Behcet's disease: An update on the pathogenesis

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ABSTRACT
Behçet's disease is a chronic inflamma - tory disorder of unknown etiology. It has long been postulated that immuno - logical abnormalities, which are possi - bly induced by microbial pathogens in genetically susceptible individuals, are important in its pathogenesis. Recent findings have both supported the signif - icance of genetic factors and better defined the nature of inflammation in Behçet's disease. Molecular genetic studies have strengthened the primary association of HLA-B51 with Behçet's disease. The exact pathogenic mecha - nism of the HLA-B51 molecule is still unknown, and its contribution to the overall genetic susceptibility to Beh - çet's disease is estimated to be less than 20%. Spontaneous and/or induced overexpression of pro-inflammatory cytokines (mainly Th1 type) from vari - ous cellular sources seems responsible for the enhanced inflammatory reac - tion in Behçet's disease, and it may be associated with the genetic susceptibil - ity. An antigen-driven immune response superimposed on this primed-state and induced by heat shock proteins or other peptides from different strains of strep - toccocci or other microbial agents has been suggested to trigger manifesta - tions of Behçet's disease. Endothelial activation/injury and the resultant oc - clusive vasculopathy may also contri - bute to the tissue damage.

Introduction
Behçet's disease is a chronic inflamma-tory disorder characterized mainly by recurrent oral aphthous ulceration, gen-ital ulceration, skin lesions and uveitis. Behçet's disease is now recognized as a multisystem vasculitis, which can also affect all types and sizes of blood ves-sels, joints, lungs, central nervous and gastrointestinal systems (1, 2).
The etiopathogenesis of Behçet's dis-ease is unknown. It has long been pos-tulated that immunological abnormali-ties, which are possibly induced by mi-crobial pathogens in genetically sus-ceptible individuals, are important in its pathogenesis (3-5). Recent findings have both supported the significance of genetic factors and better defined the nature of inflammation in Behçet's di-sease.

Genetic susceptibility to Behçet's disease
Behçet's disease has a distinct epidemi-ological feature. It is seen more preva-lently in a geographic area extending from the Mediterranean basin to Japan, between 30° and 45° latitudes North (6). This region fits well to the ancient Silk Road and also overlaps with a higher frequency of HLA-B51 in the healthy population (6, 7). It was sug gested that the genetic susceptibility to Behçet's disease might have spread via nomadic tribes or immigrating Turks along this route (7). Mitochondrial DNA studies, which indicate trading of genes bet-ween Eastern Asians and Europeans travelling on the Silk Road, also sup-port that peculiar geographic distribu-tion of Behçet's disease might have a genetic basis (8).
Behçet's disease is not a genetic dis-ease with a Mendelian inheritance model. The majority of patients are sporadic cases with no family history. However, a familial aggregation of Behçet's disease patients has long been noted, and an increased disease risk has been observed among first degree rela-tives (9, 10). Sibling recurrence risk rate was found to be 4.2% in Turkish patients, and Koné-Paut and colleagues reported a higher r ate of 10% for juve-nile patients (9, 10). Sibling recurrence risk ratio (s) of Behçet's disease was estimated to be 11.4-52.5 in Turkey (9), which indicates a strong genetic back-ground. Analysis of multicase families supported a complex genetic inheri-tance model, and a genetic anticipation in the form of earlier disease onset in the children of affected parents, was observed in some families (11, 12).
Association of HLA-B51 with Behçet's disease is the strongest evidence sup-porting the involvement of genetic fac-tors in its pathogenesis (7, 13). It has long been investigated whether HLA-B51 has a direct role in the pathogene-sis, or whether this association reflects linkage disequilibrium with a suscepti-bility gene for Behçet's disease located close to the HLA-B locus. The tumor necrosis factor (TNF) and lymphotoxin genes, which are located centromeric to HLA-B, attracted researchers as possi-ble candidates for Behçet's disease sus-ceptibility. An allelic association bet-

ween TNF locus and Behçet's disease was demonstrated using microsatellite and restriction fragment length polymorphisms (14-16). Investigation of the genomic segment between the TNF and HLA-B loci revealed a stronger association of Behçet's disease with the MHC class I chain related gene A (MICA), which is located 46-kb centromeric to HLA-B (17). The MICA gene is expressed in fibroblasts, epithelial cells, endothelial cells and monocytes. It was proposed that this expression pattern might explain the inflammation sites in Behçet's disease, and that antigen presentation by the MICA molecules to T cells, which are known to be increased in Behçet's disease, might be the pathogenic mechanism (17). However, it was shown that the polymorphism of the MICA molecule has no role in antigen presentation to T cells in the intestinal epithelium (18), and the MICA antigen does not seem to be expressed on the cell membrane of the keratinocytes and monocytes despite the detection of mRNA (19).

Fine mapping studies using highly polymorphic microsatellites confirmed the critical region for Behçet's disease as 46-kb segment between the MICA and HLA-B loci (20). Allelic association, genotypic differentiation and stratification analyses in different ethnic groups have proved that HLA-B51 is showing the strongest association with Behçet's disease, and all other associations including the MICA, is due to linkage disequilibrium with HLA-B51 (21-23). However, since the linkage disequilibrium can extend very long distances within the MHC region, it is still difficult to assess the individual supplementary effects of the MICA or other neighbouring genes on a HLA-B51 carrying haplotype to the Behçet's disease susceptibility.

