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Original article

Herpes simplex virus-induced murine dry skin model through sweating disturbance

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ABSTRACT

Background: Given that ocular glands become infected secondarily to herpes simplex virus 1 (HSV-1) keratitis, resulting in the loss of tear production, sweat glands may also be susceptible to HSV-1 infection, resulting in sweating disturbance, which is observed frequently in atopic dermatitis. However, due to the lack of sweat glands on the hairy skin of mice, the role of sweating in the maintenance of skin hydration has not been elucidated.

Objective: To determine the relationship between HSV-1 infection of sweat glands and sweating disturbance-induced dry skin.

Methods: By using the impression mold technique, we examined the sweating response together with the detection of HSV-1 DNA in the sweat glands of footpads, the only area with sweat glands in mice, after local cutaneous HSV-1 inoculation of immunocompetent mice.

Results: The sweating response and skin surface hydration were significantly decreased at 7–14 days post-infection. Sweating disturbance and dry skin was markedly enhanced when HSV-1 inoculation was followed by hyperthermic stress. Both resolved spontaneously and became resistant to a second HSV-1 inoculation, associated with increased anti-HSV-IgG antibodies. HSV-1 DNA was detected in sweat glands and dorsal root ganglia. The sweating response remained decreased after subcutaneous injection with pilocarpine, correlating histologically with marked dilatation of sweat gland lumens. These findings indicate that sweating disturbance is unlikely to be the outcome of nerve damage by HSV-1 infection.

Conclusion: Sweating disturbance could be due to HSV-induced dysfunction of sweat glands. We developed a sweating disturbance-induced dry skin mouse model by infection with HSV-1.

1. Introduction

Herpes simplex virus (HSV) and varicella zoster virus (VZV) are neurotropic and neuroinvasive viruses that persist in the human body by becoming latent in nerve cells. Although HSV infection is thought to be confined to the epidermis and nerves, the VZV can also involve the adnexa [1]. However, these differences are not absolute and HSV can sometimes involve the adnexa and present as a syringitis [2]. Under controlled conditions, experimental inoculation of small animals, including mice, with HSV has provided a wealth of information about the pathogenesis of the virus, but it does not necessarily mimic all of the features of primary HSV infection in humans. Although a number of pathological processes in the human skin suggest the presence of VZV in sweat glands [3–5], no attention has been given to the presence of HSV-1 in the sweat glands of humans and mice. In part, this may be due to the negative results obtained by routine immunohistochemical analysis of diseased skin in humans [2,6]. However, the organ distribution of the virus may provide clues about how HSV infection has an impact on the function of sweat glands. Indeed, a recent report demonstrated the presence of herpetic syringitis in an immunocompetent patient, although HSV DNA was not detected in sweat glands [7].

Dryness of the stratum corneum is often linked to the dysfunction of the skin barrier observed in patients with atopic dermatitis (AD), while ignoring the beneficial role of sweating on skin dryness [8]. Humans are among the limited number of mammals that have eccrine glands on hairy and glabrous skin. In contrast, mice have eccrine glands only on the footpads. Due to the lack of sweat glands

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on hairy skin in mice and given that their function is limited to skin hydration, the function of footpad sweat glands has rarely been examined in previous studies. In contrast, the involvement of ocular glands during corneal HSV-1 infection was only recently described [9,10]; ocular glands were shown to be susceptible to secondary infection with HSV-1. Given that the surface moisture of the eye is provided by ocular glands and that decreased glandular function may result in dry eye, as shown in the skin of patients with AD, it follows that sweat glands might also be involved in HSV-1-induced cutaneous changes through their susceptibility to HSV-1 infection, thereby impairing their function. We therefore wondered whether there might be a possible link between HSV infection in the sweat gland and sweating disturbance with the subsequent risk of developing dry skin.

To consider this hypothesis, we examined whether cutaneous HSV-1 inoculation of the thigh of wild-type immunocompetent C57BL/6J mice could lead to infection of sweat glands, thereby causing sweating disturbance. Shimoda et al. and we previously evaluated the sweating response in humans by the impression mold technique (IMT) and skin surface hydration [8,11]; the sweating response in murine footpads can also be evaluated using the IMT. We also examined the presence of HSV-1 DNA in sweat glands using a laser-micro-dissection method and real-time quantitative PCR (qPCR). Our results confirmed the presence of HSV-1 DNA in sweat glands. Furthermore, the observed sweating

