

# Learning from HSV-infected mice as a model of Behçet's disease

S. Sohn<sup>1</sup>, E.-S. Lee<sup>2</sup>, D. Bang<sup>3</sup>

<sup>1</sup>Laboratory of Cell Biology, and

<sup>2</sup>Department of Dermatology, Ajou University School of Medicine, Suwon, Korea;

<sup>3</sup>Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea.

Seonghyang Sohn, PhD

Eun-So Lee, MD, PhD

Dongsik Bang, MD, PhD

Please address correspondence to:

Seonghyang Sohn,  
Laboratory of Cell Biology,  
Ajou University Institute for  
Medical Sciences,  
Suwon 443-721, Korea.

E-mail: sohnsh@ajou.ac.kr

Received on September 19, 2011; accepted  
in revised form on November 29, 2011.

Clin Exp Rheumatol 2012; 30 (Suppl. 72):  
S96-S103.

© Copyright CLINICAL AND  
EXPERIMENTAL RHEUMATOLOGY 2012.

**Key words:** Behçet's disease,  
animal model, herpes simplex virus,  
application

## ABSTRACT

Several animal models of Behçet's disease (BD) have been proposed according to putative etiology. Among these models, the herpes simplex virus (HSV)-induced model produced the most similar disease attributes observed in patients. Inoculation of HSV type 1 to the scratched earlobe of mice produced the appropriate symptoms, including oral, genital, and skin ulcers, eye lesions, arthritis, and intestinal involvement. This HSV-induced BD model is the only continuously used model, to which various therapeutic modalities have been applied.

## Introduction

Behçet's disease (BD) is a chronic, multi-systemic inflammatory disorder that involves the mucocutaneous, ocular, vascular, arthritic, gastrointestinal, and central nervous systems. This disease has a chronic course with recurrences and progressive deterioration (1). Although its etiology is unknown, both genetic and environment factors are believed to contribute to the inflammatory background (2). Furthermore, it has become clear that infection with herpes simplex virus (HSV) and Streptococci and immunological dysregulation are involved in the initiation and progression of this disease (3). The complexity of the etiology of this disease makes it difficult to develop an appropriate experimental animal model to better understand the mechanisms behind all of the BD symptoms.

## Animal models for BD

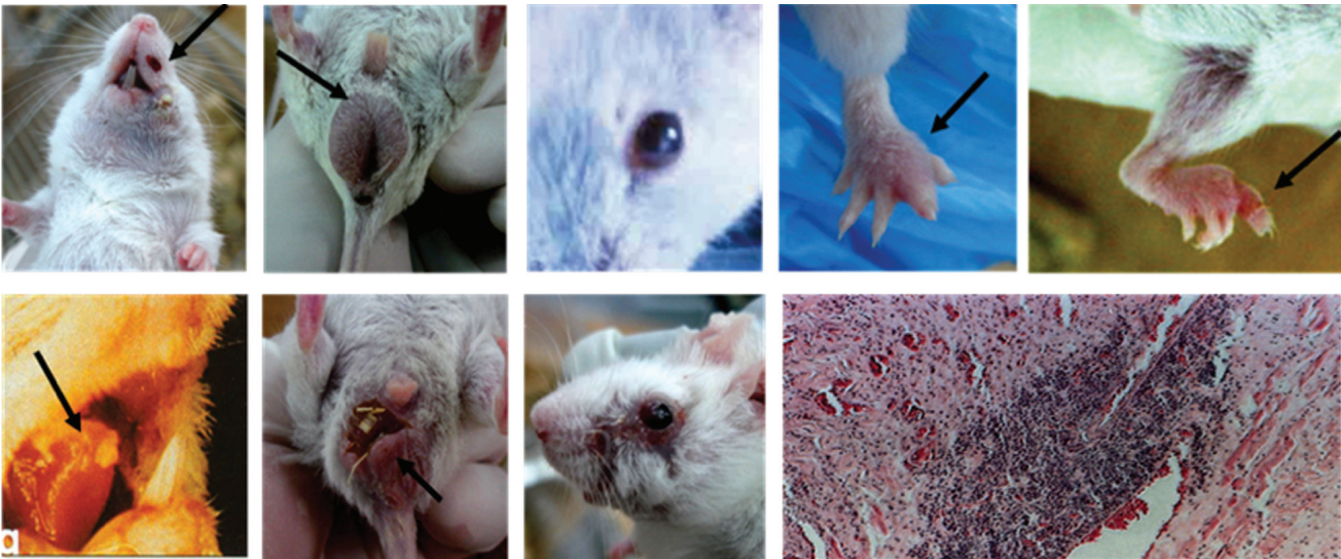
The first reported BD animal model was the Pitman-Moor miniature swine developed with administration of environmental pollutants. This model showed oral, genital and mucocutaneous lesions (4). Even though miniature swine model showed multiple BD symptoms, this has not been continu-

ously used for research. Stanford *et al.* demonstrated that mycobacterial and homologous human heat shock protein T cell peptide epitopes specific for T lymphocytes were related to the pathogenicity of BD. Therefore, human 60 kD heat shock protein-derived peptide 336-351 was injected with Freund's complete adjuvant to induce uveitis in Lewis rats (5). Uveitis was identified by clinical and histological methods. Mononuclear cell infiltration was found in and around the ciliary body and iris in rats. Takeno *et al.* developed HLA-B51 transgenic mice (6), which were shown to be closely associated with BD (7). HLA-B51 transgenic mice also exhibited excessive superoxide production from peripheral blood neutrophils without any BD symptoms. Baharav *et al.* induced inflammation in the skin, joints, and eyes of Lewis rats using  $\alpha$ -trophomyosin, a component of the contractile apparatus of the muscle (8). These induced symptoms were typical features of BD, and inflammation in the leg joints persisted for 6 months. TNF- $\alpha$  up-regulation was observed in the primary splenocytes cultures isolated from  $\alpha$ -trophomyosin vaccinated rats compared to naïve rats. Recently, administration of retinal soluble antigen (S antigen) was shown to result the development of uveitis confirmed by histology in Wistar rats (9). In addition, rats immunised with the S antigen showed a significant increase in nitric oxide production. This model could be helpful in the development of strategies for diagnosing patients with Behçet uveitis.