Different hypotheses have been generated to explain the direct pathogenic role of HLA-B51 in Behçet's disease. HLA-B51 is one of the split antigens of HLA-B5, and it differs only 2 aminoacids from the other -B5 split antigen, HLA-B52, which is not associated with Behçet's disease. Asparagine and phenylalanine at positions 63 and 67 of the

1 helix of the HLA-B51 molecule are replaced with glutamic acid and serine at the same positions in the HLA-B52 (13). These 2 aminoacids constitute the B pocket of the antigen binding groove, and determine the motif of the peptides that can bind. Presentation of certain Behçet's disease-associated peptides from different microbial antigens with HLA-B51 might be a mechanism in the pathogenesis. No HLA-B51 restriction could be demontrated so far in experiments using peptides which are thought to be Behçet's disease-specific (24).

Twenty-four different HLA-B51 alleles (HLA-B*5101-B*5124) have been described by now. They all share the same aminoacid sequence at the B pocket of the antigen binding groove except B*5107 and B*5120. Molecular HLA-B51 typing in different ethnic groups suggest that –B51 alleles in patients with Behçet's disease are not different from those in healthy controls (16, 25-28).

Cross-reactivity between HLA-B51 and organ-specific antigens is another mechanism postulated in the pathogenesis of Behçet's disease. Misfolding or aberrant assembly of HLA class I heavy chains as well as enhanced expression due to up-regulated immune response increase the possibility of presentation of HLA class I-derived peptides by HLA class II molecules. Wildner *et al.* identified a polymorphic HLA-B sequence common in HLA-B27, -B51 and several other HLA-B alleles (B27PD), which shows aminoacid homology with retinal soluble antigen (S-Ag)-derived peptide (29). This peptide can induce anterior uveitis in rats (29), and an oral tolerance could also be elicited using B27PD peptide in patients with uveitis (30). Kurhan-Yavuz and colleagues demonstrated increased T cell response against retinal S-Ag, retinal S-Ag derived peptide and B27PD peptide in Behçet's disease patients with posterior uveitis compared with those with non-Behçet anterior uveitis or Behçet's patients without eye disease (31).

Another function of HLA class I molecules has recently been described by the identification of a new family of receptors expressed mainly by natural

killer (NK) cells and also CD8+ and T cell receptor (TCR)+ cells (32-34). These killer immunoglobulin-like receptors (KIR), bind to conserved epitopes at residues 77-83 of the 1-helix, which are shared by different allellic groups of HLA class I molecules. Engagement of these receptors has been shown to be associated with selective inhibition of NK cell or T cell mediated cytotoxicity. It may be an alternative hypothesis that the pathogenic role of HLA-B51 in Behçet's disease might also involve an interaction with KIR (KIR3DL1) molecules on inflammatory cells (28, 35, 36).

HLA-transgenic animal models are quite helpful for investigation of HLA and disease associations. There is only one HLA-B*5101 heavy chain transgenic mouse model published by now. These animals did not develop any Behçet's disease-related manifestation, and only showed an increased neutrophil activity following f-Met-Leu-Phe (fMLP) stimulation compared to HLA-B35 and non-transgenic mice (37). A similar enhanced neutrophil activity was reported in HLA-B51 positive healthy individuals (37, 38). The association of HLA-B51 with neutrophil functions remains to be clarified. We still need more HLA-B51 transgenic animal models with and without human 2-microglobulin in different strains of mice and rats to explore the role of HLA-B51 in the pathogenesis of Behçet's disease.

Analysis of a small group of multicase families has confirmed the genetic linkage of the HLA-B *locus* to Behçet's disease by using the transmission disequilibrium test (39). The same study has also indicated that the contribution of the HLA-B *locus* to the overall genetic susceptibility to Behçet's disease is less than 20%, and identification of other susceptibility *loci,* which constitute the majority of genetic contribution, is awaited (39). A recent linkage study in 28 multicase families of Turkish origin has revealed a novel susceptibility *locus* for Behçet's disease in 6p22-p23, telomeric to the MHC region (40). This new *locus* needs to be fine-mapped and confirmed in other ethnic groups.

Microbial agents and Behçet's disease

Microbial pathogens have been postulated as either a causative agent or a disease-triggering factor in Behçet's disease. Professor Behçet himself suggested a viral etiology for the disease (41). Some researchers claimed that they had isolated a virus from the lesions of Behçet's disease (42-44). However, this could not be confirmed by others. The presence of *Herpes simplex* virus (HSV) type 1 genome within the peripheral blood lymphocytes of Behçet's disease patients was demonstrated by hybridization of viral DNA probes to the complementary RNA or amplification by polymerase chain reaction (PCR) (45,46). A 289-bp genomic HSV type 1 sequence was also amplified by PCR from the saliva, genital and gastrointestinal ulcers of patients with Behçet's disease (47, 48). Sohn and colleagues developed an animal model with the inoculation of HSV type 1 to the ICR mice, and about 30% of the mice showed various manifestations, some of which resembling to those of Behçet's disease (49). However, none of these findings are specific to Behçet's disease, and they could not support a direct causative role for HSV in the etiology of Behçet's disease.

Involvement of streptococcal antigens have also long been claimed in the pathogenesis of Behçet's disease. An increased skin reactivity to streptococcal antigens was reported, and these antigens induced systemic Behçet's disease manifestations in some patients during skin testing (50, 51). Flare of the manifestations were observed after dental treatment or extraction (50). Many observers including Behçet reported a higher frequency of the history of dental caries, periodontitis or tonsillitis in patients with Behçet's disease, possibly related to streptococcal infections (52-54). An increased percentage *Streptococcus sanguis* colonies was found in the oral flora of patients with Behçet's disease (55). Antibodies reactive with uncommon serotypes of *S. sanguis* KTH-1, KTH-2 and KTH-3 [now, all identified as *S. oralis* (56)] were detected in the sera of Behçet's disease patients and in none of the patients with rheumatoid arthritis and healthy controls (57).

