

Major Histocompatibility Complex Haplotype Is Associated with Postherpetic Pain in Mice

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Background: Postherpetic neuralgia is one of the major complications of herpes zoster caused by the reactivation of varicella-zoster virus and is characterized by severe pain. The authors previously showed the association of a human major histocompatibility complex (MHC) haplotype with postherpetic neuralgia. This study was performed to experimentally confirm the role of MHC haplotype in the development of postherpetic pain using a mouse model of postherpetic pain, which corresponds to postherpetic neuralgia.

Methods: BALB/c mice (MHC haplotype: H-2^d), C57BL/6 mice (MHC haplotype: H-2^b), and BALB/b mice, a congenic BALB/c strain with H-2^b, were used. Herpes simplex virus type I was transdermally inoculated on the hind paw. Unilaterally zosteriform skin lesion and pain-related responses (acute herpetic pain) were caused, and some mice showed pain-related responses (postherpetic pain) after the cure of skin lesions. Herpes simplex virus type I antigen and CD3-positive cells were immunostained in the dorsal root ganglion in the acute phase.

Results: The incidence (78%) of postherpetic pain in C57BL/6 mice was significantly higher than that (35%) in BALB/c mice ($P = 0.004$, odds ratio = 6.7). Furthermore, the incidence of postherpetic pain in BALB/b (H-2^b) was similar to that in C57BL/6. Herpes simplex virus type I antigen-positive cells were less in the dorsal root ganglion of C57BL/6 mice than that of BALB/c mice. CD3-positive T cells were more in the dorsal root ganglion of C57BL/6 mice than BALB/c mice.

Conclusions: These results suggest that the MHC haplotype (H-2^b) is involved in the incidence of postherpetic pain, and CD3-positive T cells may play a role in its pathogenesis.

VARICELLA-ZOSTER virus (VZV) belongs to the α -herpes virus and causes chickenpox typically in childhood; the virus establishes a lifelong latent infection of the sensory ganglia.¹⁻³ Herpes zoster is caused by the reactivation of VZV in the sensory ganglia and is characterized by a belt of vesicular eruptions on the body, which are generally limited to the dermatome innervated by a single sensory

ganglion. It is generally accompanied by severe pain.⁴⁻⁸ Approximately 15% of herpes zoster patients have constant or intermittent spontaneous pain more than 3-6 months after the vesicles disappear, and the pain is difficult to control; this condition is termed *postherpetic neuralgia* (PHN).⁹⁻¹¹ Studies on mechanisms and genes responsible for pain in humans have started recently.¹² We have recently shown that a genetic factor, *i.e.*, the human major histocompatibility leukocyte antigen (MHC) or human histocompatibility leukocyte antigen (HLA), the HLA-A*3303-B*4403-DRB1*1302 haplotype, is involved in the incidence of PHN after herpes zoster.^{13,14} Furthermore, the haplotype does not affect the onset of herpes zoster without PHN.¹⁴

Varicella-zoster virus causes herpes zoster only in humans.¹⁵ In contrast, a transdermal inoculation of mice with herpes simplex virus type I (HSV-I, human herpesvirus 1), which also belongs to the α -herpes virus, produces zoster-like skin lesions; vesicles erupt generally on the back of the trunk after its proliferation in the sensory ganglia and then spread throughout the inoculated dermatome.^{16,17} Mice with zoster-like skin lesions show spontaneous pain-like behaviors, tactile allodynia, and mechanical hyperalgesia.¹⁶⁻¹⁸ Some mice show pain-related responses for a long time after the healing of zoster-like skin lesions, and it is considered that the development to postherpetic pain in mice corresponds to PHN in humans.¹⁹

The aim of this study was to determine further mechanism in PHN incidence using a mouse model of postherpetic pain. Therefore, we investigated the incidence of postherpetic pain in BALB/c (MHC haplotype: H-2^d) and C57BL/6 (MHC haplotype: H-2^b) mice, and then in a congenic mouse strain, C.B10-H2^d/LilMcdJ (BALB/b; BALB/c strain with H-2^b instead of H-2^d), to elucidate whether the MHC haplotype is involved in the incidence of postherpetic pain as PHN in humans, which is associated with the HLA haplotype.

Deficiency in MHC class II attenuates allodynia induced by spinal nerve transection in mice, and the expression of MHC class II is increased in the spinal cord of rats given spinal nerve transection.²⁰ HSV-I inoculation induces cyclooxygenase 2 in the dorsal root ganglion (DRG) at an acute herpetic, but not postherpetic, pain phase.²¹ Because cyclooxygenase 2 is induced by mitogens and cytokines, the finding raises the possibility that neuroimmune activation is also involved in the acute herpetic pain. Therefore, to assess the possibility that T cells are involved in an acute herpetic pain and conse-

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quently postherpetic pain, we examined T cells infiltrating into the DRG at an acute herpetic pain phase.

