Virchows Archiv A Pathol Anat (1992) 420:71–76

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical study of skin lesions in herpes zoster

Ryoichi Muraki[1], Toru Baba[1], Takuya Iwasaki[2], Tetsutaro Sata[2], and Takeshi Kurata[2]

[1] Department of Dermatology, Institute of Clinical Medicine, University of Tsukuba, Tennodai, Tsukuba, Ibaraki 305, Japan
[2] Department of Pathology, National Institute of Health, Tokyo 141, Japan

Received July 30, 1991 / Accepted August 16, 1991

Summary. Thirty-seven biopsy skin tissues of herpes zoster taken from 27 patients were analysed immunohistochemically using two monoclonal antibodies detecting either nucleocapsid or glycoproteins of varicella-zoster virus (VZV) on paraffin sections of formalin fixed tissues. Skin lesions of herpes zoster were divided clinically into four stages: erythematous, vesicular, pustular and ulcerative. In the erythematous stage, VZV antigens, if detected, were found only within ballooning cells in the lower epidermis or follicular epithelium. In the vesicular stage, antigens were detected in the cells around and within the intraepidermal vesicles and in histiocytes or fibrocytes of the dermis in all cases and in the endothelial or perineural cells in 10 of 14 cases. In the pustular stage, the antigens were observed in degenerated or necrotic keratinocytes and multinucleated giant cells within pustules and some necrotic cells in the dermis. In the ulcerative stage, the viral antigens were detected only at the ulcer margin and around the hair shaft in 2 of 7 cases. These results suggest that VZV initially involves the epidermis in the erythematous stage, subsequently invades the dermis in the vesicular stage, and disappears in the early ulcerative stage.

Key words: Herpes zoster – Varicella-zoster virus – Viral protein

Introduction

While the pathogenesis of herpes zoster is not fully understood, the disease is generally considered to be caused by reactivation of varicella-zoster virus (VZV) which has persisted in the sensory ganglia of craniospinal nerves after an initial exposure (Gilden et al. 1983). On reactivation, the virus is thought to spread from the ganglia along the corresponding nerves to the skin (Gelb 1990; Straus et al. 1988).

Offprint requests to: R. Muraki

The skin lesions of herpes zoster have been classified into three stages histologically: (1) early lesions (papules or papulovesicles); (2) fully developed lesions (tense clear vesicles); and (3) late lesions (pustules, crusted vesicles and ulcers) (Ackerman and Ragaz 1984). Ultrastructural studies of the fully developed vesicular lesions revealed the presence of viral particles within the keratinocytes, fibroblasts, histiocytes, and endothelial cells (Hasegawa 1971; Orfanos and Runne 1975). Using immunofluorescence VZV antigens were detected by immunofluorescence not only in the epidermis and hair follicles, but also in fibroblasts and vascular endothelial cells in the upper dermis (Aoyama et al. 1974).

We previously examined the localization of VZV antigens in paraffin sections of formalin-fixed biopsy specimens obtained from the skin with herpes zoster after trypsin digestion, which was useful to detect viral antigens with a high specificity while decreasing non-specific reaction (Kurata et al. 1983). Monoclonal antibodies to nucleocapsid and glycoproteins of VZV were developed and successfully applied to paraffin sections of formalin-fixed specimens using the modified avidin-biotin complex method. We have now studied the skin lesions of herpes zoster, clinically divided into four stages. We wished to clarify the pathogenesis of herpes zoster with particular regard to viral replication and spread within the skin, and also to correlate the relationship between the clinical and pathological findings in the skin lesions. This study was performed immunohistochemically on paraffin sections of formalin-fixed biopsy material.

