51 has yet to be clarified, and available data suggest that there is possibly no single mechanism associated with HLA-B51. Furthermore, there is no evidence supporting the use of HLA-B51 as a diagnostic or prognostic marker for Behçet's disease (Gul and Ohno 2012).

Nevertheless, various host genetic factors, apart from HLA, have been demonstrated to modulate susceptibility and the severity of organ and tissue lesions, such as single nucleotide polymorphisms (SNPs) of Th1, Th2, and Th17 (Gregersen and Olsson 2009; Remmers et al. 2010; Cho and Gregersen 2011; Pineton de Chambrun et al. 2012; Zhou et al. 2012). Recent genome-wide studies have demonstrated associations of Th1, Th2, and Th17 SNPs with Behçet's disease

and its systemic manifestations, although other genetic studies have reported contrasting data (Gregersen and Olsson 2009; Remmers et al. 2010; Cho and Gregersen 2011; Pineton de Chambrun et al. 2012; Zhou et al. 2012).

Based on these observations, we typed Interleukin (IL)-12Bp40 (+1188A→C), IL-18 (−137G→C), IL-4RA (+1902A→G), IL-13 (−1055C→T), and IL-10 (−1082G→A, −819C→T) genetic variants in a group of Sicilian patients and age- and gender-matched controls.

Materials and Methods

Patients and Controls

Blood samples, collected in ethylenediamine tetra-acetate sterile tubes, were obtained from 39 Sicilian patients affected by Behçet's disease. They were enrolled at the time of their admission to the Rheumatologic Unit of Palermo University Hospital. All patients (ages 20–50 years; 13 men and 26 women) were diagnosed according to the criteria prepared by the International Study Group for Behcet's Disease (1999; Mignogna et al. 2000). As expected (Pipitone et al. 2004), the most frequent symptoms (Table 1) were oral aphthae and, with lesser frequency, genital aphthae. A minority of patients suffer from ocular or cutaneous involvement or arthritis. These clinical features were observed both as isolated manifestations or as complex pictures.

An age- and gender-matched control group of 128 individuals was also included in our study. They were in good health according to their clinical history and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C reactive protein, liver function tests, iron, and proteins). Furthermore, we selected a homogeneous population for ethnicity and geographic area. Patients and controls belonged to the same ethnic group, since their parents and grandparents were born in western Sicily.

Our study received approval from local ethics committees, and all participants gave their informed consent. Data were encoded to ensure patient and control protection. All measurements were performed without knowledge about the nature of the materials.

Table 1 Frequency of clinical features at disease onset in patients with Behçet's disease

Clinical feature	Frequency
Oral aphthae	71.8
Genital aphthae	23.1
Cutaneous lesions	17.9
Erythema nodosum	10.2
Inflammatory ocular involvement	20.5
Anterior uveitis	12.8
Arthritis	15.4

Genotyping of Th1 and Th2 Cytokine Gene SNPs

DNA from patient and control blood samples was prepared by proteinase K digestion and salt extraction (Miller et al. 1988).

The +1188A/C IL-12Bp40 SNP was genotyped in 35 patients and 128 controls, using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis according to Huang et al. (2000). Using nested PCR, as described by Giedraitis et al. (2001), we identified the −137G/C IL-18 SNP in 32 patients and 128 controls. Through an amplification refractory mutational system (ARMS-PCR) procedure (Cataldo et al. 2003), the +1902A/G IL-4RA SNP was identified in 36 patients and 128 controls and the −1055C/T IL-13 SNP was genotyped in 37 patients and 128 controls. Procedures previously described by Lio et al. (2004) were used to genotype the −1082G/A and −819C/T IL-10 SNPs in 39 patients and 128 controls. In addition, patients and controls were genotyped for the HLA-B51 allele as described by Pirim et al. (2004).

Statistical Analysis

Allele and genotype frequencies were evaluated by gene count. Data were tested for goodness of fit between observed and expected genotype frequencies according to Hardy–Weinberg equilibrium, by a chi-square test. Significant differences in frequencies among groups were calculated using the chi-square test and appropriate tables. Odds ratios with 95% confidence intervals and their significance were calculated.

Results

In the genotype distributions and allele frequencies, very significant differences were observed between patients with Behçet's disease and controls for the SNPs +1188A/C IL-12Bp40, +1902A/G IL-4RA, and −1055C/T IL-13 (Table 2). In particular, the frequencies of the IL-12Bp40 +1188C allele and related C-positive genotypes (+1188C/*) were higher in patients than in controls. In addition, the frequency of the +1902G/* IL-4RA genotype was higher in patients than in controls. Accordingly, overexpression of the +1902G allele was found in patients with respect to controls. We also observed in patients a very significant distribution of −1055T/* genotypes with respect to the controls, and an overexpression of the −1055T allele was found in patients.