Materials and Methods

Animals

Female BALB/c and C57BL/6 mice were obtained from Japan SLC (Shizuoka, Japan); they weighed 18–20 g and were 6 weeks old at the start of the experiment. C57BL/10 mice²² and the congenic mice C.B10-H2^b/LilMcdJ (BALB/b) were obtained from Jackson Laboratory (Bar Harbor, ME); they weighed 20–22 g and were 7–8 weeks old at the start of the experiment. BALB/b mice are a congenic strain identical to BALB/c (H-2^d) mice at all loci except for the H-2 locus, which was derived from a C57BL/10 (H-2^b) mouse. Six mice were housed per cage under controlled temperature ($22^{\circ} \pm 1^{\circ}\text{C}$) and humidity ($55 \pm 10\%$). The room was lighted from 7:00 AM to 7:00 PM and during the behavioral test. Food and water were freely available. HSV-I inoculation and behavioral experiments were performed in the infection room of Molecular Genetics Research Center, University of Toyama (Toyama, Japan). Animal experiments were conducted with the approval of the Animal Care Committee at University of Toyama and according to ethical standards for investigations of experimental pain in animals.²³

Virus Inoculation

The mice were inoculated with HSV-I as described previously.¹⁶ In brief, 3 days after depilation, the shin skin of the right hind paw (5×5 mm) was scarified using 27-gauge needles, and a 5- μl suspension containing 1×10^6 plaque-forming units of HSV-I (7401H strain) was applied to the scarified area. The contralateral hind paw was not inoculated. HSV-I inoculation and behavioral experiments were performed in the infection room of the Molecular Genetics Research Center, University of Toyama. Inoculated HSV-I infects primary afferents, passes along sensory nerves, and is replicated in the DRG. Then, HSV-I moves antidromically along primary afferents to the skin, which results in an eruption in the corresponding cutaneous dermatome.

At the stage of development of skin lesions (until the 9th day after the virus inoculation), they were scored as follows: 0 = no lesions; 2 = one or two vesicles on the back of the trunk; 4 = many vesicles on the back of the trunk, the surrounding inoculated area, or both; 6 = mild herpes zoster-like lesions; 8 = apparent zoster-like lesions, paw inflammation, or both; 10 = severe zoster-like lesions. At the stage of recovery from skin lesions (from the 10th day after the virus inoculation), they were scored as follows: 10 = severe herpes zoster-like lesions; 5 = presence of scabs flaking off from cutaneous lesions; 0 = complete recovery from lesions.^{16,19}

Drugs

The anti-herpes virus agent acyclovir (GlaxoSmithKline, Tokyo, Japan) was dissolved in water. It was administered orally to all mice at 10 mg/kg five times a day (at 9:00, 12:00, 15:00, 18:00, and 21:00) from 5 to 11 days after virus inoculation; this medication inhibits motor paralysis and death without effects on dermal rash and acute pain-related responses.¹⁹

Behavioral Test

Pain-related responses, allodynia (pain resulting from innocuous stimulation), and hyperalgesia (abnormally increased pain induced by noxious stimulation) of the hind paw were assessed using the von Frey method, as described previously.^{16,19,24} After an acclimation period of at least 15 min, von Frey filaments with a bending force of 0.16 or 1.0 g were pressed perpendicularly against the plantar skin and held for 3–5 s. The responses to these stimuli were evaluated as follows: 0 = no response; 1 = moving away from the von Frey filament; 2 = immediate flinching or licking of the hind paw. The stimulation of the same intensity was applied six times to each hind paw at intervals of 5 s, and pain-related score was calculated as follows:

$$\text{Pain-related score (\%)} = [(\text{sum of scores})/12] \times 100.$$

Assessment of Postherpetic Pain

Because normal mice showed almost no responses to a von Frey filament of 0.16-g strength, mice that showed a pain-related score of more than 25% (3 of 12 points) were considered to have allodynia. When stimulated with a von Frey filament of 1.0-g strength, normal mice showed a pain-related score of less than 33% (4 of 12 points), and mice that showed a pain-related score of more than 58% (7 of 12 points) were considered to have hyperalgesia. Mice that showed allodynia and hyperalgesia on 30 days after inoculation were considered to have postherpetic pain.

Immunohistochemical Analysis

We attempted to confirm whether HSV-I actually had reached DRG and performed an immunohistochemical analysis of T-cell infiltration into DRG on 6 days after inoculation.