Materials and methods

Thirty-seven specimens were obtained from 27 patients with herpes zoster. Patients were diagnosed at the Salvation Army Booth Memorial Hospital, the Tokyo Second National Hospital, and the Keio University Hospital, Tokyo, Japan. Three of the patients were undergoing immunosuppressive therapy for non-Hodgkin's lymphoma, adult T-cell leukaemia or ovarian carcinoma. The other 24 patients were without any underlying disease. The skin lesions in the immunosuppressed patients were increased in number compared with those of healthy patients.

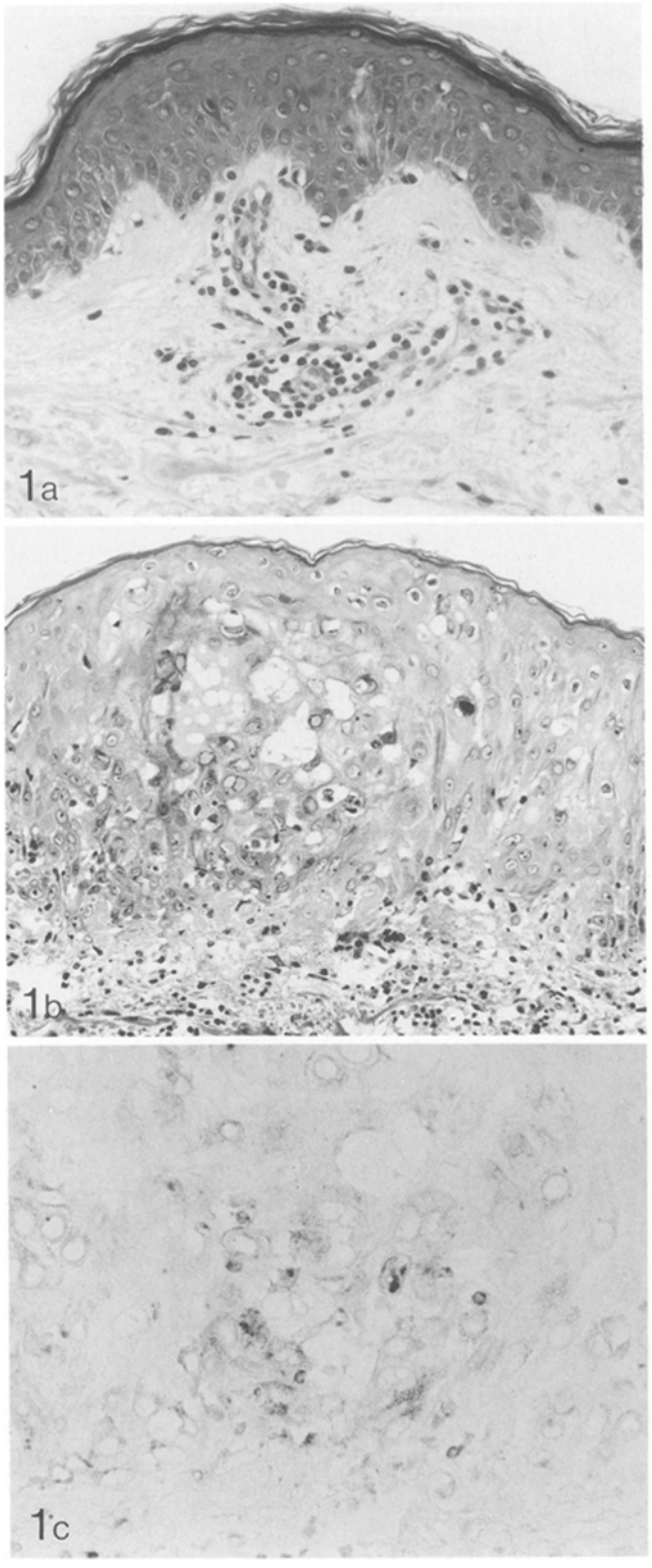


Fig. 1a–c. Erythematous stage. **a** Mononuclear cells infiltrate around the vessels in the upper dermis. Epidermal changes are not significant. Slight acanthosis is seen; however, no varicella-zoster virus (VZV) antigens are detected in immunohistochemistry. H & E, ×220. **b** Keratinocytes are swollen and acantholytic with formation of microscopic vesicles in the lower part of the epidermis. Some of these cells have intranuclear inclusions with nuclear margination. Perivascular infiltrates are found in the dermis. H & E, ×170. **c** The serial section of **b** was stained immunohistochemically for VZV antigens. The viral antigens were observed in the nuclei and cytoplasm of the swollen keratinocytes. No antigens were observed within the dermis. Immunoperoxidase, ×330

