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Hair follicle involvement in herpes zoster: pathway of viral spread from ganglia to skin

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Abstract Herpes zoster is caused by reactivation of varicella-zoster virus (VZV) persisting in dorsal root or trigeminal ganglia. To clarify the pathway of viral spread from the ganglia to skin, 16 biopsy specimens of early skin lesions of herpes zoster obtained from the face and trunk of 13 patients were studied histologically and immunohistochemically using monoclonal antibodies to the structural proteins of VZV. VZV-infected cells were detected in the hair follicles in 10 of the 16 specimens and in the epidermis in 2 specimens. Infected cells were localized in the isthmus of every involved follicle (12/12), frequently in the stem (8/10) and infundibulum (6/10), and never in the bulb. The high frequency of follicular involvement in herpes zoster suggests that VZV spreads to the area of skin innervated by myelinated nerves, which end around the isthmus of hair follicles and sebaceous glands.

Key words Varicella-zoster virus · Herpes zoster · Immunohistochemistry · Hair follicle · Sebaceous gland

Introduction

Herpes zoster (shingles) induced by varicella-zoster virus (VZV) infection is an acute disorder characterized by unilateral radicular pain and vesicular eruptions involving one to three dermatomes. The antigenic character and biological behaviour of VZV isolated from the skin lesions of herpes zoster are identical to those of VZV isolated from an initial infection with chickenpox (varicella) [21]. VZV persists latently in the dorsal root or cranial sensory ganglia after primary VZV infection [3, 7, 11], and herpes zoster is caused by reactivation of VZV. The mechanisms of latency and reactivation of VZV in the dorsal root or trigeminal ganglia and neural spread of VZV to the skin are poorly understood.

Pathological examination of autopsy cases of herpes zoster revealed characteristic cells having eosinophilic intranuclear inclusions in the corresponding ganglia with inflammatory changes and haemorrhagic necrosis [2, 6, 9]. Presence of VZV has also been confirmed by electron microscopy and by indirect immunofluorescence [4, 15, 20]. In addition, infectious virus has been isolated from acutely infected ganglia of patients dying of disseminated VZV infection [1].

In the skin lesions of herpes zoster, characteristic ballooning of keratinocytes bearing intranuclear inclusions is always found in the vesicular stage [14, 16, 17]. Viral particles have been observed not only in these keratinocytes, but also in dermal macrophages and axons [8, 19]. Previously, we reported on the sequential changes of skin lesions in herpes zoster and demonstrated that the structural proteins of VZV appear in the epidermis and follicular epithelium in the late erythematous stage, spread into the dermis in the vesicular stage, and disappear in the ulcerative stage (except in the ulcer margin and around the hair shaft in few cases) [14]. In this study, we investigated which parts of the skin were initially involved in the early lesions of herpes zoster to clarify the pathway of VZV spread to the skin.

Materials and methods

Biopsies of early skin lesions on the 3 to 8 days after the onset of skin eruptions were performed on 13 patients with herpes zoster after obtaining informed consent. These patients had no underlying diseases, except for 2 who had mild hypertension and chronic pancreatitis, respectively. For comparative study, late skin eruptions including full-blown vesicles, pustules and ulcers were biopsied from 9 cases of herpes zoster.

Histological and immunohistochemical examinations were performed on serial paraffin sections of formalin-fixed biopsy tissues according to previously described methods [14]. Briefly, after deparaffinization, sections were treated with 0.25% trypsin (Difco, Detroit, Mich.) in PBS (v/v) with 0.02% calcium chloride (w/v) at

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37° C for 30 min and immersed in 0.3% hydrogen peroxide prepared in methanol (v/v) for 30 min. Thereafter, sections were incubated overnight at 4° C with a mixture of two monoclonal antibodies which recognizes at 32 kDa capsid protein and a 64 kDa glycoprotein 3 of VZV [18] and does not cross-react with the degenerated or necrotic cells infected by herpes simplex viruses and cytomegalovirus. Biotinylated anti-mouse IgG (Vector, Burlingame, Calif.) was then applied for 45 min at 37° C, followed by avidin–biotin peroxidase complex (Vector) for 45 min at 37° C. The peroxidase reaction was developed in 0.05 M Tris buffer (pH 7.6) with 0.02% diaminobenzidine (Dojin Chemical, Kumamoto, Japan) and 0.015% hydrogen peroxidase. Nuclei were counterstained with 2% methyl green (Chroma, Stuttgart, Germany).

