Etiopathology of Behçet’s Disease; Herpes Simplex Virus Infection and Animal Model

Seonghyang Sohn

The etiology of Behçet’s disease has been proposed as being a viral, bacterial, genetic and immunological disorder. After Hulusi Behçet’s viral etiological hypothesis, many investigators have tried to confirm this. Scrapings and fluids from patients were applied to subculture in chorioallantoic membranes of fertilized eggs as well as in mice and rabbits by direct intracerebral injection. Since the 1980s, in situ hybridization, blotting, and polymerase chain reaction has also been applied to identify the herpes simplex virus DNA and RNA in patients. Animal models were developed based on environmental pollutants, bacterial and human heat shock protein derived peptides and virus injection. Using these animal models separately and/or concurrently, allows for a more effective investigation into Behçet’s disease.

Key Words: Behçet’s disease, etiology, herpes simplex virus, animal model

The etiology of Behçet’s disease is unclear and viral infection has long been postulated as one of the etiologic factors. Previously, several researchers found a virus in patients with Behçet’s disease.

Hulusi Behçet proposed that the disease was caused by a special virus. Although he was unable to demonstrate one, he had observed intracellular inclusion-like forms in smears from the hypopyon of the anterior chamber and aphthae (Behçet, 1937). In 1953, Sezer was the first to isolate the virus from ocular fluid and serially cultivate it in chorioallantoic membrane of fertile eggs. He inoculated the material from the ocular fluid of patients into the brains of mice. The manifestations of inoculated mice included roughening of the coat, inactivity or hyperactivity, tremor, circling, paralysis, encephalitis, thrombophlebitis and swelling. Inoculated rabbits also showed paralysis in the hind leg, cloudy vitreous and hair loss on the back (Sezer, 1953). Evans et al. also isolated the virus from the eye and brain of a patient who died of the disease (Evans et al. 1957). Mortada and Imam found inclusion bodies from scrapings of the scrotal and buccal ulcers, as well as from the hypopyon fluid. When the fluid from the scrapings of scrotal and buccal ulcers and hypopyon was inoculated into the chorioallantoic membrane of 10-day old chick embryos and incubated for 2 days, whitish plaques were seen. These plaques showed inclusion bodies exactly like those seen in scrapings. The filtrates from plaques were inoculated intracerebrally into 3-week-old white Swiss mice. Seventeen out of 21 mice died while 5 control mice inoculated with saline remained alive (Mortada and Imam, 1964).

Eglin et al. using the method of in situ hybridization, detected RNA complementary to Herpes Simplex Virus (HSV) type 1 in the mononuclear cells of patients with Behçet’s disease. Hybridization between HSV type 1 DNA and the complementary RNA in mononuclear cells was significantly greater in 10 of 20 patients with Behçet’s syndrome than
in controls. In 14 controls, none showed positive results (Eglin et al. 1982): HSV type 1 DNA was detected in the whole blood by Bonass et al. with dot blotting technique. Fourteen out of 34 patients were positive, compared with only 1 out of 23 rheumatoid arthritis patients and 3 out of 42 healthy controls. Both the group of patients with Behçet's syndrome and the group with rheumatoid arthritis had more than 80% of individuals positive for HSV 1 antibodies, whereas only 43% of the healthy controls had HSV 1 antibodies (Bonass et al. 1986). Denman and colleagues detected HSV DNA with southern hybridization using Eco R1 digested DNA from the peripheral blood mononuclear cells of patients with Behçet's disease. In this experiment, 7 out of 32 patients were positive (Denman et al. 1989). In 1991, Studd et al. detected HSV-1 DNA by polymerase chain reaction in peripheral blood leukocytes of Behçet's disease patients with recurrent oral ulcers. Fifty percent of Behçet's syndrome showed positive results compared to 13.6% healthy controls. By using the same method, HSV DNA was not detected in 3 biopsy samples taken from oral tissue with Behçet's disease (Studd et al. 1991). Since Hulusi Behçet proposed a viral etiology in 1937, many clinicians have observed a similarity in viral infection of patients with Behçet's disease. Recently, herpes simplex viral DNA has been confirmed in blood cells of Behçet's disease patients.