The skin eruptions were classified clinically into four stages: (1) erythematous (oedematous erythema including papules)-7 cases; (2) vesicular (fresh vesicles, vesicles with an erythematous halo or confluent vesicles in disseminated cases)-14 cases; (3) pustular (pustules with or without haemorrhage)-9 cases; and (4) ulcerative (ulcer with a crust)-7 cases. As a control, skin free from eruptions was also biopsied from 2 cases with oedematous erythema, with the informed consent of the patients.

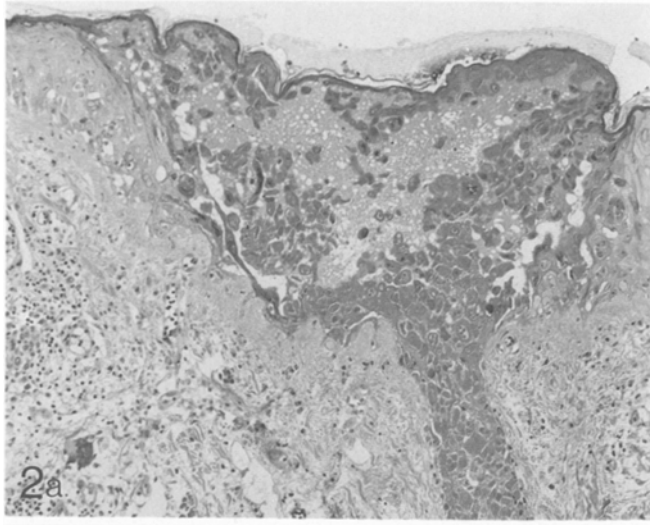
Histological and immunohistochemical examinations were performed using serial paraffin sections of formalin-fixed materials. After deparaffinization, the sections were treated with 0.25% trypsin (Difco, Detroit, Mich., USA) PBS (v/v) with 0.02% calcium chloride (w/v) at 37° C for 2 h and immersed in 0.3% hydrogen peroxide methanol solution (v/v) for 30 min to block endogenous peroxidase activity. After application of normal goat serum for 10 min, the sections were reacted with monoclonal antibodies (Okuno et al. 1983) for the nucleocapsid and glycoproteins of VZV overnight at 4° C. After washing in PBS, the biotinylated anti-mouse IgG (Vector, Burlingame, Calif., USA) was 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 HCl buffer (pH 7.6) with 0.02% diaminobenzidine (Dojin Chemical, Kumamoto, Japan) and 0.015% hydrogen-peroxide for 8 min at room temperature. The nuclei were counterstained with 2% methyl green (Chroma, Stuttgart, FRG) in veronal acetate buffer.