The potential role of viral infection in BD was first proposed by Hulusi Behçet in 1937 (10). Since 1937, many researchers, including Sezer (11), Evans *et al.* (12), Mortada and Imam (13), Eglin *et al.* (14), Bonass *et al.* (15), Denman *et al.* (16), Studd *et al.* (17), and Lee *et al.* (18), have reported

*Funding:* this study was supported by grant A100535 from the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs and grant 2010-0011130 from the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

*Competing interests:* none declared.



**Fig 1.** HSV-induced Behçet's disease (BD)-like symptoms in mice. Histology shows lymphocyte accumulation in the perivascular area of the cutaneous lesions in mice.

that viral particles and herpes simplex virus (HSV) are related to BD. In 1998, our team developed a BD mouse model by inoculation of HSV into scratched earlobes of mice (19). The HSV-induced symptoms included oral, genital, and skin ulcers, eye lesions and arthritis. These symptoms were shown to be very similar to the symptoms observed in BD patients. Inflammatory cells accumulated in the perivascular area of the lesional skin of mice (Fig. 1). The process of validating animal models should involve reliable measures of the human disease. The HSV-induced BD mouse model was shown to be reliable, consistent and valid. The BD-like symptoms have been shown to remain constant in the HSV-induced BD model after HSV inoculation. The severity score can be calculated using a similar counting method as conducted on human patients. Therefore, the controversial issues associated with the etiology and various therapeutic modalities of BD have been resolved using the HSV-induced BD mouse model.

**To understand immunological abnormalities**  
*a. Contribution of MHC (including HLA-G/Qa-2)*  
It has been postulated that human leukocyte antigen (HLA)-B51 is associated with BD (6). To examine the correlation between viral infection and ge-

netic factors in the development of this disease, several inbred mouse strains – B10.BR, B10.RIII, C57BL/6, C3H/He, and Balb/c – which had different haplotypes of major histocompatibility complex (MHC), were inoculated with HSV (20). These mouse strains manifested single or multiple symptoms of BD including oral, genital, skin ulcers, uveitis, and arthritis, which are clinically significant for the diagnosis of BD. More than 40% of B10.BR (*k*), B10.RIII (*r*) and C57BL/6 (*b*) showed BD-like symptoms, compared to 2% of C3H/He (*k*) and Balb/c (*d*) (Table I). C3H/He (*k*), which had a common haplotype to the B10.BR (*k*) strain and showed very low incidence of BD-like symptoms. A HLA-B51 transgenic mouse was generated by Takeno and co-workers (6). This transgenic mouse showed neutrophil hyperfunction but did not show any clinical manifestations that mimic BD. This indicated that the HLA-B51 molecule alone was not sufficient to induce clinical BD. This result

also suggests that other important factors besides MHC types are involved. HLA-G protein is involved in maintaining tolerance of the maternal immune system towards the fetus (21-23). HLA-G is able to inhibit NK and T-cell cytotoxicity in addition to T-cell proliferation (24). Furthermore, HLA-G inhibits the transendothelial migration of NK cells (25), shifts the cytokine balance towards Th2 dominance (26), and suppresses the proliferation of allogeneic CD4<sup>+</sup> T lymphocytes (27, 28). These combined results clearly indicate that HLA-G has specific inhibitory effects on immune cells. Park *et al.* (29) previously reported that the frequency of haplotype containing HLA-G 3741\_3754 14 base pair insertion and 1597\*delC was increased in BD patients. Individuals homozygous with the 3741\_3754\*ins14/\*ins14 genotype were found to have a 2.7-fold greater risk of BD than controls. An HLA-G 3741\_3754 14-base pair insertion allele was also significantly more frequent in

**Table I.** Therapeutic efficacy of TNF-α siRNA in BD mice.

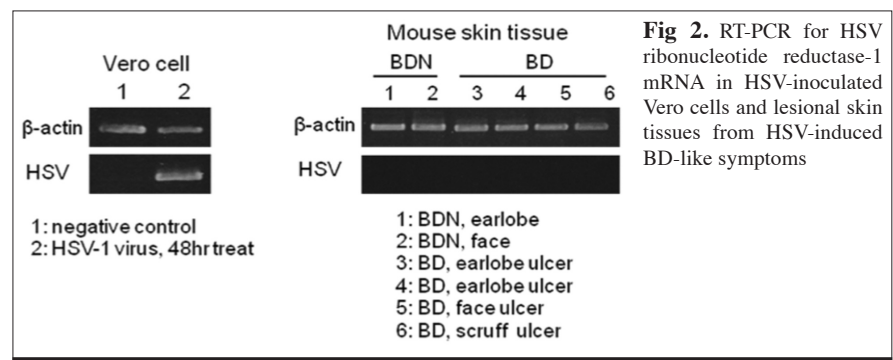
Treatment	Improved mice / Total number of mice (%)
TNF-α siRNA	18/32 (56.3%)*
Scrambled siRNA	2/19 (10.5%)
Infliximab	11/27 (40.7%)
Etanercept	9/25 (36.0%)

\**p*<0.005 (TNF-α siRNA vs. Scrambled siRNA).