Three infected mice were killed during diethyl ether anesthesia on 6 days after inoculation; noninoculated mice were used as a control. The DRGs at L4 and L5 levels were isolated and frozen in a tissue-freezing medium on dry ice and stored at -20°C until sectioning. The samples were sectioned at 10 μm using a cryotome (CM1800; Leica, Heidelberg, Germany), placed on gelatin-coated slide glasses, and stored at -20°C until use.²⁵ Before staining, sections were allowed to equilibrate to room temperature, fixed in cold ethanol for 10 min, and rehydrated in phosphate-buffered saline (PBS). For HSV-I

antigen staining, the ethanol-fixed sections were incubated with a rabbit anti-HSV-I antibody (DakoCytomation, Copenhagen, Denmark) at 4°C overnight. After being washed in PBS, the sections were reacted with a Cy3-conjugated anti-rabbit immunoglobulin G antibody (DakoCytomation) for 2 h at room temperature. HSV immunoreactivity was observed under a laser microscope (Nikon, Tokyo, Japan). For the staining of CD3-positive cells, endogenous peroxidase was depleted by incubating sections for 30 min in 0.3% hydrogen peroxide in methanol. After being washed in PBS to reduce nonspecific staining, the sections were incubated for 30 min at room temperature in a blocking solution, which was 5% normal rabbit serum in PBS containing 0.3% Triton X-100. The blocking solution was removed, and then the sections were incubated overnight at 4°C with the primary antibody, the goat polyclonal anti-mouse CD3 ϵ antibody (1/1,000; Santa Cruz Biotechnology Inc., Santa Cruz, CA). The sections were washed in PBS and further incubated with the biotinylated rabbit anti-goat immunoglobulin G antibody (Vector Laboratories, Burlingame, CA) for 2 h at room temperature. The sections were washed in PBS and incubated with the avidin-biotin-horseradish peroxidase complex (Vectastain ABC Elite Kit; Vector Laboratories) for 2 h at room temperature. The sections were washed three times in PBS and reacted with 3,3'-diaminobenzidine-nickel (DAB kit; Vector Laboratories). After a sufficient deposition was achieved, the reactions were stopped by washing in saline. Sections were counterstained with hematoxylin. Immunoreactivities were observed under a light microscope (Nikon).

Statistical Analyses

The means of data are presented together with SEMs. Data on the pain-related score were analyzed by Mann-Whitney rank sum test. Statistical differences in the incidence of postherpetic pain and mortality between mouse strains were analyzed using the chi-square test and Fisher exact probability test, respectively. Statistical differences in skin lesion score and body weight between mouse strains were analyzed using the Student *t* test. The α level was set at 0.05.

Results

Comparison of Zosteriform Skin Lesions and Pain-related Responses in Different Mouse Strains

The first series of experiments were performed to confirm whether the incidence of postherpetic pain is different between BALB/c (H-2^d) and C57BL/6 (H-2^b) mice without differences in other symptoms. Zoster-like skin lesions unilaterally developed on the HSV-I inoculated side in all BALB/c and C57BL/6 mice as shown in our previous studies.¹⁶⁻¹⁹ The skin lesions erupted on 6

Table 1. Differences in Incidence of Postherpetic Pain between BALB/c and C57BL/6 Mice

| Strain | BALB/c | C57BL/6 | P Value |
|-------------------------------------|----------------|----------------|---------|
| Number of mice inoculated | 25 | 24 | |
| Body weight,* g | 18.6 \pm 0.2 | 17.9 \pm 0.2 | |
| Pain-related score,* % | 25 \pm 7 | 29 \pm 6 | |
| Skin lesion score† | 7.7 \pm 0.4 | 7.6 \pm 0.3 | |
| Mortality rate‡ (%) | 5/25 (20.0) | 1/24 (4.2) | NS§ |
| Incidence of postherpetic pain‡ (%) | 7/20 (35) | 18/23 (78) | < 0.01 |

* Determined at inoculation; mean \pm SEM. † Determined six days after inoculation; mean \pm SEM. ‡ Determined 30 days after inoculation. § Not significant (Fisher exact probability test). || Chi-square test.

days after inoculation, peaked approximately 8 days after inoculation, and almost healed by 15 days after inoculation. There were no skin lesions, paw inflammation, or paralysis on the side contralateral to the side of virus inoculation in any of the mice. There were no differences in weight at the beginning of the experiment and the score of skin lesions between the two strains on 6 days after inoculation (table 1). They also showed no differences in average weight or the score of skin lesions during any phase of the experiment (data not shown). Mortality is shown in table 1. Five of 25 BALB/c mice (20%) and 1 of 24 C57BL/6 mice (4.2%) died, probably because of encephalitis during the acute phase of HSV-I infection, and the difference did not reach a significant level.