Lee et al. tried to detect HSV DNA in saliva of patients with Behçet's disease, and to evaluate whether the presence of HSV in saliva is associated with the presence of an intraoral ulcer, and to investigate any possible relationships between HSV and Behçet's disease using the polymerase chain reaction (Lee et al. 1996b). The primers were designed to detect 289 base pairs in HSV DNA polymerase sequence. This sequence is common in HSV type 1 and 2 (Tsurumi et al. 1987), with no crosslinking to other herpes virus groups (Cao et al. 1989) (Fig. 1a). They confirmed primary PCR products with double amplification, southern hybridization and restriction endonuclease assay (Fig. 1b). The second primers were designed as 92 base pairs used for double amplification. Positive PCR results were also positive in southern blot and negative PCR results also appeared negative. In restriction endo-

![Fig. 1. a. Identification of HSV DNA in PCR products from HSV inoculated and manifested mouse tissue. lane 1, 123 DNA ladder as a size marker; lane 2, positive control; lane 3, negative control; lane 4-6, samples from patients. b. lane 1,5, 123 DNA ladder as a size marker; lane 2-4, 92 base pairs secondary PCR products; lane 6-8, 153 and 136 base pairs PstI restriction assay products.](image)

<table>
<thead>
<tr>
<th>1</th>
<th>2908</th>
<th>2950</th>
<th>2970</th>
<th>3038</th>
<th>3075</th>
<th>3166</th>
<th>3196</th>
<th>~3767</th>
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<tr>
<td>TTT</td>
<td>TAC</td>
<td>GGA</td>
<td></td>
<td>CAT</td>
<td>CCC</td>
<td>CCC</td>
<td></td>
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</tr>
</tbody>
</table>

![probe](image)

![Fig. 2. PCR scheme and sequences of primers in HSV DNA polymerase sequence.](image)
Herpes Simplex Virus Infection and Animal Model

nuclease assay, restriction enzyme PstI can cut 289 base pairs to 153 and 136 base pairs. The PCR product of 289 base pairs involves the PstI restriction site (Fig. 2). The results from saliva showed that almost 40% of patients were positive for HSV DNA, compared to 14% of healthy controls.

Lee ES and colleagues investigated the gastrointestinal involvement of Behçet's disease. GI Behçet is occasionally seen. Ulcerative lesions may occur in many portions of the digestive tract from the esophagus to the rectum, and they are not easy to differentiate from Crohn's disease. Histopathologic comparison of Crohn's disease and Behçet's disease showed that Behçet's disease has a deeper, rounder and more punched-out ulcer than Crohn's disease. The experimental purpose was to study the relationship of intestinal ulceration of Behçet's disease and herpes simplex virus. PCR results were all positive in 7 specimens; whereas, in Crohn's disease, 2 out of 13 were positive. In American patients of Crohn's disease, HSV DNA was not detected in all 10 patients (Lee et al. 1996a).

Bang et al. experimented to detect HSV DNA from ulcerative genital tissue of patients with Behçet's disease. They applied 8 cases and all showed HSV bands. In contrast, episiotomy tissue specimens which had no HSV infection history as a control were all negative (Bang et al. 1996). From these reports, we can conclude that HSV DNA was detected in saliva and the positive rate had no relation to oral ulcer, gastrointestinal ulcer, and genital ulcer. Therefore, there is a relationship between HSV DNA and ulceration of various epithelial tissue of Behçet's disease (Table 1).

Animal models are very important and necessary in most fields of research. Many investigators have tried to develop an animal model for use in Behçet's disease. But we did not have an animal model for Behçet's disease except Hori et al.'s miniature swine study reported in 1979. Hori and colleagues created typical Behçet's disease-like lesions, including folliculitis, cutaneous and subcutaneous nodules, erosion, genital ulcer and oral aphthae, in Pitman-Moor's strain miniature swine treated with agricultural chemicals such as organophosphate, organochloride and inorganic copper (Hori et al. 1979).