Results

Sixteen skin biopsy specimens obtained from 13 patients with herpes zoster appeared as macroscopically erythematous lesions with or without papular changes. These lesions were designated as the early skin lesions. Three lesions each showing vesicular, pustular and ulcerative changes, from 9 patients were defined as late skin lesions.

Early lesions

VZV-infected cells were detected in the hair follicles in 10 of 16 specimens, and in the epidermis in 2 specimens. In the 4 remaining lesions neither viral antigens nor cytological changes suggestive of VZV infection were observed (Table 1). Viral involvement was detected in 17 (58%) of 27 hair follicles in the 10 specimens showing follicular involvement. Macroscopically, the early skin lesions of herpes zoster were frequently associated with papular changes. Histological and macroscopical correlation revealed that these papules represented follicular involvement in most of the cases.

The infected cells in both hair follicles and epidermis showed cytological characteristics of VZV infection, such as intranuclear inclusions, nuclear margination and multinucleated giant cells. However, VZV-infected cells in follicles did not show obvious acantholytic changes (Fig. 1a, c) except in superficial parts. These cells were frequently associated with necrotic changes, especially in the stem. In the epidermis, a cluster of ballooning cells observed in the lower layers often showed vesicular formation, but hardly any necrotic changes. VZV antigens were detected in these necrotic or ballooning cells in hair follicles (Fig. 1b, d) and epidermis.

It was rather difficult to determine the anatomical localization of VZV infection precisely, because the hair follicles were not always sectioned longitudinally and only parts of them were observed in one section. Nevertheless, it was apparent that the isthmus was involved in every follicle, while the stem and infundibulum were less frequently involved, and the bulb was never infected (Table 2). In addition, the outer root sheath was more involved in the isthmus and stem than in the centre (Fig. 1a, b).

In early skin lesions with follicular involvement, VZV-infected cells were always recognized in the perifollicular tissue. These follicles showed slight to mild destruction of the basement membrane, spongiosis and granulocytic infiltration. VZV antigens were detected in macrophages, fibroblasts and endothelial cells of small vessels. In one third of the follicular lesions, perifollicular involvement was remarkable and viral antigens were detected in the perineurium (Fig. 2a, b). In the remaining two thirds, perifollicular involvement was slight, and only a few fibroblasts and macrophages were infected. Neither nerve bundles nor blood vessels were involved where only mild to moderate lymphocytic infiltration was observed (Figs. 2c, d).

VZV infection was observed in nine of ten sebaceous glands (90%). Multinucleated cell formation and necrotic changes were more prominent in the periphery of glands than in the centre, with a similar distribution of viral antigens (Fig. 3).

Late lesions

Vesicular formation was only recognized in the upper parts of hair follicles, and was never found in the stem, bulb and sebaceous glands. The infected stem and sebaceous glands showed prominent necrotic changes without obvious acantholysis. Neutrophilic infiltrates were seen around small vessels near the infected follicles. In the pustular lesions, hair follicles and sebaceous glands were destroyed, with marked cellular infiltrates particularly in the isthmus and stem. Prominent necrotic changes with leucocytoclasia and cellular infiltrates were occasionally recognized in the venules and capillaries around

Table 1 Biopsy specimens of herpes zoster with follicular and/or epidermal involvement[a] in the erythematous stage of herpes zoster

Presence of infected cells in		No. of specimens (n=16)
Hair follicles	Epidermis	
–	–	4
+	–	10
–	+	2
+	+	0

[a] VZV involvement was determined by histological findings of VZV-infected cells and/or the immunohistological detection of the GP and NP antigens of VZV

Table 2 Localization of VZV infected cells within the hair follicle in the erythematous lesions

Subdivision	No.[a]	Infected cells[b]
Infundibulum	10	6 (60%)
Isthmus	12	12 (100%)
Stem	10	8 (80%)
Hair bulb	9	0 (0%)

[a] Observed number of each part in the total 17 hair follicles
[b] VZV infected cells were detected by the antibodies and/or cellular changes, such as ballooning or intranuclear inclusions