BD patients with ocular, arthritis, and CNS symptoms than in controls, and this insertion was found to be related to lower serum levels of HLA-G. Park *et al.* suggested that these HLA-G allelic variants are genetic risk factors for BD. In addition, HLA-G\*010101 alleles have been shown to be significantly lower in frequency in BD patients than in control subjects (30). Furthermore, genome-wide association studies have also reported HLA-G involvement (31). Therefore, it is important to investigate whether or not HLA-G contributes to the pathogenesis of BD. To this end, expression of Qa-2, the functional homolog of HLA-G in mice, was identified and modulated using Qa-2 siRNA. Qa-2 mRNA expression and Qa-2-positive granulocytes were lower in BD mice than in normal mice. After down-regulation of Qa-2 by injection of Qa-2 siRNA, deterioration in arthritis was observed in BD mice, and the disease severity score increased. This study confirmed that a decreased Qa-2 level was related to changes in the disease pattern, which was accompanied by the deterioration of HSV-induced BD-like symptoms.

#### *b. T cell abnormality* – *Th1/Th2 balance*

A possible polarisation of T lymphocytes toward the Th1 type in BD has been suggested based on experimental evidence of uveoretinitis and in humans (32). Th1 cells may play an important role in the immunopathogenesis of BD, although others have reported that the cytokine production profile contains a mix of Th1/Th2 cell types in active BD (33). Macrophages induce cellular immunity by activating Th1 cell responses and by suppressing Th2 responses (34), and macrophage depletion in mice shifted the Th1 response to a Th2 response (35). This study attempted to determine whether inactivation of macrophages modulated Th1/Th2 polarity and influenced the development of BD and modulation of BD symptoms (36). Liposome encapsulated Clodronate (lip-Cl<sub>2</sub>MDP) - engulfed macrophages were killed by apoptosis. Lip-Cl<sub>2</sub>MDP injected mice up-regulated Th2 cytokine IL-4



**Fig 2.** RT-PCR for HSV ribonucleotide reductase-1 mRNA in HSV-inoculated Vero cells and lesional skin tissues from HSV-induced BD-like symptoms

and did not induce BD-like symptoms when combined with HSV injection (0 of 50 mice), which is in stark contrast to HSV injection only (14 of 50 mice). The transfer of splenocytes isolated from Th2 adjuvant OVA-alum injected mice into BD mice showed improvements compared to the transfer of splenocytes from Th1 adjuvant OVA-CFA injected mice. Even though this mouse model was induced by inoculation of HSV, the disease pattern was very similar to human BD patients when evaluated in immune modulation experiments. In the skin lesion of mice, HSV ribonucleotide reductase 1 mRNA was not detected by PCR, but was detected in Vero cells inoculated with HSV (Fig. 2). This means that the symptoms were not derived from HSV infection itself but from dysregulated immune response triggered by HSV.

#### – *Regulatory T cells*

Regulatory T cells (Treg) are comprised of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells (37). CD4<sup>+</sup>CD25<sup>+</sup> cells are heterogeneous T cell populations that prevent harmful immune responses to self and non-self antigens. Foxp3 protein is currently considered to be the most specific marker of Treg cells (38). Regulatory T cells play a role in the pathogenesis of autoimmune disorders, such as arthritis and lupus (39). These autoimmune disorders can be prevented by the infusion of Treg cells (40). Recently, the frequencies of Treg cells were shown to be reduced in the peripheral blood of patients with BD prior to ocular attack (41). According to Shim *et al.* (42), the levels of CD4<sup>+</sup>CD25<sup>+</sup>T cells in BD mice are significantly lower than those in BDN mice. In HSV-induced BD mice, infusion of Treg cells was conducted

to determine if disease progress could be inhibited. For adoptive transfer of Treg cells to BD mice, CD4<sup>+</sup>CD25<sup>+</sup>T cells from normal healthy mice were isolated and cultured with stimulators and then transferred to BD mice. After transfer, the disease severity score significantly decreased in a cell number-dependent manner when compared to the CD4<sup>+</sup>CD25<sup>-</sup> control cell transfer group. CD4<sup>+</sup>CD25<sup>+</sup>T cell transfer also up-regulated IL-10 and TGF-β levels and down-regulated IFN-γ, TNF-α, IL-6, and IL-17 levels. According to genome-wide association studies, the IL-10 variant (the rs1518111 A allele) was associated with diminished mRNA expression and low protein production in BD patients (31, 43). The decrease level of Treg cells might be related to the low expression levels of IL-10 (44, 45). To confirm the efficacy of Treg cells in alleviating BD symptoms, CD25 was blocked using an anti-CD25 antibody. Anti-CD25 antibody injection deteriorated BD-like symptoms in mice. Therefore, the low level of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells was a factor in the etiopathogenesis of BD.