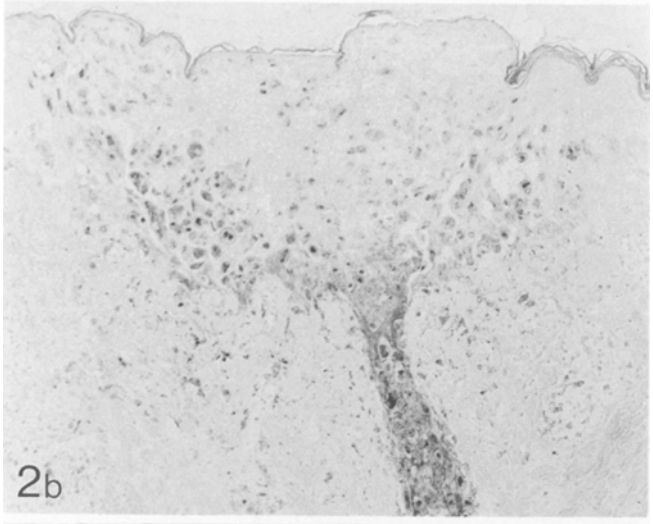
For electron microscopic observation the specimens taken from fresh vesicles (three cases) were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. After selection of appropriate areas in semi-thin sections stained with toluidine blue, ultra-thin sections were prepared and stained with uranyl acetate and lead citrate. Ultra-thin sections were observed with a JEM 1200EX electron microscope (Jeol, Tokyo, Japan).

Results

The microscopical appearances of each of the skin lesions from the four stages correlated well with the macroscopic findings; however, one lesion in the erythematous stage showed a microscopic vesicle in the epidermis.

For all 7 erythematous cases examined, moderate numbers of inflammatory cells, mainly lymphocytes, infiltrated around the dilated capillaries and venules in the upper part of the dermis (Fig. 1a). Skin free of any eruptions was devoid of perivascular inflammation. In 5 of the 7 cases, the epidermis showed minimal changes, such as acanthosis with slight nuclear enlargement and dispersed chromatin. In 1 case, a cluster of ballooning cells was found in the lower half of the epidermis. The nuclei of these cells were enlarged with obscured nucleoli and chromatin margination. Two types of intranuclear inclusions were also occasionally observed in these cells. The full and Cowdry A types of intranuclear inclusions showed an eosinophilic ground glass and fine granular appearance, respectively. Full type intranuclear inclusions were prominent in the epidermal lesions. Spongiotic changes were focally observed with minute vesicular formation (Fig. 1b). In the other case, the follicular epi-





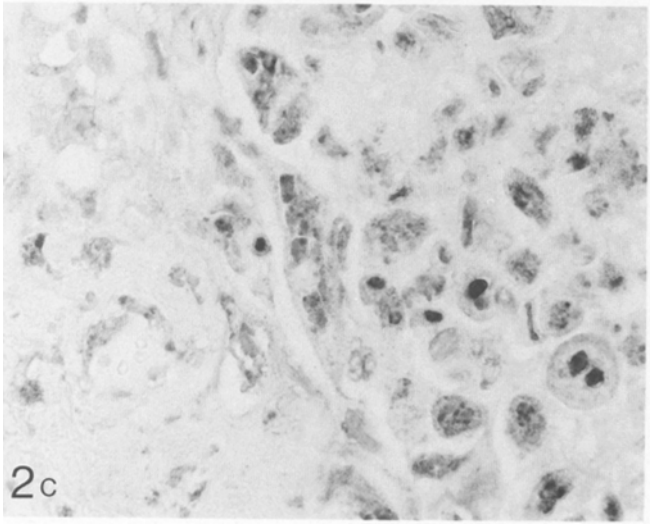
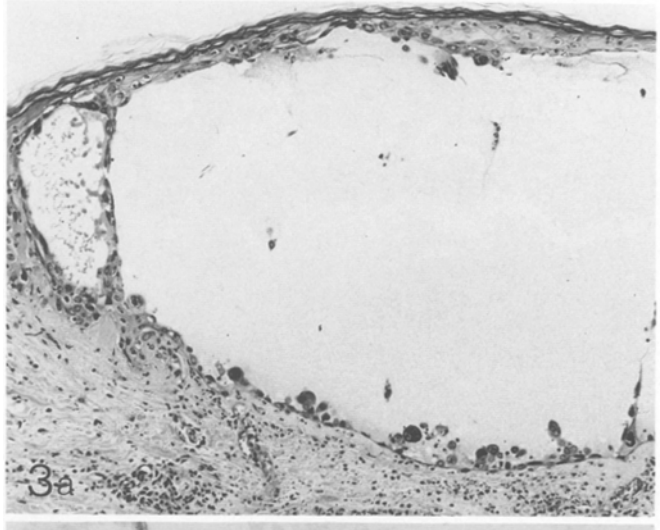