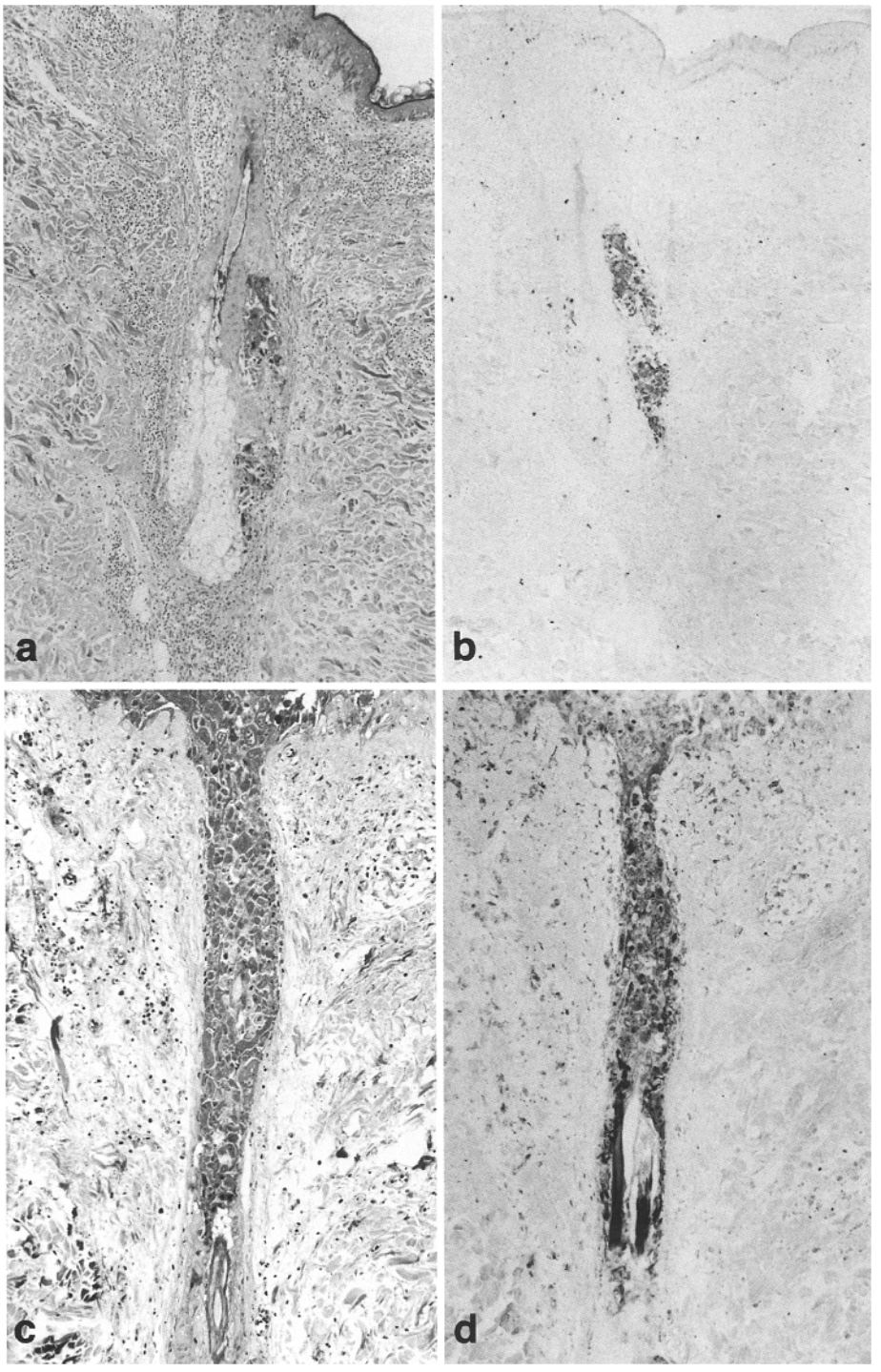

Fig. 1a–d VZV-infected lesions in hair follicles in the early lesions of herpes zoster. A small necrotic lesion was found in the isthmus (**a** HE, ×40), in which VZV antigens were detected (**b** immunohistochemistry, ×40). Marked involvement of VZV was observed throughout the follicle except for the bulb (**c** HE, ×132, **d** immunohistochemistry, ×132)

infected follicles. In the ulcerative lesions, all structures except the bulb were completely destroyed. Collagen bundle disruption, cellular infiltrate and fibrosis were also observed in the perifollicular tissue.

VZV antigens were only detected in necrotic follicles. In the late vesicular lesions, the antigens were recognized not only in the follicles and sebaceous glands showing necrotic changes, but also in the fibroblasts/macrophages and endothelial cells and the perineurium. In pustular lesions, VZV antigens were detected in degenerated or necrotic cells of hair follicles, while in ulcerative lesions, antigens were found in only one around the hair shaft.