#### **To evaluate conventional and potential therapies**

##### *a. Cytokines as therapeutic targets*

##### – *TNF-α*

Anti-TNF-α Ab has been used to treat inflammation in patients (46, 47). However, more effective and safer drugs still need to be developed. Synthetic small interfering RNA (siRNA) can interfere with the expression of specific genes and have been applied to biomedical research and drug development. RNA interference (RNAi) is a recently discovered process that utilises either endogenous or exogenous dou-



**Table II.** The efficiency of various therapeutic modalities in HSV-induced BD mice.

Treatment	Improved mice /Total number of mice (%)	Decision day after treatment	References
TNF- $\alpha$ siRNA	18/32 (56.3%)*	~14 d	49
IL-6 siRNA	7/9 (77.8%)	~26 d	57
Famciclovir	10/25 (40.0%)	~42 d	63
Gemcitabine	7/12 (36.0%)	~5d	65
Colchicine	4/5 (80.0%)	~20 d	73
Combined with Colchicine and Rebamipide	5/6 (83.3%)	~20d	73
Thalidomide	8/10 (80.0%)	~10 d	74

ble-stranded RNAs to inhibit the expression of genes in a highly sequence-specific manner. In mammals, RNAi can be invoked by introducing short (19-21 nucleotide), double-stranded RNA oligonucleotides, referred to as small interfering (siRNAs) or silencing RNA molecules, with sequences complementary to that of the target gene. siRNAs are bound by an RNA-inducing silencing complex in the cytoplasm and can silence the expression of the target mRNA. Therefore, RNAi holds promise for use as a novel therapeutic agent and may be used as a tool in functional genomics studies to elucidate genes controlling disease pathways (48). To inhibit RNA expression of TNF- $\alpha$ , siRNA was administered *in vivo* to HSV-induced BD mice. TNF- $\alpha$  siRNA ameliorated BD-like symptoms. Infliximab and Etanercept were also effective in treating BD mice (49). Comparatively, 1 week after TNF- $\alpha$  siRNA treatment, the severity score was significantly decreased compared to that by Infliximab treatment. TNF- $\alpha$  siRNA effectively decreased BD symptoms in 18 of 32 cases (56.3%). On the other hand, scrambled siRNA treatment only decreased BD symptoms in 2 of 19 cases (10.5%). Infliximab was effective in 11 of 27 cases (40.7%), while Etanercept was also effective in 9 of 25 cases (36.0%) at the end of week 2 (Table II). The serum level of TNF- $\alpha$  was lower in siRNA-treated subjects than the scrambled siRNA-treated controls. Thus, TNF $\alpha$  siRNA showed a therapeutic effect in HSV-induced BD mice.

#### – IL-6

IL-6 is a multifunctional cytokine secreted by lymphoid cells as well as many nonlymphoid cells, including macrophages, fibroblasts, keratinocytes,

and endothelial cells, and is involved in the regulation of inflammation (50-53). IL-6 was shown to be highly elevated in the culture supernatant of PBMCs from patients with active BD (54) as well as the cerebrospinal fluid of patients with neuro-BD (55) and ocular BD (56). To reduce overexpression of IL-6, IL-6 siRNA was injected into HSV-induced BD mice (57). Reduced IL-6 improved BD-like symptoms (Fig. 3) by decreasing the production of proinflammatory cytokines such as IL-6, IL-17, and TNF- $\alpha$  as well as by increasing TGF $\beta$ , an anti-inflammatory cytokine. The frequencies of Foxp3<sup>+</sup> cells, which is a marker of regulatory T cells, also increased, and the disease severity score was significantly decreased. These results highlight the potential of using systemic siRNA for therapeutic gene silencing.

#### b. Antiviral agent: Famciclovir

Famciclovir is an anti-viral compound that acts against HSV, varicella-zoster virus, and hepatitis B virus. It is metabolised into penciclovir after oral administration (58). Penciclovir is selectively phosphorylated by viral thymidine kinase in virus-infected cells to yield high intracellular concentrations of penciclovir triphosphate, which then inhibits viral replication by interacting with viral DNA polymerase (59). Treatment with the anti-viral agent, acyclovir, failed to alleviate the frequency and severity of orogenital ulceration or other disease features of BD (60). Famciclovir was more effective than aciclovir in preventing viral replication (61). *In vitro*, famciclovir has a two- to 50-fold stronger and more prolonged intracellular half-life than that of acyclovir (62). We administered famciclovir to HSV-induced BD mice

to determine whether or not this treatment improved BD-like symptoms. Treatment was effective in 22 of 25 (88%) BD mice, but in improved BD mice, recurrence occurred at a rate of 54.5%. In single symptomatic mice, improvement was observed in 20 of 25 (77%) mice, and the rate of recurrence was only 19.2% (63). Higher recurrence after famciclovir treatment might be related to the immunological effects produced by the BD symptoms rather than viral infection itself.

#### c. Gemcitabine (20,20-difluorodeoxycytidine, dFdC)

The nucleoside analog gemcitabine (2',2'-difluorodeoxycytidine, dFdC) is a new immunosuppressive agent that may be useful for the treatment of not only graft-versus-host disease (GVHD) but also autoimmune diseases. Gemcitabine affects the pyrimidine pathway. After phosphorylation by deoxycytidine kinase to its active form, gemcitabine is incorporated into DNA and this process is most likely the major mechanism by which gemcitabine causes cell death (64). Gemcitabine has been widely used for the treatment of solid malignancies and refractory hematologic malignancies. In addition, *in vitro* studies have revealed an inhibitory effect of gemcitabine on lymphocytes and bone marrow progenitors (65), and an immunosuppressive effect was described in a rat cardiac transplantation model (66). Therefore, this study hypothesised that gemcitabine may also be a promising alternative for the treatment of BD lesions. Over 5 consecutive days, treatment with 0.06  $\mu$ g/day/mouse of gemcitabine ameliorated cutaneous manifestations by more than 60% in BD mice (67). Low doses of gemcitabine were safe and showed promising effects in reducing cutaneous lesions in HSV-induced BD mice.