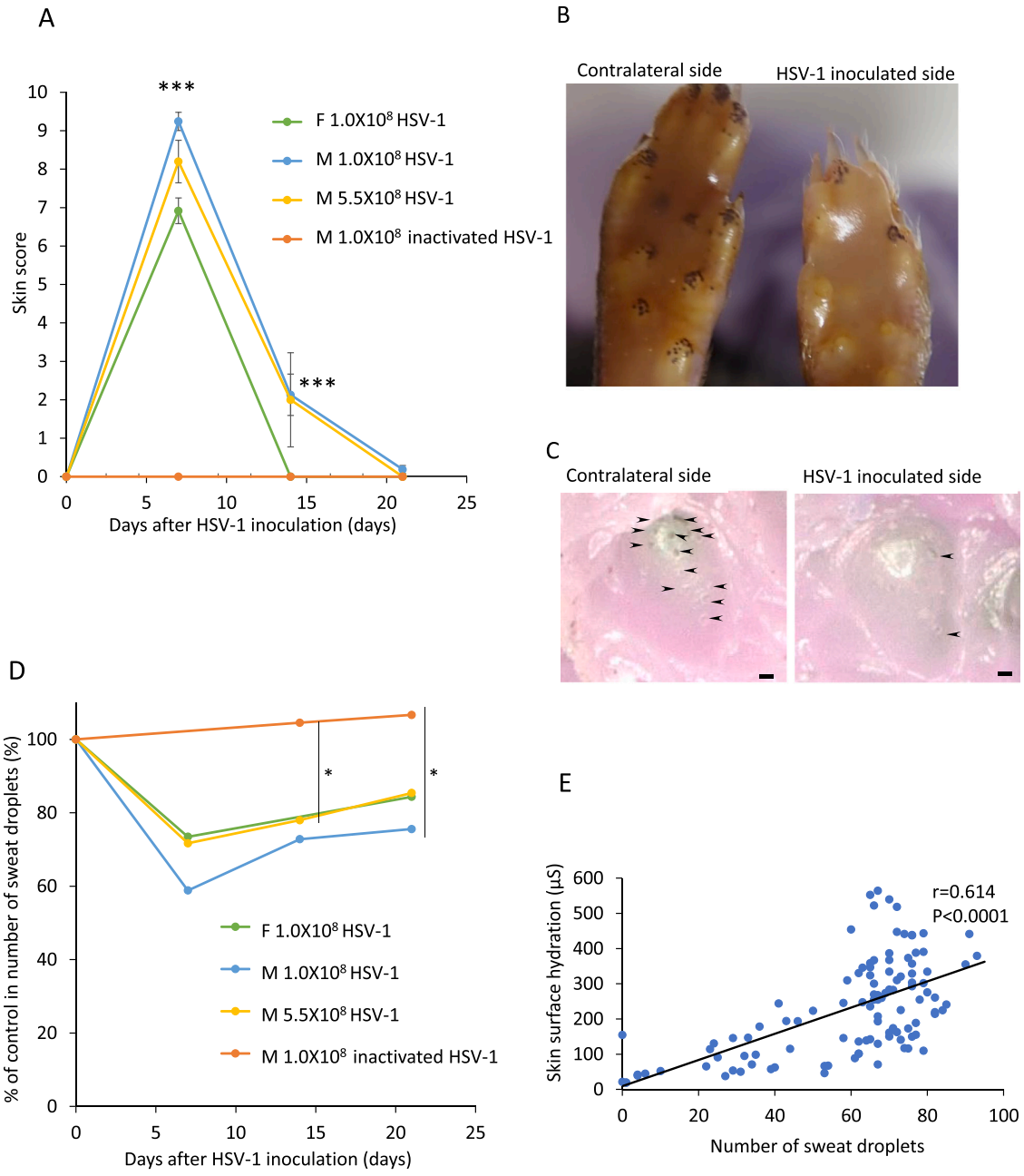

Fig. 1. HSV-1 induced zoster-like inflammation and decreased sweating responses in mouse footpad. A. Alterations in skin score over time after HSV-1 inoculation. Data are expressed as the mean and standard error (N = 6–16). Differences between four groups were examined by the Steel–Dwass test. *** $P < 0.001$. M, male;F, female. **B**. Representative sweating responses on the plantar skin of the hind paw on the HSV-1-inoculated side and contralateral side determined using Minor's iodine-starch test.**C**. Representative sweating responses in the footpad on the HSV-1-inoculated side and contralateral side determined using the impression mold technique. Sweating droplets are indicated by arrow heads. Bar,100 μm. Differences between four groups were examined by the Tukey–Kramer test. **D.** Induction of sweating disturbance over time after HSV-1 inoculation. Data are expressed as the mean and standard error (N = 6–16). * $P < 0.05$ M, male; F, female. **E**. The correlation between the number of sweat droplets and skin surface hydration is examined by Pearson's product-moment correlation coefficient.

disturbance was markedly worsened when infection was followed by hyperthermic stress. Our findings provide a mechanistic link between HSV infection and dry skin due to sweating disturbance, which is typically observed in AD skin [8].

2. Materials and methods

C57BL/6J mice were anesthetized, and mild scratching of the thigh skin was carried out with a 27-gauge needle to disrupt the epidermal barrier. Following this, 1.0 or 5.5 × 10^8 plaque-forming units (pfu)/mL HSV-1 [12] was applied topically to the skin surface in 10 μL phosphate-buffered saline (Suppl. Fig. 1). All animal experiments were carried out in accordance with "Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan, and the experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Kawasaki Medical School. Additional details are included in Supplementary Materials and Methods.

3. Results

3.1. Sweating disturbance induced by HSV inoculation

We first performed cutaneous HSV-1 inoculation in C57BL/6J mice. The mice were inspected on a regular basis for cutaneous lesions by investigators (YA, HI) unaware of the experiment, and the severity of lesions with zoster-like inflammation was scored on a 0–10 scale, with 0 corresponding to no visible lesions and 10 corresponding to the most severe cutaneous lesions [12] (Supplementary Materials and Methods, Suppl. Fig. 1). As early as day 3 post-infection (p.i.), the mice started to present with signs of inflammation, which peaked on day 7 p.i. (Fig. 1A). No significant inflammation was detected in mice inoculated with heat-inactivated HSV-1 (Fig. 1A). HSV-1 inoculation produced a significant reduction in the sweating response as evidenced by a reduction in the number of sweat droplets on footpads, as detected using the IMT (Fig. 1B–D and Suppl. Fig. 2) and iodine starch method (Fig. 1B). Because each footpad is composed of six small pads, we examined which small pads were preferentially susceptible to HSV-1-induced sweating disturbance. As shown in Suppl. Fig. 3, the pattern of sweating disturbance was not associated with the innervation area and randomly distributed, suggesting that HSV-1-induced sweating disturbance would occur totally independently of innervation. The sweating response of HSV-1-inoculated mice was significantly decreased at 7–21 days p.i. compared with those treated with heat-inactivated HSV-1 (Fig. 1D). The decreased sweating response was reflected in skin surface hydration levels ($r = 0.614$, $P < 0.0001$, Fig. 1E). There was no disturbance of the sweating response in contralateral sweat glands (Fig. 1B and C), indicating that the impaired sweating response was restricted to the area of infection. Day 7 p.i. was selected as the optimal time point for HSV-1-induced inflammation and pathological changes in susceptible mice. Female mice were more resistant to HSV-1 infection compared with male mice (Fig. 1A and D). Thus, male mice were used for the following experiments.