Fig. 2a–c. Hair follicle in the erythematous stage. **a** Follicular infundibulum is necrotic with formation of a tiny intra-epidermal vesicles. H & E, ×100. **b** VZV antigens were detected in these necrotic epithelium. Peroxidase reaction, ×130. **c** Nuclei and cytoplasm of ballooned or multinucleated cells are positive for VZV antigen in the periphery of hair follicles. Immunoperoxidase, ×380

Table 1. Localization of varicella zoster virus antigens in the skin lesions

	Stages			
	Erythematous $n=7$	Vesicular $n=14$	Pustular $n=9$	Ulcerative $n=7$
Epidermis	1	14	9	1[b]
Hair follicle	1	6 (4[a])	5 (4[a])	1[b]
Eccrine duct	0	1	0	0
Fibroblasts/histiocytes	0	14	6[c]	0
Vascular endothelium	0	10	3[c]	0
Perineurium	0	4 (2[a])	1[c]	0

[a] Not examined
[b] Only in the superficial layers
[c] Antigen-positive cells were degenerated



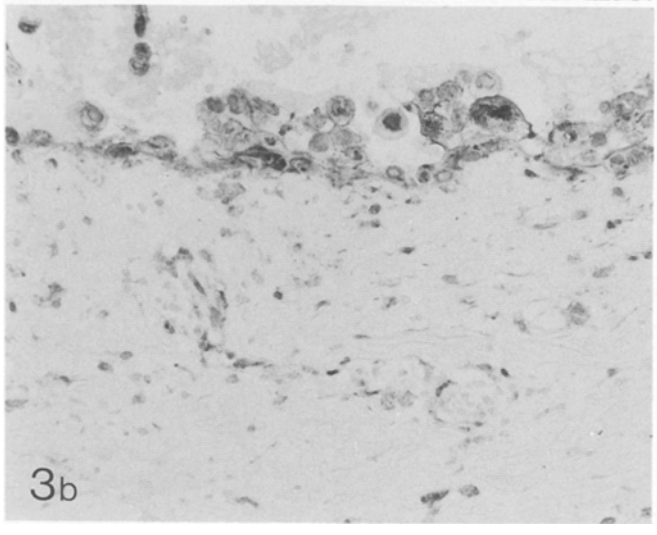


Fig. 3a, b. Vesicular stage. **a** An intraepidermal vesicle is formed with marked acantholytic changes of ballooning epidermal cells. Moderate perivascular infiltrates, mainly neutrophils, are observed in the upper dermis. H & E, ×80. **b** VZV antigens are revealed not only in the epidermal cells within and around vesicles, but also in the fibrocytes, histiocytes and endothelial cells beneath the vesicle. Immunoperoxidase, ×220

thelium showed necrotic changes with microscopic vesicle formation (Fig. 2a). Ballooning cells and multinucleated giant cells were observed in the periphery of hair follicles. In these 2 cases, VZV antigens were shown at the areas of the ballooning or necrotic epithelial cells (Table 1; Figs. 1c, 2b). VZV antigens were also detected in the nucleus and cytoplasm of the cells with inclusions (Fig. 2c). No VZV antigens were observed in the dermis. The other 5 cases were negative for VZV antigens.