Involvement of heat shock protein (hsp) in the pathogenesis of Behçet's disease was proposed as a common denominator between different microbial etiological factors which also show significant homology with human mitochondrial hsp. Certain epitopes of microbial hsp were suggested as antigens triggering a specific immune response and producing a cross-reacting inflammatory reaction (57). Four peptides from 65-kD mycobacterial hsp (111-125, 154-172, 219-233, 311-325) and their homologous peptides from human 60-kD hsp (136-150, 179-197, 224-258, 336-351) were identified by T-cell epitope mapping using short term cell lines from British patients with Behçet's disease (24). An increased T-cell response against these peptides has later been shown in the Japanese and Turkish patients (58, 59). The hsp peptides induced anterior uveitis in Lewis rats by subcutaneus immunization in complete Freund adjuvant with intraperitoneal *Bordatella pertussis* (60). The most uveitogenic peptide was the 336-351 from the human 60-kD hsp. This peptide elicited uveitis also by oral or nasal mucosal administration, and anti-CD4 antibodies prevented the development of uveitis (61). However, none of the rats developed a posterior uveitis and/or retinal vasculitis similar to uveitis of Behçet's disease, and no other finding was detected in the mouth, skin and external genitalia of the animals.

Immunological abnormalities

Non-specific hyperactivity (The pathergy phenomenon)

Non-specific increased inflammatory response is an important feature of Behçet's disease. The classical example is the skin pathergy reaction, which is characterized by the development of a papule or pustule following a simple needle prick to the skin, which is similar to those appearing spontaneously in the disease (62). This increased responsiveness to minor trauma or other stimuli is not unique to skin, and the pathergy phenomenon can be observed at other body sites or even at the cellular level as an up-regulated inflammatory response (63).

Increased expression of several cytokines from lymphocytes and monocytes is reported in Behçet's disease (4, 58, 63-66). Over-secretion of mainly Th1 type proinflammatory cytokines is prominent during especially active phase of the disease (66). However, increased secretion of interleukin-1 (IL-1), IL-6, TNF and IL-8 from monocytes following lipopolysaccharide (LPS) stimulation compared with healthy and/or disease controls can also be observed even in inactive patients (64).

Activation of neutrophils with increased chemotaxis and superoxide generation, and enhanced adhesion molecule expression was long suggested as the main pathogenic mechanism in Behçet's disease (4, 37, 67, 68). Enhanced superoxide generation after fMLP stimulation suggests that neutrophils are primed *in vivo* in Behçet's disease (4,37). Increased production of some cytokines such as IL-8, TNF and IL-1 from lymphomononuclear cells and/or endothelial cells may have a regulatory role in neutrophil functions and might explain this primed state. It has recently been demonstrated that neutrophils from patients with Behçet's disease constitutively express TNF- mRNA and produce increased amounts of TNF- with LPS stimulation (4, 69, 70). Behçet's disease neutrophils also produce IL-12 and IL-18 spontaneously, which may play a key role in the generation of Th1 immune responses. Enhanced TNF- production might help to auto-prime neutrophils and prolong their own life-span, which might result in accumulation of activated neutrophils in the site of inflammation (70) Although the relationship of HLA-B51 with neutrophil functions is not clear, genetic factors seem to play an important role in the non-specific enhanced inflammatory reaction in Behçet's disease.

Antigen-driven immune response

Recent studies has revealed the central role of T-cell mediated immune response in the pathogenesis of Behçet's disease (24, 58, 59, 63, 65, 66, 71-74). Histopathological investigations de-

monstrated mainly T-cell dominated perivascular infiltrates in involved tissues (4, 62, 75, 76). Several phenotypical and functional abnormalities were reported in both + and + T-cells in Behçet's disease. Although there are conficting reports about the number of CD4+ and CD8+ T-cells in the peripheral blood of Behçet's disease patients, an increase in the proportions of T-cells and CD8+ T-cells was consistently found (65, 71-74).

Intracytoplasmic cytokine expression of individual cells by flow cytometry showed increased percentage of IL-2 and IFN- producing T-cells in active patients with Behçet's disease (66,77). High serum levels of IL-12 in parallel with an increased frequency of peripheral IL-2 and IFN- producing T cells support a strong, polarized Th1 immune response *in vivo* (66). A decrease of Th1 type T cells was detected after the treatment of active patients with immunosuppressant drugs. On the other hand, no increase in Th2 type IL-4 producing T cells was observed during inactive stage of patients with Behçet's disease (66, 77).

Analysis of + T-cell population also revealed that significantly increased proportion of + T-cells were in activated stage and producing both IFN- and TNF- (65, 78). In contrast to the + T-cells, the chain of IL-2R (CD25) expression on + T-cells was found to be increased. Yamashita *et al.* reported that CD45RA$^+$ + T-cells produced more TNF- and TNF- than CD45RO$^+$ + T-cells, while both subsets were producing equal amounts of IL-8, but no IL-4 at all (72).