#### d. Colchicine

Colchicine inhibits microtubule polymerisation by binding to tubulin, one of the main constituents of microtubules in T cells (68). Microtubules are an essential structural component during mitosis. Colchicine is a natural product that can be extracted from two plants



**Fig. 3.** IL-6 siRNA improved HSV-induced BD-like symptoms. For each mouse, 1.5  $\mu$ g of IL-6 siRNA in 200  $\mu$ l of a 5% glucose solution was injected intraperitoneally four times at 3 day intervals.

of the lily family, the genus *Colchicum* (usually called *Colchicum autumnale*) and *Gloriosa superba* (69). Colchicine is one of the most frequently prescribed medicines for treatment of BD (70), and it has also been used to treat severe mucocutaneous lesions and prevent mucocutaneous relapse in BD (71). Functional assays previously demonstrated significantly increased NADPH oxidase activity in BD patients (72). NADPH oxidase is made of six subunits and generates superoxide. Treatment of HSV-induced BD mice with colchicine down-regulated the expression of NADPH oxidase subunits, including p40, p67, and gp91, and decreased the severity score compared to before administration (73). Combined treatment with colchicine and rebamipide (free radical scavenger) was more effective in decreasing the severity score and down-regulating these NADPH oxidase subunits. This result suggests that colchicine acted as a free radical scavenger to protect against inflammation by lowering the level NADPH oxidase in HSV-induced BD mice.

#### e. Thalidomide

Despite its inherent teratogenic risk, more than 100 papers have reported that thalidomide is effective for treating the mucocutaneous lesions of BD since 1982. We applied thalidomide to our HSV-induced mouse model to confirm its efficacy as well as to evaluate its possible mechanisms of action. Thalidomide treatment improved HSV-

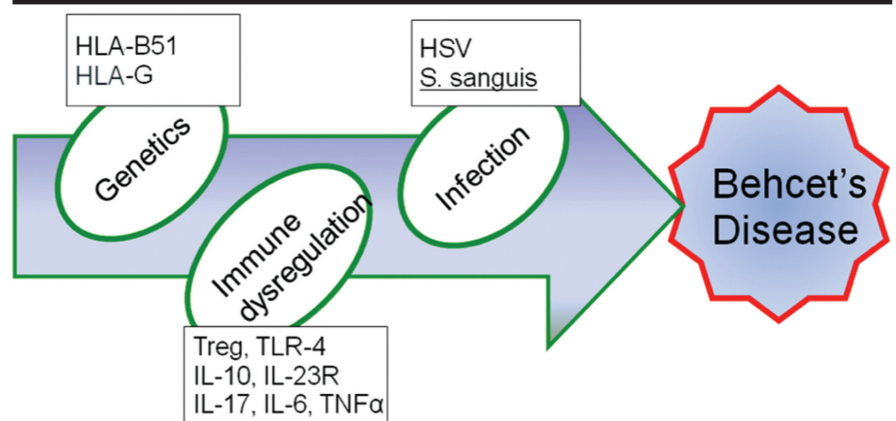
induced mucocutaneous BD-like symptoms in 80% of treated mice (74). Thalidomide also decreased inflammation through the down-regulation of IFN- $\gamma$  and TNF- $\alpha$  as well as up-regulation of MIP-1 $\alpha$ , perforin, Fas, and Fas ligand. *In vitro* treatment of a splenocyte culture from BD mice with thalidomide enhanced propidium iodide (PI) and annexin V staining compared to non-treated cultures. Thus, thalidomide induced cell death. Cell death might also down-regulate excessively proliferated inflammatory lymphocytes. (The original publication is available at [www.springerlink.com](http://www.springerlink.com)).

#### f. Vitamin D3

Recent studies have shown the anti-inflammatory effects of vitamin D3 in Kawasaki disease (75) and psoriasis (76). Reduction of vitamin D is associated with increased renal in-

flammation (77) as well as BD (78). Vitamin D3 treatment has been shown to decrease chemokine synthesis and monocyte trafficking (79), while also down-regulating expression of Toll-like receptor (TLR) 2 and 4 in monocytes (80). The immunomodulatory effects of 1,25(OH) $_2$ D $_3$  in T cell subsets significantly decreased IFN- $\gamma$  and TNF- $\alpha$  cytokine production in patients with *Mycobacterium tuberculosis* (81). Vitamin D3 supplementation has been shown to produce therapeutic effects in animal models of rheumatoid arthritis, lupus, inflammatory bowel disease, and type 1 diabetes (82).

Choi *et al.* investigated whether or not vitamin D3 can ameliorate HSV-induced BD-like symptoms in a mouse model also induce changes in TLR expression (83). Serum 25(OH)D levels in BD mice significantly decreased compared to those in control mice. The frequen-



**Fig. 4.** Hypothesised etiopathogenetic scheme of BD.

cies of TLR2-positive cells and TLR4-positive cells were higher in BD mice than in BDN mice (BD normal, HSV-inoculated but no symptomatic mice). Oral administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> increased the serum levels of 25(OH)D in a dose-dependent manner in normal mice. Treatment of BD mice with 10 µg/kg/day of 1,25(OH)<sub>2</sub>D<sub>3</sub> for 10 consecutive days was effective in down-regulating TLR2 and TLR4 expression. In addition, the serum levels of IL-6 and TNF-α decreased in response to 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment, and the disease severity score also decreased. Combined treatment with 2 µg/mouse of colchicine did not produce an additive effective in down-regulating TLR2 and TLR4. In addition, the serum levels of IL-6 and TNF-α also decreased in response to D3 alone or in combination with colchicine. Combination treatment additively down-regulated the serum level of TNF-α.

## Conclusion

While there may be no perfect animal models, the HSV-induced BD model produces symptoms that are closest to BD patients and constitutes a useful tool for the study of pathogenesis and pharmacotherapies. According to the results using the HSV-induced BD model, we hypothesise that genetic background, immunological dysregulation, and pathogens cooperatively induced and exacerbated BD (Fig. 4). In subsequent studies, specific genetic factors, such as micro RNA and epigenetic relationships, as well as pathogenic and immunological correlations should be applied to BD research.