In contrast, no statistically significant differences in genotype distributions or allele frequencies were observed for the −137G/C IL-18, −1082G/A, and −819C/T IL-10 SNPs (Table 2). In addition, no statistically significant differences were observed stratifying our results for the presence of the HLA-B51 allele between patients and controls (data not shown). We also evaluated the frequencies of the combined genotypes. Considering all possible combinations, significant differences were observed for only two combinations (Table 3). We found significantly higher frequencies of the IL-12Bp40 +1188C/* IL-18-137GG combination (43 vs. 18%,

Table 2 Genotype distributions and allele frequencies of six SNPs in Behçet's disease patients and matched controls

Gene	Genotype and allele	Behçet's patients	Matched controls	p	OR (95% CI)
IL-12Bp40		$N = 35$	$N = 128$		
	+1188AA	0.38	0.68	0.0005	2.92 (1.6–5.2)
	+1188C/*	0.62	0.32		$p = 0.0005$
	+1188A	0.63	0.83	0.0002	
	+1188C	0.37	0.17		
IL-18		$N = 32$	$N = 128$		
	−137GG	0.60	0.52	NS	
	−137C/*	0.40	0.48		
	−137G	0.75	0.73		
	−137C	0.25	0.27		
IL-4RA		$N = 36$	$N = 128$		
	+1902AA	0.20	0.63	0.000005	2.6 (1.5–4.6)
	+1902G/*	0.80	0.37		$p = 0.001$
	+1902A	0.60	0.80		
	+1902G	0.40	0.20	0.0005	
IL-13		$N = 37$	$N = 128$		
	−1055CC	0.16	0.68	$1.6 \times e^{-7}$	3.57 (2–6.2)
	−1055T/*	0.84	0.32		$p < 0.0001$
	−1055C	0.58	0.83		
	−1055T	0.42	0.17	0.000005	
IL-10		$N = 39$	$N = 128$		
	−1082GG	0.44	0.37	NS	
	−1082A/*	0.56	0.63		
	−1082G	0.60	0.61	NS	
	−1082AG	0.40	0.39		
	−819C/*	0.80	0.90	NS	
	−819TT	0.20	0.10		
	−819C	0.63	0.66		
	−819T	0.37	0.34	NS	

$p = 0.002$) and the IL-4RA +1902G/* IL-13 −1055T/* IL-10 −1082GG combination (23 vs. 9%, $p = 0.03$) in patients.

Discussion

Genetic predisposition and immune deregulation are considered crucial factors for Behçet's pathogenesis, although cellular and molecular mechanisms remain unclear. In particular, the exact role of the cytokine network in the pathophysiology of the disease needs further elucidation (Kapsimali et al. 2010). Recent findings of in vivo or in vitro studies show the promotion of different Th (Th1, Th2, and Th17)

Table 3 Frequency of genotype combinations among patients with Behçet's disease and matched controls

Combined Genotypes	Positive BD patients N(%)	Negative BD patients N(%)	Positive Controls N(%)	Negative Controls N(%)	p	OR (95% CI)
IL-12Bp40 +1188C/* IL-18 −137GG	15 (43%)	20 (57%)	23 (18%)	105 (82%)	0.002	4.4 (1.7–7.6) $p = 0.003$
IL-4RA +1902G/* IL-13 −1055T/* IL-10 −1082GG	8 (23%)	27 (77%)	12 (9%)	116 (91%)	0.03	3.8 (1–7.9) $p = 0.03$

BD Behçet's disease

responses in the complex pathophysiology and its organ and tissue lesions (Kapsimali et al. 2010; Kuchroo et al. 2012; Pineton de Chambrun et al. 2012; Zhou et al. 2012). In addition, several associations have been demonstrated of the Th1, Th2, and Th17 genetic variants with Behçet's disease risk and severity of ocular, pulmonary, vascular, neurological, and intestinal lesions, as extensively and recently reported by Zhou et al. (2012).

In the present study, we investigated some genetic variants of Th1/Th2 cytokine genes in Sicilian Behçet's patients and age- and gender-matched controls. In particular, six functional SNPs were investigated in the promoter or exon regions of the *IL-12Bp40, IL-18, IL-13, IL-4RA,* and *IL-10* genes, which are able to modify cytokine production or Th1/Th2 polarization. SNPs that affect cytokine expression represent disease modifiers and influence the severity or progression of immune-mediated and chronic inflammatory diseases. Cytokine SNPs have been associated with common diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, allergies, asthma, and autoimmune diseases, such as Behçet's disease (Ollier 2004; Hollegaard and Bidwell 2006; Dilek et al. 2009; Pineton de Chambrun et al. 2012). These are all multigenic and multifactorial diseases, involving interactions between genetic, physiological, and environmental factors. Thus, the identification of possible genetic risk factors for Behçet's disease might improve diagnostic accuracy, as well as permit disease stratification for risk assessment and treatment selection (McCarthy et al. 2008; Plomin et al. 2009; Gregersen and Olsson 2009; Remmers et al. 2010; Cho and Gregersen 2011). Individually, however, most disease-associated alleles carry very modest degrees of risk.