Discussion

In the previous study we demonstrated that the structural antigens of VZV appeared in the epidermis with formation of intranuclear inclusions and acantholysis. These

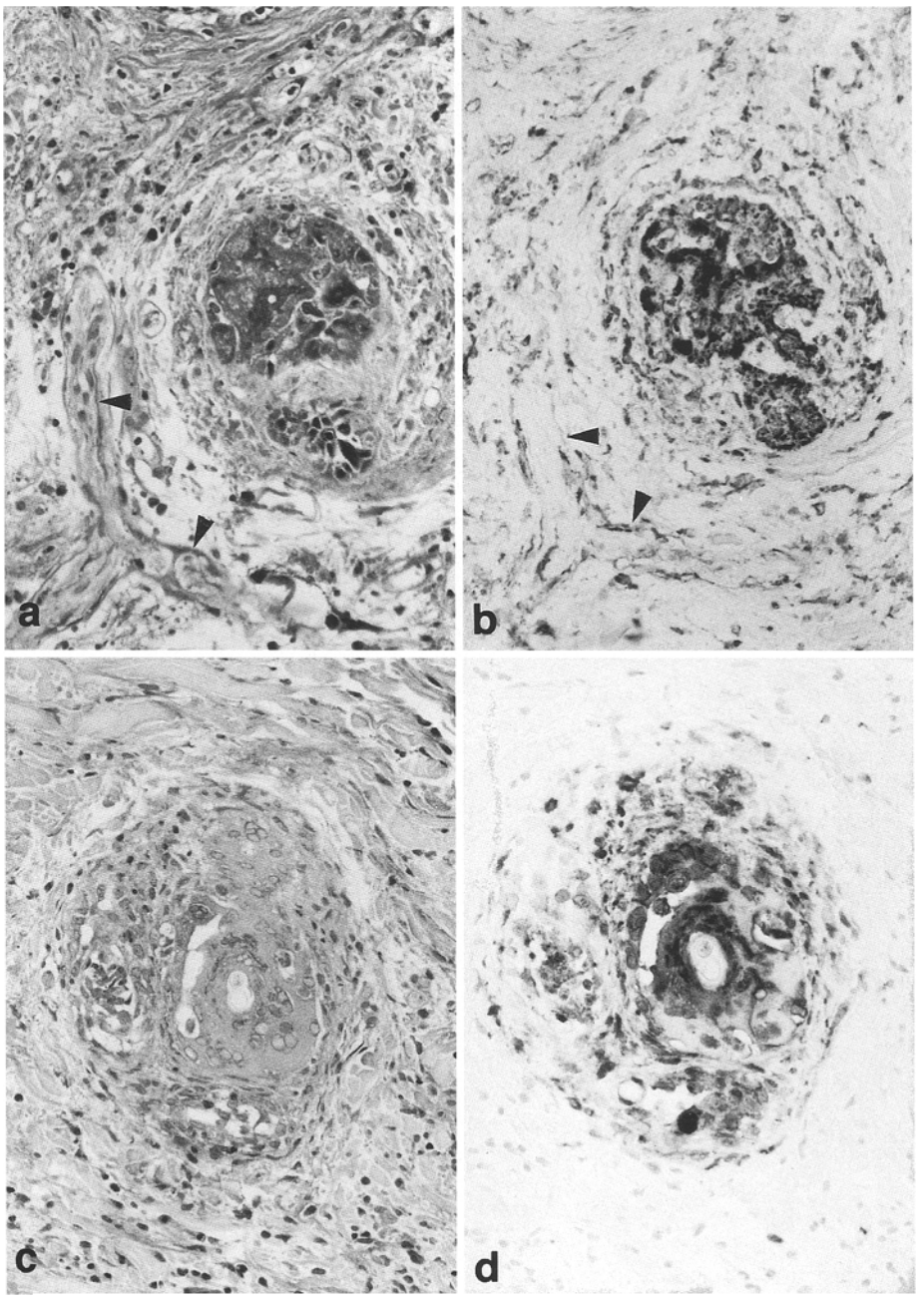

Fig. 2a–d VZV-infected lesions around the VZV-infected follicles. Follicular epithelium shows necrosis with mild perivascular neutrophilic infiltrate (**a** HE, ×264). VZV antigens were observed not only in the follicle, but also in the macrophages/fibroblasts, vascular endothelial cells and perineurium (*arrowheads*) of the innervating nerves (**b** immunohistochemistry, ×264). Mild lymphocytic infiltrate was observed around an involved follicle (**c** HE, ×264). VZV antigens were mainly observed in the follicle (**d** immunohistochemistry, ×264)

antigens could not be detected in the epidermis of most earlier lesions, where only moderate numbers of lymphocytes infiltrate around the vessels in the papillary dermis. We also noticed that VZV involves the hair follicles even in these early lesions. In this study, we found that the hair follicles were much more heavily involved than the epidermis in the early lesions of herpes zoster. This susceptibility of hair follicles to VZV infection is well correlated with a high incidence of herpes zoster on the trunk and face, which have abundant follicles, a low incidence on the limbs, and rare incidence on the palms and soles, which have no hair follicles [10].