## Acknowledgement

We would like to express our gratitude to Prof. Sungnack Lee, who gave us the opportunity to develop this animal model to study Behçet's disease.

## References

- ALPSOY E, ZOUBOULIS CC, EHRLICH GE: Mucocutaneous lesions of Behçet's disease. *Yonsei Med J* 2007; 48: 573-85.
- HORI Y, MIYAZAWA S, NISHIYAMA S, MIYATA M, ISHIKAWA S: Experimental Behçet's disease and ultrastructural x-ray microanalysis of pathological tissues. *J Dermatol* 1979; 6: 31-7.
- 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.
- 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.
- 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.
- BAHARAV E, MOR F, HALPERN M, QUINTANA F, WEINBERGER A: Tropomyosin-induced arthritis in rats. *Clin Exp Rheumatol* 2007; 25 (Suppl. 45): S86-92.
- DIERABA Z, ARROUL-LAMMALI A, MEDJEBER O *et al.*: Nitric oxide, biomarker of experimental autoimmune uveitis induced by S antigen. *J Fr Ophtalmol* 2010; 33: 693-700.
- BEHÇET H: Ueber rezidivierende, aphthoese durch ein Virus verursachte Geschwüre am Mund, am Auge und an den Genitalien. *Dermatol Wochenschr* 1937; 105: 1152-7.
- SEZER FN: The isolation of a virus as the cause of Behçet's diseases. *Am J Ophthalmol* 1953; 36: 301-15.
- 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; 273: 349-53.
- MORTADA A, IMAM IZ: Virus aetiology of Behçet's syndrome. *Br J Ophthalmol* 1964; 48: 250-9.
- 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.
- BONASS WA, BIRD-STEWART JA, CHAMBERLAIN MA, HALIBURTON IW: Molecular studies in Behçet's syndrome: Recent advances in Behçet's disease. *London, Royal Society of Medicine Services Limited* 1986; 37-41.
- DENMAN AM, PELTON BK, HYLTON W, PALMER RG, TOPPER R: Herpes simplex virus and the rheumatic diseases. *Rheumatol Int* 1989; 9: 143-6.
- 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.
- 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.
- 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.
- SOHN S, LEE ES, LEE S: The correlation of MHC haplotype and development of Behçet's disease-like symptoms induced by herpes simplex virus in several inbred mouse strains. *J Dermatol Sci* 2001; 26: 173-81.
- ELLIS SA, SARGENT IL, REDMAN CW, MICHAELAJ: Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunology* 1986; 59: 595-601.
- KOVATS S, MAIN EK, LIBRACH C, STUBBLEBINE M, FISHER SJ, DEMARS R: A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 1990; 248: 220-3.
- MCMASTER MT, LIBRACH CL, ZHOU Y *et al.*: Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* 1995; 154: 3771-8.
- RITEAU B, ROUAS-FREISS N, MENIER C, PAUL P, DAUSSET J, CAROSELLA ED: HLA-G2, -G3 and -G4 isoforms expressed as non-mature cell-surface glycoproteins inhibit NK and antigen-specific CTL cytotoxicity. *J Immunol* 2001; 166: 5018-26.
- DORLING A, MONK NJ, LECHLER RI: HLA-G inhibits the transendothelial migration of human NK cells. *Eur J Immunol* 2000; 30: 586-93.
- KANAI T, FUJII T, UNNO N *et al.*: Human leukocyte antigen-G-expressing cells differentially modulate the release of cytokines from mononuclear cells present in the decidua versus peripheral blood. *Am J Reprod Immunol* 2001; 45: 94-9.
- RITEAU B, MENIER C, KHALIL-DAHER I *et al.*: HLA-G inhibits the allogeneic proliferative response. *J Reprod Immunol* 1999; 43: 203-11.
- BAINBRIDGE DR, ELLIS SA, SARGENT IL: HLA-G suppresses proliferation of CD4<sup>+</sup> T lymphocytes. *J Reprod Immunol* 2000; 48: 17-26.
- PARK KS, NAM JH, LEE ES, CHOI JS, BANG D, LEE S: Increased risk of human leukocyte antigen-G gene variants in Behçet's disease. *Clin Exp Rheumatol* 2006; 24: S126-7.
- PARK KS, PARK JS, NAM JH, BANG D, SOHN S, LEE ES: HLA-E\*0101 and HLA-G\*010101 reduce the risk of Behçet's disease. *Tissue Antigens* 2007; 69: 139-44.
- MIZUKI N, MEGURO A, OTA M *et al.*: Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. *Nat Genet* 2010; 42: 703-6.
- EMMI L, BRUGNOLO F, MARCHIONE T: Pathogenesis and therapy of Behçet's disease. *Ann Ital Med Int* 1997; 12: 20-5.
- RAZIUDIN S, AL-DALAN A, BAHABRI S, SIRAJ AK, AL-SEDAIRY S: Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. *J Rheumatol* 1998; 25: 329-33.
- DESMEDT M, ROTTIERS P, DOOMS H, FIERIS W, GROOTEN J: Macrophages induce cellular immunity by activating Th1 cell responses and suppressing Th2 cell responses. *J Immunol* 1998; 160: 5300-8.
- JUN HS, SANTAMARIA P, LIM HW, ZHANG ML, YOON JW: Absolute requirement of macrophages for the development and activation of beta-cell cytotoxic CD8<sup>+</sup> T-cells in T-cell receptor transgenic NOD mice. *Diabetes* 1999; 48: 34-2.
- SOHN S, LEE ES, KWON HJ, LEE SI, BANG D, LEE S: Expression of Th2 cytokines decreases the development of and improves Behçet's disease-like symptoms induced by herpes simplex virus in mice. *J Infect Dis*