Intraepidermal vesicles with one to three layers of degenerating cells in the rooves were observed in the vesicular stage (Fig. 3a). Follicular epithelium became necrotic in some hair follicles. Cellular ballooning with acantholysis was found within and around the vesicles and in necrotic follicles. Multinucleated giant cells were also observed in the lower part of vesicles. In all 14 cases, VZV antigens were detected not only in the ballooning cells around and within the vesicles in the epidermis and follicular epithelium around necrosis, but also in histiocytes and fibrocytes in the dermis beneath the vesicles. Multinucleated cells were also positive for viral antigens (Fig. 3b). In these cells VZV antigens were localized both in the nucleus and cytoplasm. Moreover, VZV antigens were observed in endothelial linings of the capillaries, venules and lymphatics in 10 cases and the perineurium of peripheral nerves in the upper dermis in 4 cases. In these cases, neutrophil infiltrates were occasionally observed around the vessels of the upper dermis. Eccrine ducts also showed involvement by the virus in a fatal disseminated case with non-Hodgkin's lymphoma.

In the pustular stage, neutrophils were found in the vesicles of the epidermis, in which necrotic or degenerated keratinocytes and multinucleated giant cells were also

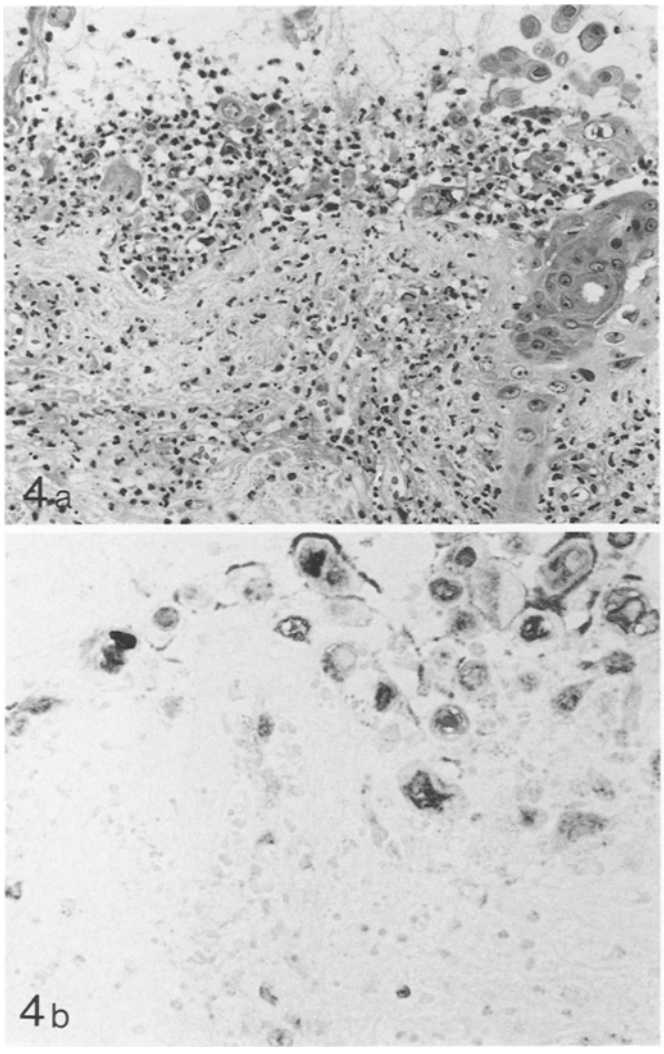


Fig. 4a, b. Pustular stage. **a** Epidermal cells within and around the vesicle are necrotic and intermingle with neutrophils and nuclear debris. Inflammatory infiltrate, with macrophages phagocytosing nuclear debris, is observed around and within the vessels. H & E, ×190. **b** VZV antigens were detected in these necrotic keratinocytes within a pustule and degenerated cells in the upper dermis. Immunoperoxidase, ×380