We next examined whether hyperthermic stress after HSV inoculation could enhance HSV-induced sweating disturbance because a recent study demonstrated that a single bout of prolonged exercise impairs antiviral immunity to HSV-2 intravaginal infection in mice [13]. Hyperthermic stress at 20 hr after HSV inoculation (HT post 20 hr), but not at 4 hr before HSV inoculation (HT pre 4 hr), induced a more robust inflammatory response, as evidenced by a slight increase in inflammation scores, compared with HSV-1-inoculated mice without hyperthermic stress (Fig. 2A and B). Reflecting the robust inflammatory response, significantly more severe sweating disturbance was observed in HSV-1-inoculated mice with hyperthermic stress at day 7 p.i. (Fig. 2C). The disturbance of sweating function was more intense and sustained until day 21 p.i. in HSV-1-inoculated mice with hyperthermic stress.

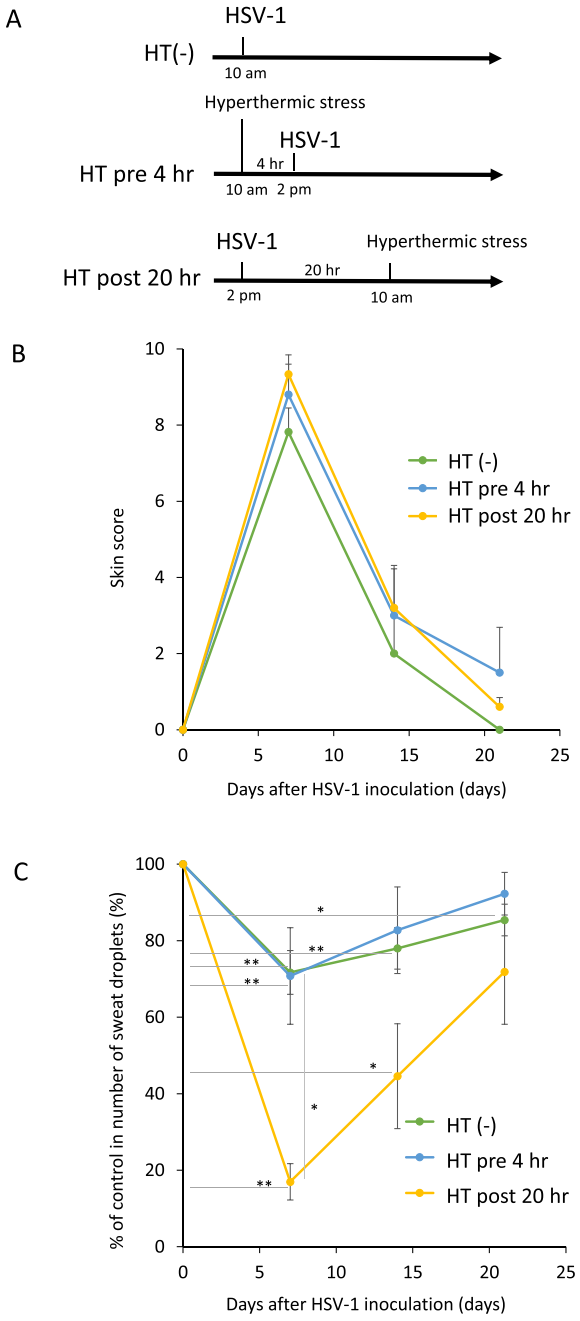

Fig. 2. Enhancement of HSV-1 induced sweating disturbance in the footpad of mice inoculated with HSV-1 followed by hyperthermic stress. A. Experimental design. Mice received hyperthermic stress either at 4 hr before (HT pre 4 hr) or 20 hr after HSV infection (HT post 20 hr). **B**. Alterations in skin score over time in mice after HSV-1 inoculation followed by hyperthermic stress. Data are expressed as the mean and standard error (N = 5–11). Differences between three groups are examined by the Steel–Dwass test. **C**. Sweating disturbance over time in mice after HSV-1 inoculation followed by hyperthermic stress. Differences between baseline and days 7–21 were examined with a *t*-test. Differences between three groups are examined by the Tukey–Kramer test. N = 5–11. $^{*}P < 0.05$, $^{**}P < 0.01$.

3.2. Histological changes in HSV-inoculated mice

Hematoxylin and eosin staining of footpad tissue sections revealed the greatest dilatation of the lumen of sweat glands with inflammatory cell infiltrates in HSV-1-inoculated mice with and