The histological responses of follicles to VZV are quite different from that of epidermis with respect to vesicular formation. This difference is probably caused by their structural differences: the follicles are surrounded by the tight collagenous tissue. Thus, infected follicles could not show vesicular changes except in their superficial parts; destruction occurs in subsequent stages. Macroscopically, the follicular involvement may be recognized as an erythematous lesion with or without papular changes, irrespective of its severity. Alopecia, which is often associated with old lesions of severe herpes zoster, is considered to be a final state, as is follicular destruction.

In the previous study we demonstrated that dermal involvement was only observed in the dermis beneath the vesicles, and VZV was considered to spread from epider-

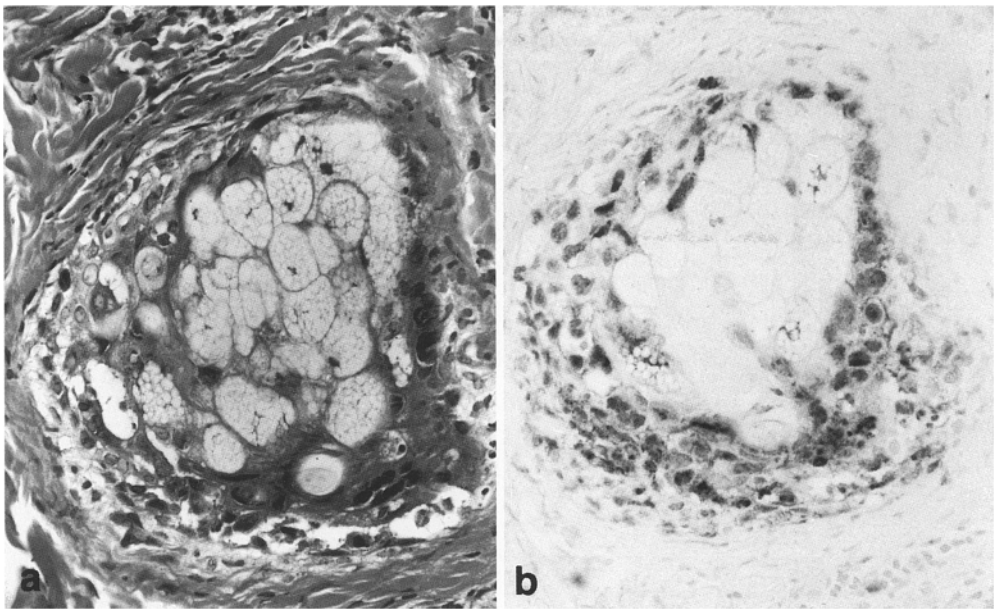

Fig. 3a, b VZV infected cells in a sebaceous gland. Ballooning or necrotic cells localized mainly in periphery of sebaceous gland (**a** HE, ×264). VZV antigens were detected in these areas (**b** immunohistochemistry, ×264)

mal lesions into the dermis [14]. In this study we clarified that VZV infects the outer part or periphery of the hair follicles and sebaceous glands and spreads from the infected follicles to connective tissue cells such as fibroblasts, macrophages and nerve bundles.

The highly susceptible sites in herpes zoster are the hair follicles, especially the isthmus, and sebaceous glands. On reactivation of latent VZV in ganglia, infectious virus spreads antidromically down the sensory efferent nerve and reaches the skin [5]. Hair follicles and sebaceous glands are innervated by large myelinated sensory nerve endings [13, 22], in contrast to the epidermis, which is innervated by small non-myelinated nerve endings except for the epidermal disc (Haarscheibe), a special part of epidermis considered to be a sensory organ. On the basis of these findings, VZV is suspected to reach the hair follicles and sebaceous glands through the myelinated nerve endings, and cause infection. In addition, the early epidermal lesions might start in the epidermal disc, as it is innervated by myelinated nerve endings, although we could not confirm the presence of myelinated nerve fibres beneath and Merkel cells in these epidermal lesions. This correlates with the ratio of hair follicles and epidermal discs (4:1) [12] and the ratio of follicular and epidermal lesions in this study (10:2). From these data it is suggested that the myelinated nerves may play an important part in the route of reactivated VZV from the ganglion to skin. Further studies will be required to confirm this role of myelinated nerves in the spread of reactivated VZV in the ganglion.

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