Oligoclonal T-cell expansions which correlate with clinical activity of the patients have proved the contribution of the antigen driven immune response to the immunopathogenesis of Behçet's disease (58, 71). Several antigens have been found to stimulate T-cells in Behçet's disease. T-cell can be stimulated by streptococcal antigens to produce IL-6, IFN- or neutrophil potentiating factors (79, 80). Also, hsp-derived peptides can stimulate T-cell proliferation in Behçet's disease (24). Hasan and colleagues reported a significant proliferative response to four mycobacterial hsp-derived peptides in specifically subset of T-cells, and also observed a correlation between disease activity and T-cell response (74). They suggested that these + T-cells may have a regulatory role on + T-cells. Kaneko *et al.* found an antigen-driven oligoclonal expansion in CD4+ subset of T-cells in response to the 336-351 peptide from the human 60-kD hsp in Japanese patients with Behçet's disease (58). An association between the T-cell proliferative response to this peptide and the presence of ocular lesions was also observed (58). Proliferating T-cells expressed proinflammatory cytokine mRNAs, including IL-8, TNF- , TNF- (58). It has been suggested that hsp-stimulated T-cells may cross react with oral mucosal or retinal tissues because of a molecular mimicry between bacterial and self hsp molecules (57, 81).

Hirohata *et al.* showed that this hypersensitivity of Behçet's disease T cells is not restricted to the disease-specific hsp peptides (63). Low concentrations of staphylococcal enterotoxins could stimulate Behçet's disease T-cells through TCR chain for IFN- production, much more effectively than normal or rheumatoid arthritis T-cells (63).

Although their total number was within normal range, some functional abnormalities were defined in B-cells in Behçet's disease. Elevated numbers of spontaneously immunoglobulin secreting B-cells, increased percentage of activated and memory B-cells and IgA isotype of B-cells were found in active patients (73, 82, 83). B-cell epitope mapping with the 65-kD mycobacterial hsp in Behçet's disease showed significant increases in IgA and IgG antibodies against the peptides overlapping with the T cell epitopes (84).

Some investigators have reported increased number of NK cells in patients with clinically active Behçet's disease (85, 86). Suzuki and colleagues showed increased percentages of both NK (CD16 + CD56 +) cells and CD56+ T-cells (86). On the other hand, Eksioglu-Demiralp *et al.* found increased CD4+CD16+ and CD4+CD56+ T cell subsets in Behçet's disease, but could not demonstrate elevated levels of CD16+CD56+ NK cells in their series (87). And Hamzaoui and colleagues observed a decrease in CD16+ cells in active patients (88). NK cell activity measured by K562 cytotoxicity was found to be significantly low, and it was explained by the increase of immature NK cells in active patients. The expression of CD16 and CD56 antigens on many different cells, the activity and type of manifestations, immunosuppressant drugs and the heterogeneity in the genetic and triggering factors might partly explain the conflicting results, and it needs to be further investigated in longitudinal studies. Data about the KIR expression on NK and CD56+ T-cells and their interaction with HLA molecules are also lacking in Behçet's disease, and it may be important in the pathogenesis.

Endothelial activation/injury

Behçet's disease is recognized as an unclassified systemic vasculitis, which affects all types and sizes of blood vessels. A thrombotic tendency with a predilection for the venous side of the vasculature is well-known feature of Behçet's disease. Hemostatic investigations have shown findings not specific for Behçet's disease, but consistent with both activation of coagulation system and fibrinolytic activity, reflecting endothelial activation and/or injury (89-92). It has been suggested that activation of endothelial cells by perivascular infiltrates composed of activated mononuclear cells and neutrophils is the origin of thrombotic tendency of Behçet's disease. However, endothelial activation markers do not differ between Behçet's disease patients with and without thrombosis.

The presence of procoagulant mutations, such as the factor V Leiden or the prothrombin gene mutation increases the risk of thrombosis in Behçet's disease (93-95). Circulating anticardiolipin and anti-endothelial cell antibodies were reported in some series (96-99). The contribution of these antibodies to the endothelial injury and/or thrombotic tendency of Behçet's disease is speculative, and many believe that they reflect disease activity and

develop secondary to tissue injury.

Hormonal factors

Hormonal factors may also have a place in the pathogenesis of Behçet's disease which remains to be identified. Although the number of female patients is almost equal to the number of males in many series, Behçet's disease has a more severe disease course in men (100, 101). It can be argued that female hormones may have a protective role on the endothelial activation, and may prevent the progression of Behçet's disease (102, 103).

Conclusions

Behçet's disease is a chronic inflammatory disorder with exacerbations in the oral and genital mucosa, skin, eyes, joints, blood vessels, lungs, brain and intestines. Recent studies have supported the direct role of HLA-B51 in the pathogenesis of Behçet's disease. The exact mechanism of action of HLA-B51 molecule is still unknown, and its contribution to the overall genetic susceptibility to Behçet's disease is estimated to be less than 20%. Spontaneous and/or induced over-expression of pro-inflammatory Th1 type cytokines from various cellular sources seems responsible for the enhanced inflammatory reaction in Behçet's disease, and it may be associated with the genetic susceptibility. An antigen-driven immune response superimposed on this primed-state and induced by hsp (or other) peptides from different strains of streptoccocci or other microbial agents has been suggested to trigger manifestations of Behçet's disease. Endothelial activation/injury and the resultant occlusive vasculopathy may also contribute to the tissue damage.