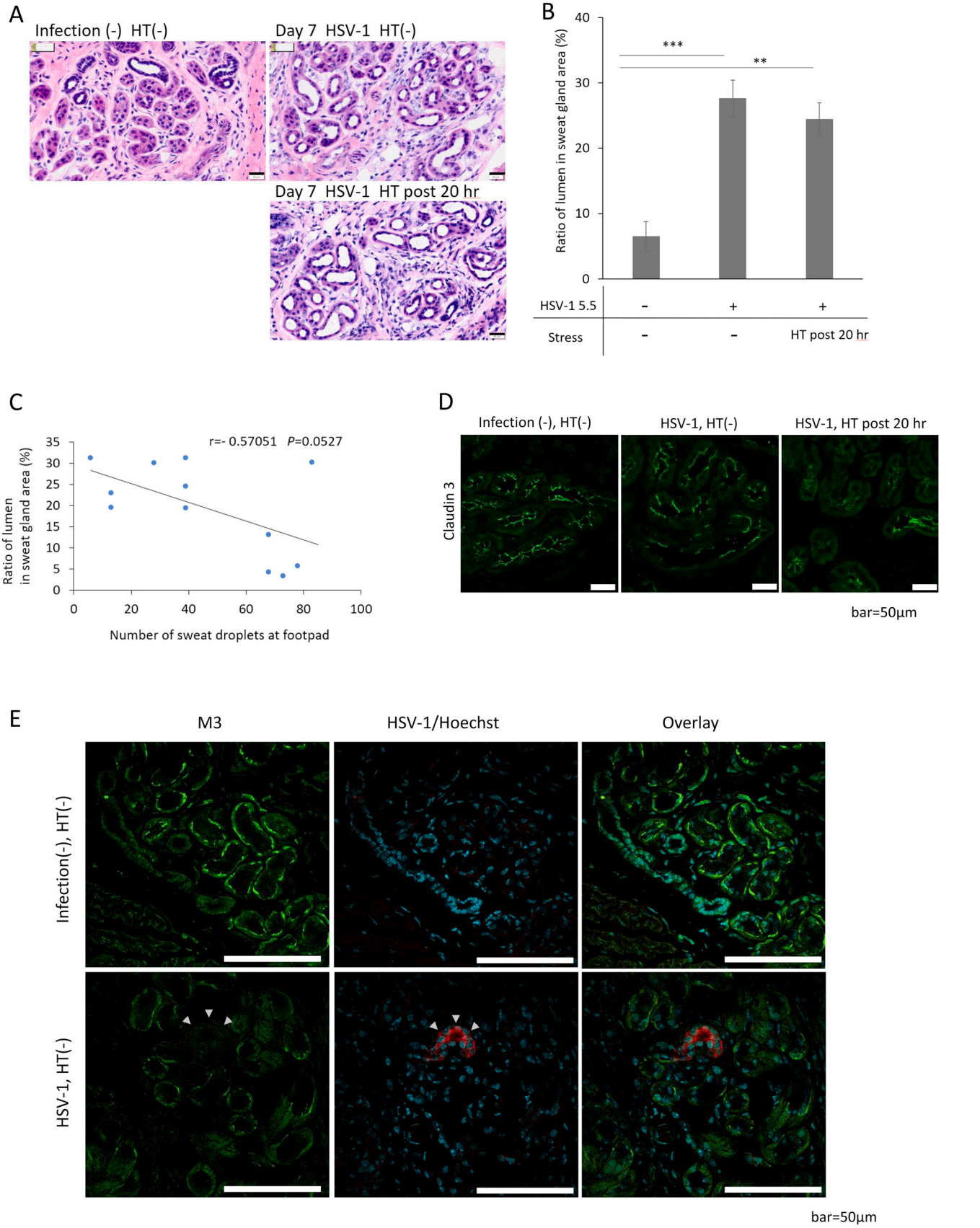

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Fig. 3. Enhancement of the dilatations of the lumen in the sweat glands of the footpad of mice inoculated with HSV-1 followed by hyperthermic stress. **A.** Hematoxylin and eosin staining of footpad sweat glands after HSV-1 infection. Control mice neither received hyper-thermic stress nor HSV-1 inoculation. The sweat ducts of control mice and mice with and without hyperthermic stress after HSV-1 infection are compared. No dilatation of the lumen of sweat glands in control mice. Dilatation of the lumen of sweat glands in mice inoculated with HSV-1 with or without hyperthermic stress is noted. Scale bar = 20 μm. **B.** Percentage of the area of the lumen in total area of sweat glands. Data are expressed as the mean and standard error ($n = 4$). The area of sweat glands and lumens are measured with cellSens imaging software (ver. 1.14; Olympus). Differences between three groups are examined by the Tukey–Kramer test. ** $P < 0.01$, *** $P < 0.001$. **C**. The correlation between the number of sweat droplets and percentage of the area of the lumen in total area of sweat glands is examined by Pearson's product-moment correlation coefficient. **D.** Immunostaining of claudin 3 of sweat glands in footpad 7 days after HSV-1 infection. Control mice neither received hyper-thermic stress nor HSV-1 inoculation. N = 3; Bar 50 μm. **E.** Immunostaining for HSV-1 and muscarinic acetylcholine receptor M3 in sweat glands in footpad 7 days after HSV-1 infection. Control mice neither received hyper-thermic stress nor HSV-1 inoculation. N = 3; Bar 50 μm.

without hyperthermic stress, but not in those without HSV-1 inoculation (Fig. 3 A and B); sweating disturbance was associated with extensive architectural damage to sweat glands as evidenced by gland dilatation and flattening of luminal cells. The magnitude of luminal dilatation was reflected in the severity of sweating disturbance. As shown in Fig. 3 C, a positive relation was found between the magnitude of luminal dilatation and the severity of sweating disturbance. Because there were no keratotic plugs in the ductal opening of the footpad of HSV-1-inoculated mice, it is unlikely that keratotic plugs are responsible for the HSV-1- induced sweating disturbance.

We next performed immunohistochemical analysis with tight junction-related proteins localized around sweat glands, claudin 3, to investigate whether this protein expression was decreased in association with HSV-induced sweating disturbance (Fig. 3D). Claudin 3 expression was profoundly decreased in the HSV-inoculated mouse footpads, especially in HSV-inoculated mice followed by hyperthermic stress, demonstrating a decrease in a functionally required marker for sweat secretion. Interestingly, a decrease in claudin 3 expression was associated with the lumen dilatation of sweat glands, suggesting that the lumen dilation of sweat glands may reflect a decrease in claudin 3 expression.