Lehner et al. reported that uveitis could be induced in Lewis rats by 4 peptides (amino acids 111-125, MPLGLKRGIEKAVEK; 154-172, QSIGDLIAFMADKVGNLEV; 219-233, LVSSKVGTVK-DLLP; and 311-325, DLSLGKARKVVTKDK) derived from the sequence of the mycobacterial 65kD heat shock protein, which stimulate specifically TCR γδ lymphocyte from patients with Behçet's disease and human 65 kD heat shock protein (hsp) derived peptides (336-351, QPHDLKGVGEVIVTKD) (Lehner et al. 1993; Lehner et al. 1996).

The total number of animals used for the development of an animal model by Hori and colleagues was 8. Though all of the experimental animals showed Behçet's disease-like symptoms after a 1-year administration of the chemicals, it is not feasible to breed as a large group for use in other experimental designs.

Lehner group's Lewis rats showed only single eye symptom. Patients' symptoms of Behçet's disease are multiple, chronic or recurrent. If additional symptoms were to appear in heat shock protein (hsp) derived peptide stimulated Lewis rats, this animal model would be more useful.

Sohn and colleagues experimented to develop an animal model for use in Behçet's disease using herpes simplex virus (HSV) infection which was hypothesized to be one of the etiologic or triggering factors in Behçet's disease by Lee (Lee et al. 1996b). To test the HSV infection hypothesis and to develop an animal model, ICR mice were inoculated with HSV. Using the method of Hirata and colleagues (Hirata et al. 1993), the earlobes of 258 mice of ICR strain, aged 5 to 6 weeks, were scratched with a needle, then inoculated with \(10^6\) plaque forming units (pfu)/mL of HSV type 1 (KOS strain) solution. As a control, 30 mice were inoculated in the same site with a culture medium. Four

Table 1. PCR results from various tissues of Behçet's disease patients

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Patients Positive/Total</th>
<th>Control subjects Positive/Total</th>
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<tbody>
<tr>
<td>saliva</td>
<td>26/66 (39.4%)</td>
<td>12/87 (normal healthy)</td>
</tr>
<tr>
<td>G-I tissue</td>
<td>7/7</td>
<td>2/13 (Crohn's disease)</td>
</tr>
<tr>
<td>Genital tissue</td>
<td>8/8</td>
<td>0/8 (episiotomy)</td>
</tr>
</tbody>
</table>

Number 6
weeks later, a second inoculation was performed using the same method, followed by 16 weeks of observation. After the induced infection, 86 mice (33.3%) died, 77 (29.8%) showed Behçet's disease-like symptoms (two or more symptoms in one mouse was considered an indication of Behçet's disease-like syndrome), and 95 (36.8%) had a healthy normal appearance or a single symptom. The symptoms included skin ulcers on the earlobe, scruff, abdomen, back or face (57.1%); eye syndromes (39.0%); partial hair loss (33.8%); genital ulcer (19.5%); bullae (11.7%); arthritis (5.2%); gastrointestinal ulcer (5.2%); and tongue ulcer (3.9%) (Fig. 3) (Sohn et al. in press). The induced Behçet's disease-like symptoms were similar to the clinical manifestations of ulcers, uveitis, and arthritis which have been significant in diagnosing Behçet's disease in patients. Aside from hair loss, the most frequent symptoms affecting mice were skin ulcers, eye syndromes, and genital ulcers. The frequency of these symptoms were similar to that of patients with Behçet's disease.

The polymerase chain reaction was used to detect HSV DNA sequences in DNA extracted from the lesions of mice with Behçet's disease-like symptoms. The methods were almost the same as those used in the patient experiments. HSV DNA sequ-

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**Fig. 3.** a. Facial hair loss and conjunctivitis, b. Skin ulcer around mouth, c. Eye symptom (keratitis), d. Genital ulcer, e. Skin ulcer in the scruff, f. Arthritis.

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**Fig. 4.** Pathological finding of lesional skin tissue (H&E stain) showing many inflammatory cells accumulated around the blood vessel (×100).
ferences were detected in the lesional skin and gastrointestinal track, but not in normal healthy skin area. Abdominal skin lesions stained with hematoxylin and eosin showed that many inflammatory cells had accumulated around the blood vessel (Fig. 4). Vasculitis was also common in intestinal, oral, earlobular, and genital epithelial lesions. These findings were very similar to typical morphological changes in human Behçet's disease (Haim et al. 1976; Chun et al. 1990). These experiments proved that it was possible to induce Behçet's disease-like symptoms such as ulceration and vascular inflammation in ICR mice by inoculating them with HSV. So, these mice could be used as an animal model of Behçet's disease.