References

1. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999; 341: 1284-91.
2. YAZICI H, YURDAKUL S, HAMURYUDAN V: Behçet's syndrome. *Curr Opin Rheum* 1999; 11: 53-7.
3. LEHNER T: The role of heat shock protein, microbial and autoimmune agents in the aetiology of Behçet's disease. *Int Rev Immunol* 1997; 14: 21-32.
4. SAKANE T, SUZUKI N, NAGAFUCHI H: Etiopathology of Behcet's disease: immunological aspects. *Yonsei Med J* 1997; 38: 350-8.
5. LEHNER T: Immunopathogenesis of Behcet's disease. *Ann Med Interne* (Paris) 1999; 150: 483-7.
6. VERITY DH, MARR JE, OHNO S, WALLACE GR, STANFORD MR: Behçet's disease, the Silk Road and HLA-B51:Historical and geographical perspectives. *Tissue Antigens* 1999; 54: 213-20.
7. OHNO S, OHGUCHI M, HIROSE S, MATSUDA H, WAKISAKA A, AIZAWA M: Close association of HLA-Bw51 with Behçet's disease. *Arch Ophthalmol* 1982; 100: 1455-8.
8. COMAS D, CALAFELL F, MATEU E, *et al.*: Trading genes along the Silk Road: mtDNA sequences and the origin of Central Asian populations. *Am J Hum Genet* 1998; 63: 1824-38.
9. GÜL A, INANÇ M, OCAL L, ARAL O, KONIÇE M: Familial aggregation of Behçet's disease in Turkey. *Ann Rheum Dis* 2000; 59: 622-5.
10. KONÉ-PAUT I, GEISLER I, WECHSLER B, *et al.*: Familial agregation in Behçet's disease: High frequency in siblings and parents of pediatric probands. *J Pediatr* 1999; 135: 89-93.
11. BIRDSTEWART JA: Genetic analysis of families of patients with Behçet's syndrome: Data incompatible with autosomal recessive inheritance. *Ann Rheum Dis* 1986; 45: 265-8.
12. FRESKO I, SOY M, HAMURYUDAN V, *et al.*: Genetic anticipation in Behçet's syndrome. Ann Rheum Dis 1998; 57: 45-8.
13. MIZUKI N, INOKO H, OHNO S: Pathogenic gene responsible for the predisposition to Behçet's disase. *Intern Rev Immunol* 1997; 14: 33-48.
14. MIZUKI N, INOKO H, SUGIMURA K, *et al.*: RFLP analysis in the TNF- gene and the susceptibility to alloreactive NK cells in Behçet's disease. *Invest Ophthalmol Vis Sci* 1992; 33: 3084-90.
15. MIZUKI M, OHNO S, SATO T, *et al.*: Microsatellite polymorphism between the tumor necrosis factor and HLA-B genes in Behçet's disease. *Hum Immunol* 1995; 43: 129-35.
16. VERITY DH, WALLACE GR, VAUGHAN RW, *et al.*: HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behçet's disease. *Tissue Antigens* 1999; 54: 264-72.
17. MIZUKI N, OTA M, KIMURA M, *et al.*: Triplet repeat polymorphism in the transmembrane region of the MICA gene: A strong association of six GCT repetitions with Behçet's disease. *Proc Natl Acad Sci* USA 1997; 94: 1298-303.
18. GROH V, STEINLE A, BAUER S, SPIES T: Recognition of stress-induced MHC molecules by intestinal epithelial cells. *Science* 1998; 279: 1737-40.
19. ZWIRNER NW, DOLE K, STASTNY P: Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes. *Hum Immunol* 1999; 60:323-30.
20. OTA M, MIZUKI N, KATSUYAMAY, *et al.*: The critical region for Behçet's disease in the human major histocompatibility complex is reduced to a 46-kb segment centromeric of HLA-B, by association analysis using refined microsatellite mapping. *Am J Hum Genet* 1999; 64: 1406-10.
21. MIZUKI N, OTA M, KATSUYAMA Y, *et al.*: Association analysis between the MIC-A and HLA-B alleles in Japanese patients with Behçet's disease. *Arthritis Rheum* 1999; 42: 1961-6.
22. WALLACE GR, VERITY DH, DELAMAINE LJ, *et al.*: MIC-A allele profiles and HLA class I associations in Behçet's disease. *Immunogenetics* 1999; 49: 613-17.
23. MIZUKI N, OTA M, YABUKI K, *et al.*: Localization of the pathogenic gene of Behcet's disease by microsatellite analysis of three different populations. *Invest Ophthalmol Vis Sci* 2000; 41: 3702-8.
24. PERVIN K, CHILDERSTONE A, SHINNICK T, *et al.*: T cell epitope expression of mycobacterial and homologous human 65-kilodalton heat shock protein peptides in short term cell lines from patients with Behçet's disease. *J Immunol* 1993; 151: 1-10.
25. MIZUKI N, INOKO H, ANDO H, *et al.*: Behçet's disease associated with one of the HLA-B51 subantigens, HLA-B*5101. *Am J Ophthalmol* 1993; 116: 406-9.
26. MIZUKI N, INOKO H, ISHIHARA M, *et al.*: A complete type patient with Behçet's disease associated with HLA-B*5102. *Acta Ophthalmologica* 1994; 72: 757-8.
27. MIZUKI N, OHNO S, ANDO H, *et al.*: A strong association of HLA-B*5101 with Behçet's disease in Greek patients. *Tissue Antigens* 1997; 50: 57-60.
28. KERA J, MIZUKI N, OTA M, *et al.*: Significant associations of HLA-B*5101 and B*5108, and lack of association of class II alleles with Behçet's disease in Italian patients. *Tissue Antigens* 1999; 54: 565-71.
29. WILDNER G, THURAU SR: Cross-reactivity between an HLA-B27-derived peptide and a retinal autoantigen peptide: a clue to a major histocompatibility complex association with autoimmune disease. *Eur J Immunol* 1994; 24: 2579-85.
30. THURAU SR, DIEDRICHS-MÖHRING M, FRICKE H, ARBOGAST S, WILDNER G: Molecular mimicry as a therapeutic approach for an autoimmune disease: oral treatment of uveitis patients with an MHC-peptide cross-reactive with autoantigen-first results. *Immunol Letters* 1997; 57: 193-201.
31. KURHAN-YAVUZ S, DIRESKENELI H, BOZKURT N, *et al.*: Anti-MHC autoimmunity in Behçet's disease:T cell responses to an HLA-B-derived peptide cross-reactive with restinal-S antigen in patients with uveitis. *Clin Exp Immunol* 2000; 120: 162-6.
32. LITWIN V, GUMPERZ J, PARHAM P, PHILLIPS JH, LANIER LL: NKB1: A natural killer cell receptor involved in the recognition of polymorhic HLA-B molecules. *J Exp Med* 1994; 180: 537-43.
33. CELLA M, LONGO A, FERRARA GB, STROMINGER JL, COLONNA M: NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. *J Exp Med* 1994; 180: 1235-42.
34. LANIER LL: NK cell receptors. *Ann Rev Immunol* 1998; 16: 359-93.
35. MINGARI MC, MORETTA A, MORETTA L: Regulation of KIR expression in human T cells: a safety mechanism that may impair protective T cell responses. I*mmunol Today* 1998; 19: 153-7.
36. GÜL A, UYAR FA, INANÇ M, *et al.*: A weak