By day 21 p.i., all mice showed recovery from HSV-1 infection as evidenced by the resolution of inflammation; sweating function also improved spontaneously following recovery from HSV-1 infection. Nevertheless, sweating disturbance induced by HSV inoculation followed by hyperthermic stress was not completely restored by day 21 p.i (Fig. 2C). An HSV-1 inoculation dose of 5.5×10^8 pfu/mL was well tolerated by naive C57BL/6J mice and no mice died. On the use of a lower viral dose (1.0×10^8 pfu/mL), a similar pattern of results was also obtained (Fig. 1D).

3.3. Detection of HSV-DNA in the skin, sweat glands, and dorsal root ganglia (DRG)

Next, we asked whether HSV-1 DNA could be detected in murine sweat glands with sweating disturbance as well as the DRG, and whether the inflammatory response in the cutaneous lesions was associated with the disturbance of sweating function. Tissues were harvested from at least eight mice at the indicated times during acute infection; each sample was measured in duplicate, and the number of copies/μg of DNA was calculated as the average and median (1st and 3rd quartiles) of these measurements. The sensitivity of our qPCR assay was < 17 copies/μg DNA. At days 3–14 p.i., viral loads were quantified in the footpad skin, sweat glands, and DRG from the mice by qPCR, as previously described [14]. At the peak of the clinical symptoms at day 7 p.i., high levels of HSV-1 DNA were detected in the skin, DRG, and sweat glands (Fig. 4 A-C). HSV-1 DNA levels in sweat glands ranged from low to relatively significant levels, which was probably due to differences in the amount of tissue collected and the timing of sampling. Viral DNA levels were highest at the inoculation site on day 3 p.i. HSV-1 DNA in sweat glands disappeared by day 10 p.i., except in one mouse, whereas the same level of viral DNA was detected in the DRG until day 14 p.i., suggesting that latent infection appeared to be in the DRG. HSV-1 DNA was detected persistently in the DRG even on the contralateral side, which does not innervate the site of infection (Fig. 4 D,E). Based on the kinetics of viral DNA in the tissues, we hypothesize that HSV-1 topically applied could traffic via the blanch of the DRG to the sweat glands. These results suggest that sweat glands are susceptible to HSV-1 infection, but do not support its replication. We can conclude that the virus replicates in the skin and DRG, but not in the sweat glands. The difference in viral loads between sweat glands and DRG is expected to limit the detection of HSV-1 DNA in sweat glands. In addition, the inoculated virus was cleared much faster from sweat glands than from the DRG. The HSV-1 copy number in sweat glands was markedly increased at day 7 in the hyperthermic stress group (Fig. 4 F-H), suggesting that hyperthermic stress after HSV-1 infection renders mice more permissive to persistent HSV-1 infection of sweat glands. Nevertheless, no relationship was observed between the magnitude of inflammation as evidenced by skin scores and HSV-1 DNA load (data not shown).

3.4. Sweating responses to pilocarpine

To exclude the involvement of autonomic nerve innervation in sweating disturbance, autonomic innervation of sweat glands in the HSV-infected footpad was evaluated by measuring the sweating response over 7–8 min after intraperitoneal injection of pilocarpine (0.025 w/v%), a muscarinic acetylcholine (ACh) agonist [15]. Mice, which were initially immobilized in a plastic tube while awake, were defined as an emotional sweating condition. Next, mice immobilized were anesthetized (a resting condition), ~~t~~hen followed by pilocarpine (a pilocarpine stimulation condition). Under a pilocarpine stimulation condition as well as an emotional sweating condition, mice not inoculated with HSV-1showed significant levels of sweating responses to pilocarpine, indicating that the pilocarpine administration system works. In contrast, mice inoculated with HSV-1 showed a markedly decreased sweating response to pilocarpine (Suppl. Fig. 4, Pilocarpine stimulation), indicating that the sweat disturbance in HSV-1-infected mice was not restored by administration of pilocarpine. Thus, the sweating disturbance observed in mice inoculated with HSV-1 is likely due to dysfunction of the sweat gland unit composed of sweat glands, periglandular capillaries and myoepithelial cells. Thus, HSV-1 infection of sweat gland is characterized by varying degrees of functional impairment of sweat gland cells and myoepithelial cells and the defect is a principal reason for the inability of the host to secret sweat. Muscarinic ACh receptor M3 (mAChRM3) expression as a basolateral marker of sweat glands was detected to a lower value in HSV-inoculated mouse footpads as compared with that in non-treated footpads (Fig. 3E).

To further confirm HSV-1 infection of the sweat glands, immunohistochemical staining of HSV-1 antigen was performed. As shown in Fig. 3E, some HSV-1 positive sweat gland cells were detected inside faint mAChRM3 positive cells in the footpad on day 7p.i.