We then analyzed differences in pain-related responses between these two strains. Pain-related responses to von Frey filaments of 1.0-g strength before inoculation did not significantly differ between BALB/c and C57BL/6 mice (table 1). HSV-I inoculation induced pain-related responses in both strains on 5 days after inoculation, and these effect peaked on 7 days after inoculation (fig. 1). The severities of allodynia (assessed as a response to a von Frey filament of 0.16-g bending force) and hyperalgesia (assessed as a response to a von Frey filament of 1.0-g bending force) were similar between BALB/c and C57BL/6 mice. Thirteen of 20 BALB/c mice (65%) and 5 of 23 C57BL/6 mice (21.7%) were relieved of allodynia and hyperalgesia after skin lesions healed, but allodynia and hyperalgesia persisted until at least 37 days after inoculation in the remaining mice of either strain (fig. 1). When the presence of postherpetic pain was assessed on 30 days after inoculation, 18 of 23 C57BL/6 mice (78.3%) and 7 of 20 BALB/c mice (35%) had postherpetic pain (table 1). There was a significant difference in the incidence of postherpetic pain between these two strains ($P = 0.004$, odds ratio = 6.7). There were no significant differences in body weight on 37 days after inoculation between mice with and without postherpetic pain; body weights of C57BL/6 mice with and without postherpetic pain were 20.3 \pm 0.3 g ($n = 18$) and 20.1 \pm 0.3 g ($n = 5$), respectively. Body weights of BALB/c mice with and

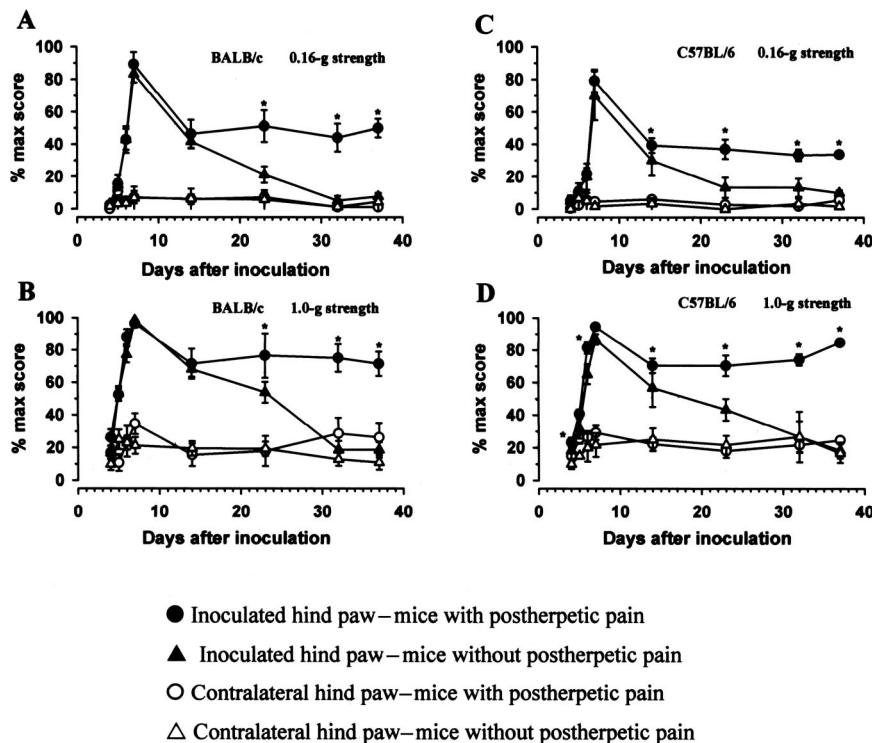


Fig. 1. Effects of herpetic inoculation on pain-related responses to von Frey filament stimuli in BALB/c and C57BL/6 mice. BALB/c (A and B) and C57BL/6 (C and D) mice were inoculated with herpes simplex virus type I on the unilateral shin. Pain-related responses of the inoculated (closed symbols) and contralateral (open symbols) hind paws to von Frey filaments of 0.16-g (A and C) or 1.0-g (B and D) strength were assessed from 4 to 37 days after inoculation. Postherpetic pain, criterion of which is described in the text, was assessed 30 days after inoculation, and mice were classified into two groups with (circles) or without (triangles) postherpetic pain. The numbers of BALB/c mice with and without postherpetic pain were 7 and 13, respectively. The numbers of C57BL/6 mice with and without postherpetic pain were 18 and 5, respectively. The data are presented as mean \pm SEM. * $P < 0.05$ as compared with the inoculated hind paw of mice without postherpetic pain (Mann-Whitney rank sum test).

without postherpetic pain were 20.6 ± 0.7 g ($n = 7$) and 20.9 ± 0.3 g ($n = 13$), respectively.