association of HLA-B*2702 with Behçet's disease in Turkey (abstract). *Yonsei Med J* 2000; 41 (Suppl): 38.

37. TAKENO M, KARIYONE A, YAMASHITA N, *et al.*: Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice. *Arthritis Rheum* 1995; 38: 426-33.

38. SENSI A, GAVIOLI R, SPISANI S, *et al.*: HLA B51 antigen associated with neutrophil hyper-reactivity. *Dis Markers* 1991; 9: 327-31.

39. GÜL A, HAJEER AH, WORTHINGTON J, BARRET JH, OLLIER WER, SILMAN AJ: Evidence for linkage of the HLA-B locus in Behçet's disease, obtained using the transmission disequilibrium test. *Arthritis Rheum* 2001; 44: 239-41.

40. GÜL A, HAJEER AH, WORTHINGTON J, OLLIER B, SILMAN AJ: Linkage mapping of a novel susceptibility locus for Behçet's disease to chromosome 6p22-23. *Arthritis Rheum* 2001 (in press).

41. BEHÇET H: Über rezidivierende, aphthöse, durch ein Virus verursachte Geschwüre am Mund, am Auge und an den Genitalien. *Dermatologische Wochenschrift* 1937; 105:1152-7.

42. SEZER FN: The isolation of a virus as the cause of Behçet's disease. *Am J Ophthalmol* 1953; 36: 301-15.

43. EVANS AD, PALLIS CA, SPILLANE JD: Involvement of the nervous system in Behçet's syndrome: report of three cases and isolation of virus. *Lancet* 1957; 2:349-53.

44. MORTADA A, IMAM IZE: Virus aetiology of Behçet's disease. *Br J Ophthalmol* 1964; 48: 250-9.

45. EGLIN RP, LEHNER T, SUBAK-SHARPE JH: Detection of RNA complementary to *herpes simplex* virus in mononuclear cells from patients with Behçet's syndrome and recurrent oral ulcers. *Lancet* 1982; 2: 1356-61.

46. STUDD M, McCANCE DJ, LEHNER T: Detection of HSV-1 DNA in patients with Behçet's syndrome and in patients with recurrent oral ulcers by the polymerase chain reaction. *J Med Microbiol* 1991; 34: 39-43.

47. LEE S, BANG D, CHO YH, LEE ES, SOHN S: Polymerase chain reaction reveals *herpes simplex* virus DNA in saliva of patients with Behçet's disease. *Arch Dermatol Res* 1996; 288: 179-83.

48. SOHN S: Etiopathology of Behçet's disease; *Herpes simplex* virus infection and animal model. *Yonsei Med J* 1997; 38: 359-64.

49. SOHN S, LEE ES, BANG D, LEE S: Behçet's disease-like symptoms induced by the *Herpes simplex* virus in ICR mice. *Eur J Dermatol* 1998; 8: 21-3.

50. MIZUSHIMA Y, MATSUDA T, HOSHI K, *et al.*: Induction of Behçet's disease symptoms following dental treatment and streptococcal antigen skin test. *J Rheumatol* 1998; 15: 1029-30.

51. THE BEHÇET'S DISEASE RESEARCH COMMITTEE OF JAPAN: Skin hypersensitivity to streptococcal antigens and the induction of systemic symptoms by the antigens in Behçet's disease-A multicenter study. *J Rheumatol* 1989; 16: 506-11.

52. MIZUSHIMA Y: Streptococcus and Behçet's disease. *In:* O'DUFFY JD, KOKMEN E (Eds.): *Behçet's Disease:Basic and Clinical Aspects*. New York, Marcel Dekker, Inc., 1991: 421-6.

53. AOKI K,OHNO S: Studies on the constitution and past history of patients with Behçet's disease. *Acta Soc Ophthalmol Jpn* 1972; 76: 1608-12.

54. COOPER C, PIPPARD EC, SHARP H, WICKHAM C, CHAMBERLAIN MA, BARKER DJP: Is Behçet's disease triggered by childhood infection? *Ann Rheum Dis* 1989; 48: 421-3.

55. ISOGAI E, ISOGAI H, YOSHIKAWA K, *et al.*: Microbial ecology of oral flora in Behçet's disease. *In:* O'DUFFY JD, KOKMEN E (Eds.): *Behçet's Disease:Basic and Clinical Aspects*, New York, Marcel Dekker, Inc., 1991: 405-14.

56. NARIKAWA S, SUZUKI Y, TAKAHASHI M, FURUKAWA A, SAKANE T, MIZUSHIMA Y: Streptococcus oralis previously identified as uncommon *Streptococcus sanguis* in Behçet's disease. *Arch Oral Biol* 1995; 40: 685-90.