- 2001; 183: 1180-6.
37. SAKAGUCHI S, SAKAGUCHI N, ASANO M, ITOH M, TODA M: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155: 1151-64.
  38. CRISPIN JC, MARTINEZ A, VARELA JA: Quantification of regulatory T cells in patients with systemic lupus erythematosus. *J Autoimmun* 2003; 21: 273-6.
  39. AMELSFORT J, JACVOBS K, BIJLSMA J, LAFFEBER F, TAAMS L: CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in rheumatoid arthritis: Difference in the presence, phenotype, and function between peripheral blood and synovial fluid. *Arthritis Rheum* 2004; 50: 2775-85.
  40. SAKAGUCHI S, SAKAGUCHI N, SHIMIZU J: Immunological tolerance maintained by CD25<sup>+</sup> CD4<sup>+</sup> regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 2001; 182: 18-32.
  41. NANKE Y, KOTAKE S, GOTO M, UJIHARA H, MATSUBARA M, KAMATANI N: Decreased percentages of regulatory T cells in peripheral blood of patients with Behçet's disease before ocular attack: a possible predictive marker of ocular attack. *Mod Rheumatol* 2008; 18: 354-8.
  42. SHIM J, LEE ES, PARK S, BANG D, SOHN S: CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells ameliorate Behçet's disease-like symptoms in a mouse model. *Cytotherapy* 2011; 13: 835-47.
  43. REMMERS EF, COSAN F, KIRINO Y *et al.*: Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. *Nat Genet* 2010; 42: 698-702.
  44. JIA L, KOVACS JR, ZHENG Y, SHEN H, GAWALT ES, MENG WS: Expansion of Foxp3-expressing regulatory T cells *in vitro* by dendritic cells modified with polymeric particles carrying a plasmid encoding interleukin-10. *Biomaterials* 2008; 29: 1250-61.
  45. RONCAROLO MG, GREGORI S, BATTAGLIA M, BACCHETTA R, FLEISCHHAUER K, LEVINGS MK: Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006; 212: 28-50.
  46. HAUGEBOERG G, VELKEN M, JOHNSEN V: Successful treatment of genital ulcers with infliximab in Behçet's disease. *Ann Rheum Dis* 2004; 63: 744-5.
  47. OHNO S, NAKAMURA S, HORI S *et al.*: Efficacy, safety, and pharmacokinetics of multiple administration of infliximab in Behçet's disease with refractory uveoretinitis. *J Rheumatol* 2004; 31: 1362-8.
  48. SCHIFFELERS RM, XU J, STORM G, WOODLE MC, SCARIA PV: Effects of treatment with small interfering RNA on joint inflammation in mice with collagen-induced arthritis. *Arthritis Rheum* 2005; 52: 1314-8.
  49. CHOI B, HWANG Y, KWON HJ *et al.*: Tumor necrosis factor alpha small interfering RNA decreases herpes simplex virus-induced inflammation in a mouse model. *J Dermatol Sci* 2008; 52: 87-97.
  50. AKDENIZ N, ESREFOGLU M, KELEŞ MS, KARAKUZU A, ATASOY M: Serum interleukin-2, interleukin-6, tumour necrosis factor-alpha and nitric oxide levels in patients with Behçet's disease. *Ann Acad Med Singapore* 2004; 33: 596-9.
  51. KALLEN KJ: The role of trans signalling via the agonistic soluble IL-6 receptor in human diseases. *Biochim Biophys Acta* 2002; 1592: 323-43.
  52. HEINRICH PC, CASTELL JV, ANDUS T: Interleukin-6 and the acute phase response. *Biochem* 1990; 265: 621-36.
  53. HIRANO T: Interleukin-6 and its relation to inflammation and disease. *Clin Immunol Immunopathol* 1992; 62: 60-5.
  54. YAMAKAWA Y, SUGITA Y, NAGATANI T *et al.*: Interleukin-6 in patients with Behçet's disease. *J Dermatol Sci* 1996; 11: 189-95.
  55. LINKSWANG CR, CHUANG CY, CHEN CY: Anticardiolipin antibodies and interleukin-6 in cerebrospinal fluid and blood of Chinese patients with neuro-Behçet's syndrome. *Clin Exp Rheumatol* 1992; 10: 599-602.
  56. BARDAK Y, ARIDOĞAN BC: The demonstration of serum interleukin 6-8, tumor necrosis factor-alpha, complement, and immunoglobulin levels in Behçet's disease with ocular involvement. *Ocul Immunol Inflamm* 2004; 12: 53-8.
  57. SHIM J, BYUN HO, LEE YD, LEE ES, SOHN S: Interleukin-6 small interfering RNA improved the herpes simplex virus-induced systemic inflammation *in vivo* Behçet's disease-like mouse model. *Gene Therapy* 2009; 16: 415-25.
  58. CIRELLI R, HERNE K, MCCRARY M, LEE P, TYRING SK: Famciclovir: review of clinical efficacy and safety. *Antiviral Res* 1996; 29: 141-51.
  59. PERRY CM, WAGSTAFF AJ: Famciclovir: a review of its pharmacological properties and therapeutic efficacy in herpes simplex infection. *Drugs* 1995; 50: 396-415.
  60. DAVIES UM, PALMER RG, DENMAN AM: Treatment with acyclovir does not affect orogenital ulcers in Behçet's syndrome: a randomized double blind trial. *Br J Rheumatol* 1988; 27: 300-2.