Our study results demonstrate associations with +1188A/C IL-12Bp40, +1902A/G IL-4RA, and −1055C/T IL-13 SNPs, but the analysis of odds ratios for single SNPs demonstrates a modest contribution of each single variant to Behçet's disease susceptibility. On the other hand, when the contemporaneous presence of at least three genetic variants was evaluated according to the Th1 or Th2 profiles, our data demonstrated significant differences between patients and controls with respect to the higher odds ratios for the IL-12Bp40 +1188C/* IL-18-137GG and IL-4RA +1902G/* IL-13 −1055T/* IL-10 −1082GG genotype combinations. Thus, our results suggest an involvement of both Th1 and Th2 cytokine pathways in the development of Behçet's disease. Furthermore, they lead to the suggestion that the disease risk increases in individuals carrying a combination of the genetic variants of *IL-12Bp40, IL-18, IL-4RA, IL-13,* and *IL-10*. Our data are partially in agreement with those obtained in a large genome-wide association study of Behçet's patients from different population backgrounds, demonstrating that variants in the IL10 and IL23R–IL12RB2 regions are associated with Behçet's disease risk (Remmers et al. 2010).

In this view, we can hypothesize that various cytokine pathways are involved in the pathogenesis of the disease, including the emerging role of the IL-23/IL-17 axis (Leng et al. 2010; Hamzaoui 2011; Kuchroo et al. 2012). These genetically influenced cytokine profiles might modulate the induction of different effector T CD4+ cell subsets involved in inflammation, tissue injury, and autoantibody production, driving the different clinical profiles that characterize Behçet's disease. The complex interplay between Th1, Th17, Th2, and Treg cell lineages and their involvement in autoimmune diseases such as Behçet's suggests that it may be possible to develop preventive

measures using specific inhibitors, such as monoclonal antibodies, against these cytokines and their receptors. On the other hand, promising data propose an elegant mechanism based on the use of cytokine inhibitors (i.e., monoclonal antibodies for TGF-β and its receptors) for regulating the pathological potential of these cytokines, particularly the cytokines of the emerging T effector subset of CD4+ cells, Th17 cells. This mechanism derives from the discovery that Th17 cells differ from Th1 and Th2 cells. Th1 and Th2 cells have been thought to represent terminal products of their respective developmental programs. Recent studies suggest that Th17 cells are less rigid. In addition to early developmental links to induced Tregs, reflecting the shared requirement for TGF-β, it is now apparent that there is substantial plasticity late in the Th17 program, which allows committed Th17 cells to transition from effectors that produce predominantly IL-17A and IL-17F to effectors that produce predominantly IFNγ. In this setting, a fine balance between RORγt and Foxp3 may be critical for immune homeostasis. This promises new insights into strategies for balancing inflammation and Th responses involved in the pathogenesis of several vascular inflammatory pathologies and autoimmune diseases, such as Behçet's disease, and raises new questions regarding the stability of epigenetic modifications that accompany induction of cytokine gene expression during T cell lineage development (Lee et al. 2009). In light of these observations, a future objective of our studies will be to investigate genetic variants of Th17 and Treg cytokine genes in a very large sample. A limitation of our present study is the small sample size, which may restrict and influence its statistical power. Nevertheless, we have studied a very homogeneous patient population born in western Sicily and clinically followed in a single center. Thus, even if larger cohort studies are necessary in order to definitively assess the true associations of the IL-12Bp40, IL-4RA, and IL-13 alleles with Behçet's disease, our data might trigger innovative therapeutic approaches based on modulation of more components of the cytokine pathways with different possibilities for immune response modulation.

References

Bluestone JA, Mackay CR, O'Shea JJ, Stockinger B (2009) The functional plasticity of T cell subsets. Nat Rev Immunol 9:811

Cataldo F, Lio D, Marino V, Scola L, Crivello A, Mulè AM, Corazza GR, Groups of the SIGEP and Club del Tenue (2003) Cytokine genotyping (TNF and IL-10) in patients with celiac disease and selective IgA deficiency. Am J Gastroenterol 98:850

Cho JH, Gregersen PK (2011) Genomics and the multifactorial nature of human autoimmune disease. New Engl J Med 365:1612

Dilek K, Ozçimen AA, Saricaoğlu H, Saba D, Yücel A, Yurtkuran M, Oral HB (2009) Cytokine gene polymorphisms in Behçet's disease and their association with clinical and laboratory findings. Clin Exp Rheumatol 27:S73