Association of an MHC Haplotype with Onset of Postherpetic Pain

BALB/c and C57BL/6 mice have different H-2 genes. Therefore, we investigated the incidence of postherpetic pain using mice of a congenic strain, C.B10-H2^b/LilMcdJ (hereafter designated as BALB/b), which is identical to BALB/c (H-2^d) at all loci except for the H-2 region derived from a C57BL/10 (H-2^b) mouse, to determine whether the susceptibility is controlled by H-2 gene region. Although body weight was higher in BALB/b mice than in other strains used, pain-related responses to von Frey filament of 1.0-g strength before inoculation and peaked skin lesion were similar among all strains used (table 2). Mortality rates were also similar among all strains used (table 2). The incidences of postherpetic

pain in BALB/c and C57BL/6 mice were reproduced again ($P = 0.025$, odds ratio = 9.3). The incidence of postherpetic pain in both C57BL/10 and BALB/b mice was 75.0% (6 of 8), which was significantly different from that (20%, 2 of 10) in BALB/c mice ($P = 0.02$, odds ratio = 12.0).

HSV-I Proliferation and Infiltration of T Cells in DRG

We attempted to confirm whether HSV-I actually had reached DRG. In BALB/c mice, HSV-I inoculation produces noticeable HSV antigen-like immunoreactivities in DRG on 5 days after inoculation but not on 3 days after inoculation.¹⁷ The immunoreactivity was markedly decreased on 7 days after inoculation¹⁷ and disappeared at a postherpetic pain phase (I. Takasaki, Ph.D., and M. Sato-Takeda, M.D., Ph.D., unpublished observation, November 2002). These findings suggest that HSV-I actively

Table 2. Differences in Incidence of Postherpetic Pain between Four Different Mouse Strains

| Strain | BALB/c | C57BL/6 | C57BL/10 | BALB/b |
|--------------------------------------|------------------|------------------|------------------|------------------|
| MHC haplotype | H-2 ^d | H-2 ^b | H-2 ^b | H-2 ^b |
| Number of mice inoculated | 12 | 11 | 9 | 9 |
| Body weight,* g | 18.6 ± 0.2 | 18.3 ± 0.2 | 18.1 ± 0.4 | 22.5 ± 0.4 † |
| Pain-related score,* % | 24 ± 7 | 25 ± 7 | 28 ± 8 | 26 ± 5 |
| Skin lesion score‡ | 7.7 ± 0.4 | 7.5 ± 0.4 | 7.3 ± 0.7 | 7.8 ± 0.8 |
| Mortality rate§ (%) | 2/12 (16.7) | 1/11 (9.1) | 1/9 (11.1) | 1/9 (11.1) |
| Incidence of postherpetic pain (%) | 2/10 (20) | 7/10 (70) | 6/8 (75) | 6/8 (75) |

* Determined at inoculation; mean \pm SEM. † $P < 0.05$ as compared with BALB/c (Student *t* test). ‡ Determined 6 days after inoculation; mean \pm SEM.

§ Determined 30 days after inoculation. || $P < 0.05$ as compared with BALB/c (chi-square test).

MHC = major histocompatibility complex.

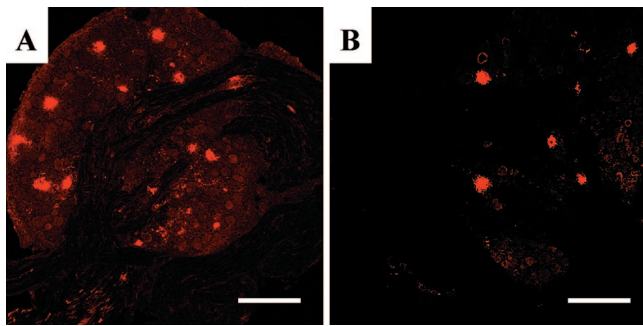


Fig. 2. Typical examples of distribution of herpes simplex virus antigen-positive cells in the dorsal root ganglion after inoculation. BALB/c (A) and C57BL/6 (B) mice were inoculated with herpes simplex virus type I on the unilateral hind paw. Six days later, immunostaining of the dorsal root ganglion (L5 level) on the inoculated side for the herpes simplex virus antigen was performed. Scale bar = 100 μ m.

proliferates in the DRG around 5 days after inoculation. In this study, we compared HSV-I proliferation in the DRG of BALB/c and C57BL/6 mice on 6 days after inoculation. We counted HSV antigen-immunoreactive infected neurons on 6 days after inoculation and found that $28.1 \pm 1.4\%$ neurons were immunoreactive in the DRG of BALB/c mice, whereas $4.5 \pm 0.5\%$ neurons were immunoreactive in that of C57BL/6 mice (figs. 2A and B). Therefore, the percentage of infected neurons in BALB/c mice was much higher than that in C57BL/6 mice.