  61. ASHTON RJ, ABBOT KH, SMITH GM, SUTTON D: Antiviral activity of famciclovir and acyclovir in mice infected intraperitoneally with herpes simplex virus type 1 SC16. *J Antimicrob Chemother* 1994; 34: 287-90.
  62. WUTZLER P, ULBRICHT A, FARBER I: Antiviral efficacies of famciclovir, valaciclovir, and brivudin in disseminated herpes simplex virus type 1 infection in mice. *Intervirology* 1997; 40: 15-21.
  63. SOHN S, BANG D, LEE ES, KWON HJ, LEE SI, LEE S: Experimental studies on the antiviral agent famciclovir in Behçet's disease symptoms in ICR mice. *Br J Dermatol* 2001; 145: 799-804.
  64. PLUNKETT W, HUANG P, GANDHI V: Preclinical characteristics of gemcitabine. *Anticancer Drugs* 1995; 6 (Suppl. 6): 7-13.
  65. SCHIRMER M, GEISEN F, TIEFENTHALER M, KONWALINKA G: Differential effects of cladribine and gemcitabine on erythrocytic progenitors from patients with chronic myeloid leukemia. *Leuk Res* 1999; 23: 1121-6.
  66. MARGREITER R, FISCHER M, ROBERTS K *et al.*: Gemcitabine- a novel immunosuppressive agent -prevents rejection in a rat cardiac transplantation model. *Transplantation* 1999; 68: 1051-3.
  67. SOHN S, LUTZ M, KWON HJ, KONWALINKA G, LEE S, SCHIRMER M: Therapeutic effects of gemcitabine on cutaneous manifestations in an Adamantiades-Behçet's disease-like mouse model. *Exp Dermatol* 2004; 13: 630-4.
  68. PERICO N, OSTERMANN D, BONTEMPELLI M *et al.*: Colchicine interferes with L-selectin and leukocyte function-associated antigen-1 expression on human T lymphocytes and inhibits T cell activation. *J Am Soc Nephrol* 1996; 7: 594-601.
  69. COCCO G, CHU DC, PANDOLFI S: Colchicine in clinical medicine. A guide for internists. *Eur J Intern Med* 2010; 21: 503-8.
  70. WECHSLER B: Colchicine and Behçet's disease: an efficacious treatment finally recognized. *Rev Med Interne* 2002; 23: 355-6.
  71. SAKANE T, TAKENO M: Novel approaches to Behçet's disease. *Expert Opin Investig Drugs* 2000; 9: 1993-2005.
  72. MELIKOGLU MA, KACAR C, CORT A, YUCEL G, ASLAN M: Oxidase and oxygenase in regulation of neutrophil redox pathways in Behçet's disease patients. *J Enzyme inhib med chem* 2011; 1-6.
  73. BANG D, CHOI B, KWON HJ, LEE ES, LEE S, SOHN S: Rebamipide affects the efficiency of colchicine for the herpes simplex virus-induced inflammation in a Behçet's disease mouse model. *Eur J Pharmacol* 2008; 598: 112-7.
  74. LEE ES, KIM YA, KWON HJ, BANG D, LEE S, SOHN S: Thalidomide upregulates macrophage inflammatory protein-1alpha in a herpes simplex virus-induced Behçet's disease-like animal model. *Arch Dermatol Res* 2004; 296: 175-81.
  75. SUZUKI Y, ICHIHAMA T, OHSAKI A, HASEGAWA S, SHIRAIISHI M, FURUKAWA S: Anti-inflammatory effect of 1alpha,25-dihydroxyvitamin D(3) in human coronary arterial endothelial cells: Implication for the treatment of Kawasaki disease. *J Steroid Biochem Mol Biol* 2009; 113: 134-8.
  76. GERRITSEN MJ, BOEZEMAN J.B, VAN VLIJMEN-WILLEMS IM, VAN DE KERKHOF PC: The effect of tacalcitol (1,24(OH)<sub>2</sub>D<sub>3</sub>) on cutaneous inflammation, epidermal proliferation and keratinization in psoriasis: a placebo-controlled, double-blind study. *Br J Dermatol* 1994; 131: 57-63.
  77. ZEHNDER D, QUINKLER M, EARDLEY KS *et al.*: Reduction of the vitamin D hormonal system in kidney disease is associated with increased renal inflammation. *Kidney Int* 2008; 74: 1343-53.
  78. DO JE, KWON SY, PARK S, LEE ES: Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behçet's disease. *Rheumatology (Oxford)* 2008; 47: 840-8.
  79. PEDERSEN LB, NASHOLD FE, SPACH KM, HAYES CE: 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. *J Neurosci Res* 2007; 85: 2480-90.
  80. SADEGHI K, WESSNER B, LAGGNER U *et al.*: Vitamin D3 down-regulates monocyte TLR

- expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. *Eur J Immunol* 2006; 36: 361-70.
81. PRABHU ANAND S, SELVARAJ P, NARAYANAN PR: Effect of 1,25 dihydroxyvitamin D3 on intracellular IFN-gamma and TNF-alpha positive T cell subsets in pulmonary tuberculosis. *Cytokine* 2009; 45: 105-10.
82. DELUCA HF, CANTORNA MT: Vitamin D: its role and uses in immunology. *FASEB J* 2001; 15: 2579-85.
83. CHOI B, LEE ES, SOHN S: Vitamin D3 ameliorates herpes simplex virus-induced Behcet's disease-like inflammation in a mouse model through down-regulation of Toll-like receptors. *Clin Exp Rheumatol* 2011; 29 (Suppl. 67): S13-9.