57. LEHNER T, LAVERY E, SMITH R, VAN DER ZEE R, MIZUSHIMA Y, SHINNICK T: Association between the 65-kilodalton heat shock protein, *Streptococcus sanguis*, and the corresponding antibodies in Behçet's syndrome. *Infect Immun* 1991; 59: 1434-41.

58. KANEKO S, SUZUKI N, YAMASHITA N, *et al.*: Characterization of T cells specific for an epitope of human 60-kD heat shock protein (hsp) in patients with Behçet's disease (BD) in Japan. *Clin Exp Immunol* 1997; 108: 204-12.

59. DIRESKENELI H, EKSIOGLU-DEMIRALP E, YAVUZ S, *et al.*: T cell responses to 60/65 kDa heat shock protein derived peptides in Turkish patients with Behçet's disease. *J Rheumatol* 2000; 27: 708-13.

60. STANFORD MR, KASP E,WHISTON R, *et al.*: Heat shock protein peptides reactive in patients with Behçet's disease are uveitogenic in Lewis rats. *Clin Exp Immunol* 1994; 97: 226-31.

61. HU W, HASAN A, WILSON A, *et al.*: Experimental mucosal induction of uveitis with the 60-kDa heat shock protein-derived peptide 336-351. *Eur J Immunol* 1998; 28: 2444-55.

62. GÜL A, ESIN S, DILSEN N, KONIÇE M, WIGZELL H, BIBERFELD P: Immunohistology of skin pathergy reaction in Behçet's disease. *Br J Dermatol* 1995; 132: 901-7.

63. HIROHOTA S, HASHIMOTO T: Abnormal T cell responses to bacterial superantigens in Behçet's disease. *Clin Exp Immunol* 1998; 112: 317-24.

64. MEGE J-L, DILSEN N, SANGUEDOLCE V, *et al.*: Overproduction of monocyte derived tumor necrosis factor, interleukin (IL) 6,IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. *J Rheumatol* 1993; 20: 1544-9.

65. FREYSDOTTIR J, LAU S-H, FORTUNE F: T cells in Behçet's disease (BD) and recurrent aphthous stomatitis (RAS). *Clin Exp Immunol* 1999; 118: 451-7.

66. FRASSANITO MA, DAMMACCO R, CAFFORIO P, DAMMACCO F: Th1 polarization of the immune response in Behçet's disease. *Arthritis Rheum* 1999; 42: 1967-74.

67. NIWA Y, MIYAKE S, SAKANE T, *et al.*: Auto-oxidative damage in Behçet's disease-endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. *Clin Exp Immunol* 1982; 49: 247-255.

68. SAHIN S, AKOGLU T, DIRESKENELI H, SEN LS, LAWRENCE R: Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease. *Ann Rheum Dis* 1996; 55: 128-33.

69. SAKANE T, SUZUKI N, TAKENO M: Innate and acquired immunity in Behçet's disease (L14). *8th International Congress on Behçet's Disease.* Reggio Emilia, Italy, 7-9 October 1998. Program and Abstracts: 56.

70. TAKENO M, SHIMOYAMA Y, SUZUKI N, SAKANE T: Prolonged survival of auto-primed neutrophils from patients with Behçet's disease (L15). *8th International Congress on Behçet's Disease*. Reggio Emilia, Italy, 7-9 October 1998. Program and Abstracts: 57.

71. ESIN S, GÜL A, HODARA V, *et al.*: Peripheral blood T cell expansions in patients with Behçet's disease. *Clin Exp Immunol* 1997; 107: 520-7.

72. YAMASHITA N, KANEOKA H, KANEKO S, *et al.*: Role of T lymphocytes in the development of Behçet's disease. *Clin Exp Immunol* 1997; 107: 241-247.

73. FORTUNE F, WALKER J, LEHNER T: The expression of T cell receptor and the prevalence of primed, activated and Ig-A bound T cells in Behçet's syndrome. *Clin Exp Immunol* 1990; 82: 326-32.

74. HASAN A, FORTUNE F, WILSON A, *et al.*: Role of T cells in the pathogenesis and diagnosis of Behçet's disease. *Lancet* 1996; 347: 789-94.

75. POULTER LW, LEHNER T: Immunohistology of oral lesions from patients with recurrent oral ulcers and Behçet's syndrome. *Clin Exp Immunol* 1989; 78: 189-95.

76. CHARTERIS DG, BARTON K, McCARTNEY ACE, *et al.*: CD4+ lymphocyte involvement in ocular Behçet' disease. *Autoimmunity* 1992; 12: 201-6.

77. SUGI-IKAI N, NAKAZAWA M, NAKAMURA S, OHNO S, MINAI M: Increased frequencies of interleukin-2- and interferon-gamma-producing T cells in patients with active Behçet's disease. *Invest Ophthalmol Vis Sci* 1998; 39: 996-1004.

78. MOCHIZUKI M, SUZUKI N, TAKENO M, *et al.*: Fine antigen specificity of human T cell lines (V 9+) established by repetitive stimulation with a serotype (KTH-1) of a gram-positive bacterium, *Streptococcus sanguis*. *Eur J Immunol* 1994; 24: 1536-43.

79. NIWA Y, MIZUSHIMA Y: Neutrophil-potentiating factors released from stimulated lymphocytes; special reference to the increase in neutrophil-potentiating factors from streptococcus-stimulated lymphocytes of patients with Behçet's disease. *Clin Exp Immunol* 1990; 79: 353-60.