3.5. Sweat glands that have recovered from sweating disturbance become resistant to a second HSV inoculation and the involvement of anti-HSV-1 IgG antibodies

At more than 30 days after the first inoculation, mice were again inoculated cutaneously with 5.5×10^8 pfu/mL HSV-1. Following this

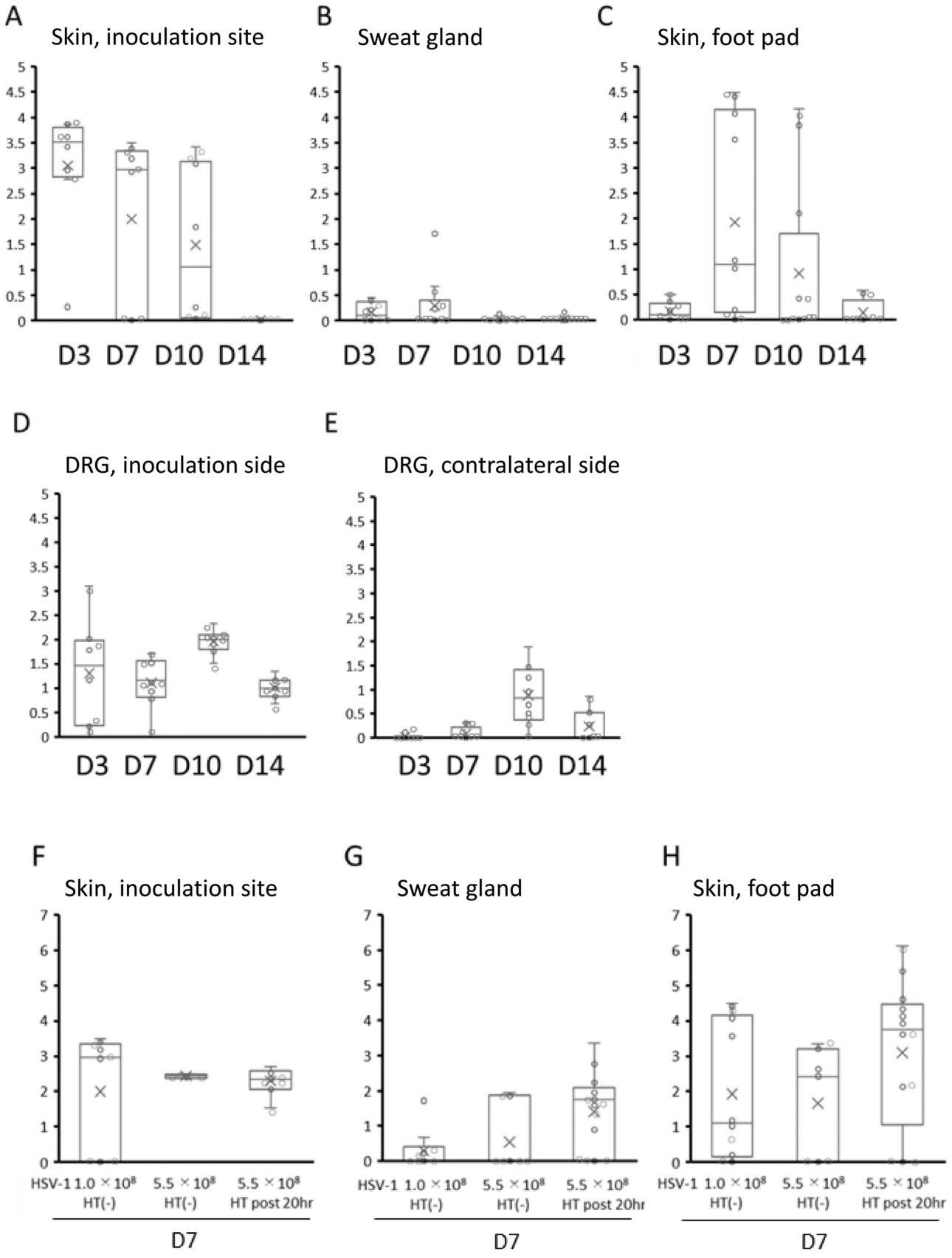

Fig. 4. HSV-1 DNA in various tissues from mice at various time points after HSV-1 inoculation. The marked enhancing effect of hyperthermic stress on the virus detection in sweat glands in mouse footpad. HSV-1 DNA levels in the skin (inoculation site, A), sweat glands (B), footpad skin (C), and dorsal root ganglia (D, inoculation side; E, contralateral side) after HSV-1 inoculation. HSV-1 DNA levels in the skin (inoculation site, F), sweat glands (G), and footpad skin (H) after HSV-1 inoculation with (HT post 20 hr) or without hyperthermic stress [HT (-)]. N = 8. Note that virus detection in sweat glands after HSV-1 administration was markedly enhanced by hyperthermic stress.

Table 1
Effect of prior infection on HSV-1-induced sweating disturbance.

Pretreatment	Infection	n	Skin score		Number of sweat droplets				*p* value
			Day 7 after infection		Before infection		Day 7 after infection		
			mean	SD	mean	SD	mean	SD	
(-)	Inactivated HSV-1 1.0	6	0.0	0.0	72.3	7.4	75.7	9.0	0.095
(-)	HSV-1 1.0	23	8.4	2.5	74.5	6.4	45.0	28.1	< 0.001
(-)	HSV-1 5.5	15	8.0	2.0	72.5	4.6	52.5	16.7	< 0.001
(-)	HSV-1 5.5 HT pre 4 hr	5	8.8	1.8	68.6	2.2	48.2	18.0	0.1
(-)	HSV-1 5.5 HT20 hr p.i.	12	9.3	1.8	73.4	5.6	17.5	20.0	< 0.001
HSV1 5.5 (left)	HSV-1 5.5 (left)	4	0.5	1.0	68.3	5.0	67.0	6.0	0.754
HSV1 1.0 (left)	HSV-1 1.0 (left)	8	4.1	0.8	62.3	7.0	61.5	8.0	0.838
HSV1 1.0 (left)	HSV-1 1.0 (right)	3	2.7	1.2	75.7	6.0	75.0	5.0	0.882
Inactivated HSV1 5.5 (left)	HSV-1 5.5 (left)	11	0.2	0.6	70.4	3.4	67.7	4.4	0.1313