As mentioned previously, HSV-1 proliferates in the DRG at an acute pain phase. At a postherpetic pain phase, latency-associated transcript, but not HSV antigen-like immunoreactivity, was observed in the DRG (I. Takasaki, Ph.D., and M. Sato-Takeda, M.D., Ph.D., unpublished observation, November 2002). In addition, cyclooxygenase 2 is induced there at an acute herpetic, but not postherpetic, pain phase.²¹ These findings suggest the neuroimmune activation at an acute pain phase. Therefore, we performed an immunohistochemical analysis of T-cell infiltration into DRG on 6 days after inoculation, an acute phase. CD3 was immunostained to examine the difference in the infiltration of T cells between BALB/c mice and C57BL/6 mice. Although there were no CD3-positive T cells in the DRG of noninoculated mice, many CD3-positive T cells were observed in the affected DRG (figs. 3 A-D). The number of CD3-positive T cells was markedly larger in C57BL/6 mice than in BALB/c mice (figs. 3C and D). Similar results were obtained from two other animals.

Discussion

In this study, we suggest the possibility that the incidence of postherpetic pain is dependent on the MHC haplotype (H-2^b). The current results support the idea that the HLA genes play an important role in the incidence of PHN in humans; the excessive immune response to VZV antigens presented by *HLA-A*3303*,

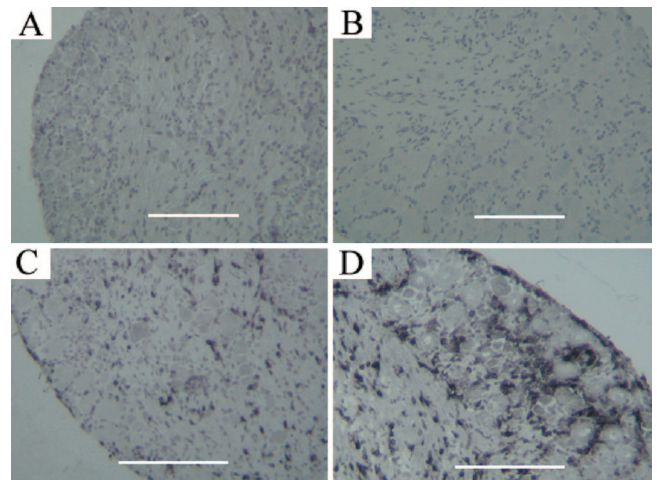


Fig. 3. Typical examples of the distribution of cells positive for CD3 in the dorsal root ganglia after herpes simplex virus type I inoculation. BALB/c and C57BL/6 mice were inoculated with herpes simplex virus type I on the unilateral hind paw. Six days later, CD3-positive T cells were immunostained in the dorsal root ganglia at the L4 and L5 levels in uninfected mice (A and B) and on the inoculated side (C and D). A and C show immunostaining of BALB/c mice, and B and D show that of C57BL/6 mice. Scale bar = 75 μ m.

*B*4403* and *DRB1*1302* or the HLA haplotype is one of the important risk factors for the incidence of PHN in humans but not for the incidence of herpes zoster.^{13,14} These support the idea that the HLA genes play an important role in the incidence of PHN in humans. These findings, taken together, suggest the existence of a common mechanism of pain development between PHN in humans and postherpetic pain in mice.

In general, PHN is a neuropathic pain that is one of the chronic pain diseases and is associated with infection or inflammation rather than with nerve trauma.^{26,27} It has been revealed recently that immune activation at DRG or dorsal roots causes hyperalgesia and that the immune system actively participates in creating and maintaining neuropathic pain of diverse etiologies.²⁷ It has been reported that trafficking of leukocytes into the central nervous system and MHC class II are associated with mechanical allodynia in rats with L5 spinal nerve transection, suggesting a role of central neuroinflammation in persistent neuropathic pain.^{20,28,29} In this respect, it has been revealed that PHN is not an exception, and immune reaction induced by the MHC system may affect its onset.

There are two possibilities regarding the association of MHC haplotype with postherpetic pain. One is that in mice possessing H-2^b, the virus easily proliferates, and the virus infection is more severe, which is a cause of postherpetic pain. An alternative possibility is that postherpetic pain results from an excessive immune response to virus antigens that may be efficiently presented by H-2^b. In this study, HSV-I proliferation in C57BL/6 (H-2^b) was less than that in BALB/c (H-2^d) mice. It is considered that C57BL/6 mice, which showed a

high incidence of postherpetic pain, have an efficient immune reaction against HSV-I, and an excessive immune response to the virus antigens rather than an inefficient immunoreaction might be the more likely mechanism, as our previous study suggested.¹⁴ This idea is supported by the results of T-cell staining in the DRG. HSV-I inoculation induced marked CD3-positive T cells in DRG innervating the inoculated region, although there were no CD3-positive T cells in the DRG of non-inoculated mice. CD3 antibody recognizes all mature T cells. Therefore, the results suggest that HSV-I inoculation causes the substantial infiltration of T cells in the infected DRG. CD3-positive T cells were markedly higher in C57BL/6 mice than in BALB/c mice. Therefore, it is possible that marked infiltration of T cells is responsible for higher incidence of postherpetic pain in C57BL/6 mice than in BALB/c mice. There is a report on a postmortem study of patients with PHN.³⁰ Patients with persistent pain showed dorsal horn atrophy, an infiltration of lymphocytes and cells, and axon and myelin loss with fibrosis in the sensory ganglion; however, this study did not mention the association between lymphocytes and PHN. Therefore, we first suggest an association of T-cell infiltration with the pathogenesis of postherpetic pain.