It has been postulated that human leukocyte antigen (HLA) B 51 is associated with Behçet's disease in Korean, Japanese and other ethnic groups (Baricordi et al. 1986; Ohno and Matsuda, 1986; Lee et al. 1988). To pursue this hypothesis, several inbred mouse strains - B10.BR, B10.RIII, C57BL/6, C3H/He-having different major histocompatibility complex (MHC), were inoculated with HSV type 1 (KOS strain) (Table 2). B10.BR, B10.RIII, and C57BL/6 showed Behçet's disease-like symptoms in more than 40%, whereas C3H/He showed only 2%. Inbred mice with different haplotype of the H-2 region showed various incidence rates of Behçet's disease-like symptom (Table 3) (Data not published). B10.BR and C3H/He strains had a common haplotype but the rate of manifestation was different. So, these results suggest that the important factors for onset of Behçet's disease symptoms are diverse, including haplotype.

### Table 2. Inbred mice strains and haplotype used in herpes simplex virus-induced Behçet's disease animal model

<table>
<thead>
<tr>
<th>Strain of mouse</th>
<th>H-2 complex</th>
<th>Haplotype</th>
<th>K</th>
<th>A</th>
<th>E</th>
<th>S</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.BR</td>
<td></td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td>B10.RIII</td>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
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<td>r</td>
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<tr>
<td>C57BL/6</td>
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<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>C3H/He</td>
<td></td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
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<td>k</td>
</tr>
</tbody>
</table>

### Table 3. Symptomatic number of mice and strains after ear-lobe inoculation with $1.0 \times 10^6$ p.f.u. of HSV

<table>
<thead>
<tr>
<th>Strains</th>
<th>B10.BR</th>
<th>B10.RIII</th>
<th>C57BL/6</th>
<th>C3H/He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom</td>
<td>27/54</td>
<td>24/49</td>
<td>12/30</td>
<td>1/49</td>
</tr>
<tr>
<td>Death</td>
<td>5/54</td>
<td>6/49</td>
<td>4/30</td>
<td>2/49</td>
</tr>
<tr>
<td>Normal</td>
<td>22/54</td>
<td>19/49</td>
<td>14/30</td>
<td>46/49</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>49</td>
<td>30</td>
<td>49</td>
</tr>
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</table>

B10.BR, B10.RIII, C57BL/6, and C3H/He strains were bred to 4 weeks old in SPF animal facility, then transferred to a conventional facility.

### CONCLUSIONS

Recently, due to developments in molecular biological techniques such as dot and southern blotting, in situ hybridization, and polymerase chain reaction, the postulation that viral etiology is one of the etiologic factors of Behçet's disease has been proven. By using agricultural pollutants, 65 kD heat shock protein (hsp) derived peptides and herpes simplex virus, animal models are being developed. Animal model developed by using agricultural pollutants showed typical Behçet's disease-like lesions, such as folliculitis, cutaneous and subcutaneous nodules, erosion, genital ulcer and oral aphthae in Pitman-Moor's strain miniature swine. 65 kD heat shock protein (hsp) derived peptides induced uveitis in Lewis rat. Herpes simplex virus-inoculated ICR mice showed skin ulcers, eye syndromes, partial hair loss, genital ulcer, bullae, arthritis, gastrointestinal ulcer, and tongue ulcer. The induced Behçet's disease-like symptoms were similar to the clinical manifestations and the frequency of these symptoms were similar to those of patients with Behçet's disease.

### REFERENCES

Bang D, Cho YH, Choi HJ, Lee S, Sohn S. Herpes simplex virus detection by polymerase chain reaction in genital ulcer of patients with Behçet's disease. The
Seonghyang Sohn

Denman AM, Pelton BK, Hylton W, Palmer RG, Topper R: Herpes simplex virus and the rheumatoid disease. Rheumatol Int 9: 143-146, 1989
Tsurumi T, Maeno K, Nishiyama Y: Nucleotide sequence of the DNA polymerase gene of herpes simplex virus type 2 and comparison with the type 1 counterpart. Gene 52: 129-137, 1987

364 Volume 38