80. HIROHATA S, OKA H,MIZUSHIMA Y: Streptococcal-related antigens stimulate production of IL6 and interferon-gamma by T cells from patients with Behcet's disease. *Cell Immunol* 1992; 140: 410-9.

81. TANAKA T, YAMAKAWA N, KOIKE N, SUZUKI J, MIZUNO F, USUI M: Behçet's disease and

antibody titers to various heat-shock protein 60s. *Ocul Immunol Inflamm* 1999; 7: 69-74.

82. SUZUKI N, SAKANE T, UEDA Y, TSUNEMAT-SU T, *et al.*: Abnormal B cell function in patients with Behçet's disease. *Arthritis Rheum* 1986; 29: 212-9.

83. EKSIOGLU-DEMIRALP E, KIBAROGLU A, DIRESKENELI H, *et al.*: Phenotypic characteristics of B cells in Behçet's disease: Increased activity in B cell subsets. *J Rheumatol* 1999; 26: 826-32.

84. DIRESKENELI H, HASAN A, SHINNICK T, *et al.*: Recognition of B-cell epitopes of the 65 kDa HSP in Behçet's disease. *Scand J Immunol* 1996; 43: 464-71.

85. KANEKO F, TAKAHASHI Y, MURAMATSU R, *et al.*: Natural killer cell numbers and function in peripheral lymphoid cells in Behçet's disease. *Br J Dermatol* 1985; 113: 313-8.

86. SUZUKI Y, HOSHI K, MATSUDA T, MIZU-SHIMA Y: Increased peripheral blood + T cells and natural killer cells in Behçet's disease. *J Rheumatol* 1992; 19: 588-92.

87. EKSIOGLU-DEMIRALP E, DIRESKENELI H, ERGUN T, FRESKO I, AKOGLU T: Increased CD4+CD16+ and CD4+CD56+ T cell subsets in Behçet's disease. *Rheumatol Int* 1999; 19: 23-26.

88. HAMZAOUI K, AYED K, HAMZA M, TOU-RAINE JL: Natural killer cells in Behçet's disease. *Clin Exp Immunol* 1988; 71: 126-31.

89. KANSU E, SAHIN G, SAHIN F, SIVRI B, SAYEK I, BATMAN F: Impaired prostacyclin synthesis by vessel walls in Behçet's disease. *Lancet* 1986; 2: 1154.

90. HAZNEDAROGLU IC, OZCEBE OI, OZDEMIR O, CELIK I, DUNDAR SV, KIRAZLI S: Impaired haemostatic kinetics and endothelial function in Behçet's disease. *J Intern Med* 1996; 240: 181-7.

91. HAZNEDAROGLU IC, OZDEMIR O, OZCEBE O, DUNDAR SV, KIRAZLI S: Circulating thrombomodulin as a clue of endothelial damage in Behçet's disease. *Thromb Haemost* 1996; 75: 974-5.

92. USLU T, EREM C, TOSUN M, DEGER O: Plasma endothelin-1 levels in Behçet's disease. *Clin Rheumatol* 1997; 16: 59-61.

93. GÜL A, ÖZBEK U, ÖZTÜRK C, INANÇ M, KONIÇE M, ÖZÇELIK T: Coagulation factor V gene mutation increases the risk of venous thrombosis in Behçet's disease. *Br J Rheumatol* 1996; 35: 1178-80.

94. GÜL A, ASLANTAS AB, TEKINAY T, KONIÇE M, ÖZÇELIK T: Procoagulant mutations and venous thrombosis in Behçet's disease. *Rheumatology* 1999; 38: 1298-9.

95. VERITY DH, VAUGHAN RW, MADANAT W, *et al.*: Factor V Leiden mutation is associated with ocular involvement in Behçet's disease. *Am J Ophthalmol* 1999; 128: 352-6.

96. HULL RG, HARRIS EN, GHARAVI AE, *et al.*: Anticardiolipin antibodies: occurrence in Behçet's syndrome. Ann Rheum Dis 1984; 43: 746-8.

97. ZOUBOULIS CC, BUTTNER P, TEBBE B, ORFANOS CE: Anticardiolipin antibodies in Adamantiades-Behcet's disease. *Br J Dermatol* 1993; 128: 281-4.

98. MADER R, ZIV M, ADAWI M, MADER R, LAVI I: Thrombophilic factors and their relation to thromboembolic and other clinical manifestations in Behçet's disease. *J Rheumatol* 1999; 26: 2404-8.

99. DIRESKENELI H, KESER G, D'CRUZ D, *et al.*: Anti-endothelial cell antibodies, endothelial proliferation and von Willebrand factor antigen in Behçet's disease. *Clin Rheumatol* 1995; 14: 55-61.

100. YAZICI H, TUZUN Y, PAZARLI H, *et al.*: Influence of age of onset and patient's sex on the prevalence and severity of manifestations of Behçet's syndrome. *Ann Rheum Dis* 1984; 43: 783-9.

101. DILSEN N, KONIÇE M, ARAL O, ÖCAL L, INANC M, GÜL A: Risk factors for vital organ involvement in Behçet's disease. *In* WECHSLER B, GODEAU P (Eds.): *Behçet's disease*. Amsterdam, Excerpta Medica, 1993: 165-9.

102. SULLIVAN TR JR, KARAS RH, ARONOVITZ M, *et al.*: Estrogen inhibits the response-to-injury in a mouse carotid artery model. *J Clin Invest* 1995; 96: 2482-8.

103. MIYAMOTO N, MANDAI M, SUZUMA I, SUZUMA K, KOBAYASHI K, HONDA Y: Estrogen protects against cellular infiltration by reducing the expressions of E-selectin and IL-6 in endotoxin-induced uve-