We used a postherpetic pain mouse model infected by HSV-I instead of VZV in this study. VZV infection to mice by the same method has not been successful to date because VZV is highly species specific, having a natural host range restricted only to humans.^{1-3,31} On the other hand, HSV-I has a low species specificity and can infect mice, rats, guinea pigs, and rabbits as well as humans.³²⁻³⁵ Both HSV-I and VZV belong to the α -herpes virus, and the genome structure and the nucleotide sequence of HSV-I are similar to those of VZV, suggesting that they are derived from a common ancestor.^{36,37} After primary infection, both VZV and HSV-I remain latent in DRG,³⁸ and then some factors reactivate the virus and produce clinical symptoms.³⁹⁻⁴² Therefore, it is considered that HSV-I is adequate for a mouse model of postherpetic pain, which currently corresponds to PHN in humans. Both VZV and HSV have been found to down-regulate MHC class I antigen expression, allowing viral dissemination to skin sites of replication that are required to achieve transmission.¹

In summary, the current study demonstrated that the MHC haplotype is associated with the susceptibility of postherpetic pain in mice and supports the idea that the HLA genes in human are responsible for the incidence of PHN. The infiltration of CD3-positive T cells in the DRG in the acute phase may be involved in the development of postherpetic pain.

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References

1. Abendroth A, Arvin A: Varicella-zoster virus immune evasion. *Immunol Rev* 1999; 168:143-56
2. Abendroth A, Arvin A: Immune evasion mechanisms of varicella-zoster virus. *Arch Virol Suppl* 2001; 17:99-107
3. Abendroth A, Arvin A: Immune evasion as a pathogenic mechanism of varicella zoster virus. *Semin Immunol* 2001; 13:27-39
4. Cluff RS, Rowbotham MC: Pain caused by herpes zoster infection. *Neurol Clin* 1998; 16:813-32
5. Portenoy RK, Duma C, Foley KM: Acute herpetic and postherpetic neuralgia: Clinical review and current management. *Ann Neurol* 1986; 20:651-64
6. Kost RG, Straus SE: Postherpetic neuralgia: Pathogenesis, treatment, and prevention. *N Engl J Med* 1996; 335:32-42
7. Weller TH: Varicella and herpes zoster: Changing concepts of the natural history, control, and importance of a not-so-benign virus. *N Engl J Med* 1983; 309:1434-40
8. Schmader K: Herpes zoster in older adults. *Clin Infect Dis* 2001; 32:1481-6
9. Dworkin RH, Portenoy RK: Proposed classification of herpes zoster pain (letter). *Lancet* 1994; 343:1648
10. Dworkin RH, Portenoy RK: Pain and its persistence in herpes zoster. *Pain* 1996; 67:241-51
11. Rowbotham MC, Fields HL: The relationship of pain, allodynia and thermal sensation in post-herpetic neuralgia. *Brain* 1996; 119:347-54
12. Belfer I, Wu T, Kingman A, Krishnaraju RK, Goldman D, Max MB: Candidate gene studies of human pain mechanisms. *ANESTHESIOLOGY* 2004; 100:1562-72
13. Sato M, Ohashi J, Tsuchiya N, Kashiwase K, Ishikawa Y, Arita H, Hanaoka K, Tokunaga K, Yabe T: Association of HLA-A*3303-B*4403-DRB1*1302 haplotype, but not of TNFA promoter and NKp30 polymorphism, with postherpetic neuralgia (PHN) in the Japanese population. *Genes Immun* 2002; 3:477-81
14. Sato-Takeda M, Ihn H, Ohashi J, Tsuchiya N, Ishikawa Y, Satake M, Arita H, Tamaki K, Hanaoka K, Tokunaga K, Yabe Y: The human histocompatibility leukocyte antigen (HLA) haplotype is associated with the onset of postherpetic neuralgia after herpes zoster. *Pain* 2004; 110:329-36
15. Sadtot-Delvaux C, Merville-Louis MP, Delree P, Piette J, Moonen G, Rentier B: An *in vivo* model of varicella-zoster virus latent infection of dorsal root ganglia. *J Neurosci Res* 1990; 26:83-9
16. Takasaki I, Andoh T, Shiraki K, Kuraishi Y: Allodynia and hyperalgesia induced by herpes simplex virus type-1 infection in mice. *Pain* 2000; 86:95-101
17. Takasaki I, Andoh T, Nitta M, Takahata H, Nemoto H, Shiraki K, Nojima Kuraishi Y: Pharmacological and immunohistochemical characterization of a mouse model of acute herpetic pain. *Jpn J Pharmacol* 2000; 83:319-26
18. Sasaki A, Takasaki I, Andoh T, Nojima H, Shiraki K, Kuraishi Y: Role of α -adrenoceptors and sympathetic nerve in acute herpetic pain induced by herpes simplex virus infection. *J Pharmacol Sci* 2003; 29:329-36
19. Takasaki I, Sasaki A, Andoh T, Nojima H, Shiraki K, Kuraishi Y: Effect of analgesics on delayed postherpetic pain in mice. *ANESTHESIOLOGY* 2002; 96:1168-74
20. Sweitzer SM, White KA, Dutta C, DeLeo C: The differential role of spinal MHC class II and cellular adhesion molecules in peripheral inflammatory *versus* neuropathic pain in rodents. *J Neuroimmunol* 2002; 125:82-93
21. Takasaki I, Nojima H, Shiraki K, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, Kuraishi Y: Involvement of cyclooxygenase-2 and EP3 prostaglandin receptor in acute herpetic but not postherpetic pain in mice. *Neuropharmacology* 2005; 49:283-92
22. Weiss EH, Golden L, Fahrner K, Mellor AL, Devlin JJ, Bullman H, Tiddens H, Bud H, Flavell R: Organization and evolution of the class I gene family in the major histocompatibility complex of the C57BL/10. *Nature* 1984; 310:650-5
23. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983; 16:109-10
24. Kuraishi Y, Takasaki I, Nojima H, Shiraki K, Takahata H: Effects of the suppression of acute herpetic pain by gabapentin and amitriptyline on the incidence of delayed postherpetic pain in mice. *Life Sci* 2004; 74:2619-26
25. Dokun AO, Chu DT, Yang L, Bendelac AS, Yokoyama WM: Analysis of in situ NK cell responses during viral infection. *J Immunol* 2001; 167:5286-93
26. Said G, Hontebeyrie-Joskowicz M: Nerve lesions induced by macrophage activation. *Res Immunol* 1992; 143:589-99
27. Watkins LR, Maier SF: Beyond Neurons: Evidence that immune and glial cells contribute to pathological pain states. *Physiol Rev* 2002; 82:981-1011
28. Sweitzer SM, Hickey WF, Rutkowski MD, Pahl JL, DeLeo JA: Focal peripheral nerve injury induces leukocyte trafficking into the central nervous system: Potential relationship to neuropathic pain. *Pain* 2002; 100:163-70
29. DeLeo JA, Yezierski RP: The role of neuroinflammation and neuroimmune activation in persistent pain. *Pain* 2001; 90:1-6