1.0, 1.0×10^8 pfu/mL; 5.5, 5.5×10^8 pfu/mL; HSV, herpes simplex virus; HT, Hyperthermic stress;n, number of mice, p.i., post infection; SD, standard deviation.

second inoculation, the mice developed mild skin lesion scores of 2 and 3 without sweating disturbance, indicating that HSV-1-inoculated mice were resistant to HSV-1 infection compared with naive mice (Table 1). Measurement of sweat droplets in the same footpads before and after the second HSV-1 inoculation showed that HSV-1-inoculated mice that then recovered from the sweating disturbance showed no significant sweating disturbance after a second HSV-1 inoculation in comparison with non-pretreated mice. Surprisingly, even sweat glands in mice inoculated initially with heat-inactivated HSV-1 became resistant to the induction of sweating disturbance by a second inoculation with live HSV-1 (Table 1); even those in the contralateral side were also resistant, suggesting the protection afforded by robust antibody responses may occur in the sweat gland system.

Because antibodies have long been appreciated as a means to protect the host against HSV-1 infection, we asked whether anti-HSV-1 IgG antibodies could be involved in the protection against sweat gland HSV-1 infection. HSV-1-inoculated mice with hyperthermic stress generated an equal level of anti-HSV-1 IgG antibodies compared with those inoculated with HSV-1 but without hyperthermic stress (mean ± SE on day 28, 103.74 ± 7.0, 108.84 ± 19.8, $P = 0.8145$). Mice initially inoculated with heat-inactivated HSV-1 did not generate a significant level of anti-HSV-1 IgG on day 21 p.i. (Fig. 5A), but when this was followed by inoculation with 5.5×10^8 pfu/mL HSV-1(Fig. 5B), a comparable level of anti-HSV-IgG was generated on day 7 p.i. (Fig. 5C), suggesting that HSV-1 proliferation helps to optimize the anti-HSV-1 humoral immune response and that anti-HSV IgG antibody production is markedly accelerated in mice initially inoculated with heat-inactivated HSV-1 followed by live HSV-1 inoculation, indicating that anti-HSV-1 IgG may play a role in protecting the sweat gland from HSV-1 infection.

4. Discussion

This study aimed to establish an in vivo model of virally induced sweating disturbance to investigate the role of HSV infection in the complex pathogenesis of sweating disturbance-related diseases, such as AD; therefore, the reduced sweat levels observed in patients with AD are likely to contribute to skin barrier dysfunction [8,16–18]. In support of our hypothesis that sweat glands are susceptible to HSV infection, leading to a disturbance of sweating function, we demonstrated that HSV infection of the skin resulted in HSV-induced functional damage to sweat glands. Decreased sweat gland function might lead to cutaneous desiccation. The decrease in sweat production in the footpads of mice percutaneously infected with HSV-1 suggests that HSV infection to sweat glands in AD patients would contribute at least in part to the development of dry skin [8,11,17,19].

Patients with AD exhibit increased susceptibility to a limited range of pathogens, namely, *Staphylococcus aureus* and HSV [20,21]. Indeed, a severe form of HSV infection, named eczema herpeticum, occurs almost exclusively in AD patients, particularly in those in whom the skin lesions are not controlled [22,23]. Patients with AD often experience decreased sweat production, especially in dry-appearing skin, suggesting prolonged dysfunction of the processes involved in sweat production. We hypothesized that sweat glands in patients with AD have increased susceptibility to HSV infection, thereby impairing their function and leading to dry skin. The acetone/ethanol/water-induced murine model has been used most widely for studying dry skin [24]. However, because there is no murine dry skin model in which sweating disturbance and viral infection are primarily involved in the induction of dry skin, our aim was to provide a convincing link between the presence of HSV-1 in sweat glands and their functional disturbance and to establish a virally induced dry skin murine model. We, for the first time, demonstrated that HSV infection to sweat glands could be one of the causes of the loss of sweat production.

By utilizing a mouse model of cutaneous HSV-1 inoculation, we found that sweat glands were functionally damaged by HSV-1 infection following percutaneous inoculation in a time-dependent fashion, the HSV-1-induced sweating disturbance recovered within 3–4 weeks, and the mice that recovered from sweat gland dysfunction became resistant to a second percutaneous inoculation with HSV-1, as shown in an HSV-lacrimal gland inflammation model [9]. Because mice inoculated with heat-inactivated HSV-1, when subsequently followed by second HSV-1 inoculation, generated comparable or more accelerated levels of anti-HSV-1 IgG antibodies (Fig. 5C), their sweat glands were protected from subsequent HSV-1 inoculation, as evidenced by the absence of sweating disturbance.

Although our data suggest that HSV-1 has a role in the initiation, progression, and exacerbation of sweating disturbance, other studies suggest no such effect. However, in our unique mouse model, the HSV-1-induced disturbance of sweating function resolved spontaneously at 3–4 weeks p.i. without any sequelae. HSV demonstrates latency in humans, while it is rapidly fatal in mice and latency occurs much less frequently in immunocompetent mice [25–28]. Therefore, it has been difficult to establish a mouse model of HSV-induced persistent sweating disturbance. Based on the similarities in the clinical symptoms and pathogenesis between HSV-1-induced sweating disturbance in our mouse model and the sweating disturbance observed in AD patients, we consider that this model is useful for understanding virally induced sweating disturbance and may lead to the development of innovative therapeutic strategies for sweating disturbance.