Giedraitis V, He B, Huang WX, Hillert J (2001) Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. J Neuroimmunol 112:146

Gregersen PK, Olsson LM (2009) Recent advances in the genetics of autoimmune disease. Annu Rev Immunol 27:363

Gul A, Ohno S (2012) HLA-B*51 and Behçet disease. Ocul Immunol Inflamm 20:37

Hamzaoui K (2011) Th17 cells in Behçet's disease: a new immunoregulatory axis. Clin Exp Rheumatol 29:S71

Hamzaoui K, Borhani Haghighi A, Ghorbel IB, Houman H (2011) RORC and Foxp3 axis in cerebrospinal fluid of patients with neuro-Behçet's disease. J Neuroimmunol 233:249
Hamzaoui K, Abid H, Berraies A, Ammar J, Hamzaoui A (2012) NOD2 is highly expressed in Behçet disease with pulmonary manifestations. J Inflamm (Lond) 9:3
Hollegaard MV, Bidwell JL (2006) Cytokine gene polymorphism in human disease: on-line databases, supplement 3. Genes Immun 7:269
Huang D, Cancilla MR, Morahan G (2000) Complete primary structure, chromosomal localisation, and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. Genes Immun 1:515
International Study Group for Behcet's Disease (1999) Criteria for diagnosis of Behçet's disease. Lancet 335:1078
Kapsimali VD, Kanakis MA, Vaiopoulos GA, Kaklamanis PG (2010) Etiopathogenesis of Behçet's disease with emphasis on the role of immunological aberrations. Clin Rheumatol 29:1211
Kuchroo VK, Ohashi PS, Sartor RB, Vinuesa CG (2012) Dysregulation of immune homeostasis in autoimmune diseases. Nat Med 18:42
Lee YK, Mukasa R, Hatton RD, Weaver CT (2009) Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol 21:274
Leng RX, Chen GM, Pan HF, Ye DQ (2010) The role of IL-23/IL-17 axis in the etiopathogenesis of Behçet's disease. Clin Rheumatol 29:1209
Lio D, Candore G, Crivello A, Scola L, Colonna-Romano G, Cavallone L, Hoffmann E, Caruso M, Licastro F, Caldarera CM, Branzi A, Franceschi C, Caruso C (2004) Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. J Med Genet 41:790
McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, Hirschhorn JN (2008) Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9:356
Mignogna MD, Fedele S, Lo Russo L (2000) International diagnostic criteria and delay of diagnosis in Behçet's disease. J Rheumatol 27:2725
Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215
Ollier WE (2004) Cytokine genes and disease susceptibility. Cytokine 28:174
Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D (2012) New insights into the pathogenesis of Behçet's disease. Autoimmun Rev 11:687
Pipitone N, Boiardi L, Olivieri I, Cantini F, Salvi F, Malatesta R, La Corte R, Triolo G, Ferrante A, Filippini D, Paolazzi G, Sarzi-Puttini P, Restuccia G, Salvarani C (2004) Clinical manifestations of Behçet's disease in 137 Italian patients: results of a multicenter study. Clin Exp Rheumatol 22:S46
Pirim I, Atasoy M, Ikbal M, Erdem T, Aliagaoglu C (2004) HLA class I and class II genotyping in patients with Behcet's disease: a regional study of eastern part of Turkey. Tissue Antigens 64:293
Plomin R, Haworth CM, Davis OS (2009) Common disorders are quantitative traits. Nat Rev Genet 10:872
Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, Le JM, Yang B, Korman BD, Cakiris A, Aglar O, Emrence Z, Azakli H, Ustek D, Tugal-Tutkun I, Akman-Demir G, Chen W, Amos CI, Dizon MB, Kose AA, Azizlerli G, Erer B, Brand OJ, Kaklamani VG, Kaklamanis P, Ben-Chetrit E, Stanford M, Fortune F, Ghabra M, Ollier WE, Cho YH, Bang D, O'Shea J, Wallace GR, Gadina M, Kastner DL, Gül A (2010) Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R–IL12RB2 regions associated with Behçet's disease. Nat Genet 42:698
Shimizu J, Takai K, Fujiwara N, Arimitsu N, Ueda Y, Wakisaka S, Yoshikawa H, Kaneko F, Suzuki T, Suzuki N (2012) Excessive CD4 + T cells co-expressing interleukin-17 and interferon-γ in patients with Behçet's disease. Clin Exp Immunol 168:68
Zhou ZY, Chen SL, Shen N, Lu Y (2012) Cytokines and Behcet's disease. Autoimmun Rev 11:699
Zygmunt B, Veldhoen M (2011) T helper cell differentiation more than just cytokines. Adv Immunol 109:159