30. Watson CPN, Deck JH, Morshead C, Van der Kooy D, Evans RJ: Postherpetic neuralgia: Further post-mortem studies of cases with and without pain. *Pain* 1991; 44:105-17
31. Straus SE, Ostrove JM, Inchauspe G, Felser JM, Frifel A, Croen KD, Sawyer MH: Varicella-zoster virus infections. Biology, natural history, treatment, and prevention. *Ann Intern Med* 1988; 108:227-37
32. Andoh T, Shiraki K, Kurokawa M, Kuraishi Y: Paresthesia induced by cutaneous infection with herpes simplex virus in rats. *Neurosci Lett* 1995; 190:101-4
33. Schaeffer HJ, Beauchamp L, de Miranda P, Elion GB, Bauer DJ, Collins P: 9-(2-Hydroxyrhanoxymethyl) guanine activity against viruses of the herpes group. *Nature* 1978; 272:583-5
34. Collins P, Oliver NM: Acyclovir treatment of cutaneous herpes in guinea pigs and herpes encephalitis in mice. *Am J Med* 1982; 73:96-9
35. Simmons A, Nash AA: Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. *J Virol* 1984; 52:816-21
36. Davidson AJ, Scott JE: The complete DNA sequence of varicella zoster virus. *J Gen Virol* 1986; 67:1759-816
37. Davidson AJ, Scott JE: DNA sequence of the major capsid protein gene of herpes simplex virus type-1. *J Gen Virol* 1986; 67:2279-86
38. Warren K, Brown SM, Wroblewska Z, Gilden D, Koprwski H, Subak-Sharpe J: Isolation of latent herpes simplex virus from the superior cervical and vagus ganglions of human beings. *N Engl J Med* 1978; 298:1068-9
39. Selmanowitz VJ: Neurocutaneous herpes simplex. *Int J Dermatol* 1971; 10:227-32
40. Krohel GB, Richardson JR, Farrell DF: Herpes simplex neuropathy. *Neurology* 1976; 26:596-7
41. Whitley RJ, Roizman B: Herpes simplex virus infection. *Lancet* 2001; 357:1513-8
42. Nahmias AJ, Roizman B: Medical progress: Infection with herpes simplex viruses 1 and 2. *N Engl J Med* 1973; 289:781-8