The clinical diagnosis of sweating disturbance in inflammatory skin disease, even if coupled with measurements of the sweating response, such as with the IMT, remains challenging given that

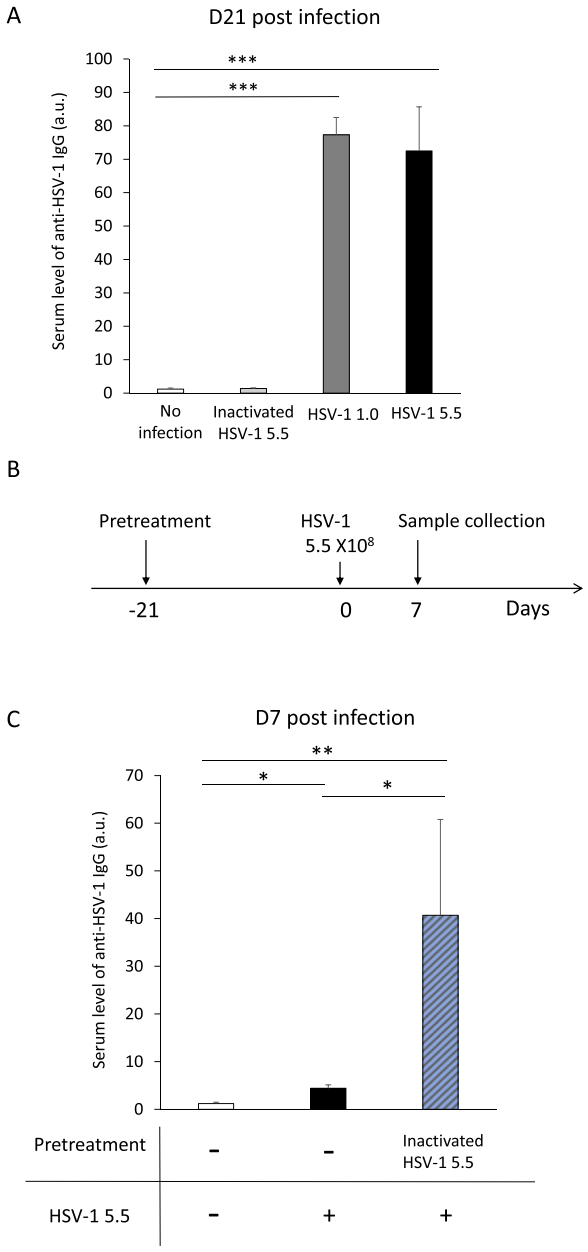

Fig. 5. Effect of heat-inactivated HSV-1 inoculation on serum levels of anti-HSV-1 IgG. A. Serum anti-HSV-1 IgG antibody levels on days 21 after heat-inactivated HSV-1 or live HSV-1 inoculation. N = 3–12. B. Experimental design. Mice were pretreated with heat inactivated HSV-1 21 days before live HSV-1 infection. C. Serum anti-HSV-1 IgG antibody levels on day 7 after HSV-1 inoculation.Note that pretreatment with heat-inactivated HSV-1 followed by live HSV-1 inoculation at a dose of 5.5×10^8 pfu/mL generated a comparable or more increased levels of anti-HSV-1 IgG in the early phase (day 7 p.i.). N = 4–10.

clinical and histological findings alone may not provide an accurate diagnosis. In this regard, the dilatation of sweat glands observed in mouse footpads infected with HSV-1may be informative for the histological diagnosis of sweating disturbance. Indeed, similar histological findings are observed in the sweat glands of footpads with repeated administration of topical corticosteroids and those in aged footpads, in which a marked disturbance of sweating function is observed [29]. Thus, the dilatation of sweat glands associated with the flattening of luminal cells may serve as a histological hallmark of sweating disturbance. Given that decreased claudin 3 expression in sweat glands is accompanied by sweat leakage in AD patients [30], we reasoned that decreased claudin 3 expression by HSV-1 infection of sweat glands would be responsible for luminal dilatation of sweat gland as a histological marker for dysfunction of processes involved in sweat secretion/production.

We recognize several limitations of our study. First, the power to detect statistically significant associations may have been limited to a subset of mice, owing to the small sample size. Second, we were unable to reactivate HSV by hyperthermic stress in HSV-1-inoculated mice and those that had recovered from HSV-1 infection (data not shown), unlike in human HSV-1-related diseases. Consistent with this, the spontaneous reactivation of latent HSV-1 in immunocompetent mice is reported to be limited. Therefore, if HSV-1 can be reactivated in our HSV-1 cutaneous inoculation model, interventions focused on preventing the development of sweating disturbance and treatment of HSV infection could serve to improve the trajectory of sweating disturbance over time in the general population, especially AD patients.

Here, we provide a model of HSV-1-induced sweating disturbance at early time points after infection, even in immunocompetent mice. The preferential presence of HSV-1 in the excretory system suggests underrecognized viral shedding through eccrine glands in humans. Although it remains unknown how sweat glands that have recovered from functional damage by primary HSV-1 inoculation became resistant to subsequent HSV-1 inoculation, HSV DNA may be detected in sweat glands and sweat even from humans if the samples are obtained at early time points after HSV-1 infection. Due to the paucity of studies investigating the role of HSV-1 in sweating dysfunction and dry skin, considerable research remains to be performed. Our mouse model provides a better understanding of the processes underlying HSV-1-induced sweating disturbance and may lead to the development of new therapeutic strategies for virally induced sweating impairment. In addition to genetic factors and frequent use of topical corticosteroids [28], HSV may be one of the leading causes of atopic dry skin.

CRediT authorship contribution statement

Yumiko Asanuma: Investigation, Formal analysis, Writing – original draft. **Hironobu Ishimaru:** Investigation, Formal analysis, Visualization, Methodology. **Tetsuko Sato**: Methodology. **Takenobu Yamamoto:** Conceptualization, Methodology, Writing – review & editing. **Yumi Aoyama:** Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Conflict of interest

H.I. is an employee of Maruho Co., Ltd. Others declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2022.09.001.

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