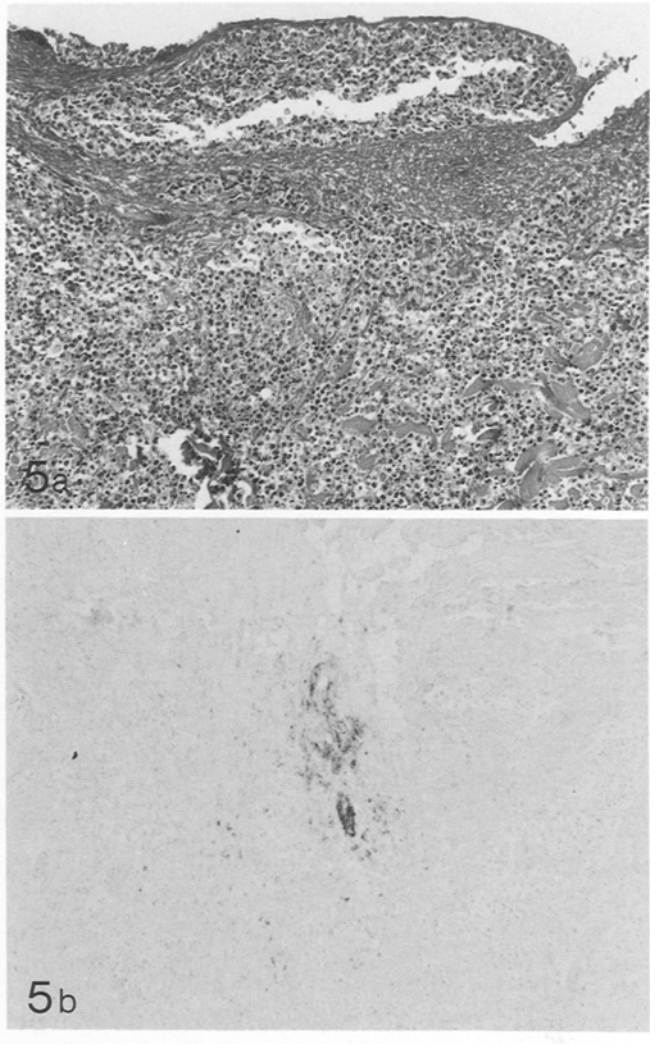


Fig. 5a, b. Ulcerative stage. **a** No epidermal cells, except for some remaining follicular epithelium are observed in ulcer bed. Lymphocytes, plasma cells and histiocytes infiltrate the dermis beneath the necrotic tissue. H & E, ×130. **b** VZV antigen was negative, except in remaining follicular epithelium around hair. Immunoperoxidase, ×130

prominent (Fig. 4a). The epidermal cells at the bases of pustules were exfoliated from the basement membrane of the epidermis. Necrotic changes with neutrophilic infiltration and nuclear debris were observed within and around the vascular walls of capillaries and venules in the dermis beneath the pustules. Occasionally, vascular lumina were obliterated with fibrin thrombi. In all 9 cases, VZV antigens were detected in the necrotic or degenerated keratinocytes and in multinucleated giant cells in the pustules, and fibrocytes, histiocytes and some disintegrated cells in the dermis showed VZV antigens in 6 cases (Fig. 4b).

In the ulcerative stage the follicular epithelium and upper part of dermis were destroyed, as well as the epidermis. Marked inflammatory infiltrates, consisting of lymphocytes, plasma cells and histiocytes, were also observed in all layers of the dermis beneath the ulcer (Fig. 5a). VZV antigens were detected in the epidermis only at the ulcer margin and around the hair shaft in 2 of 7 cases (Fig. 5b). The other 5 cases were negative for VZV antigen.

On electron microscopy marked dilatation of intercellular space was observed in the epidermis of samples obtained from the fresh vesicular stage. Viral particles of the herpes virus type, 180 nm in diameter, were found both in intercellular space and in enlarged keratinocytes (Fig. 6). In addition, the virions were also seen in macrophages and endothelial cells in the upper dermis beneath the vesicles. Nucleocapsids, 100 nm in size, were observed in the nuclei of macrophages. Enveloped viral particles were also noted in the cytoplasm, along with budding processes from nuclear membrane. Margination of heterochromatin and swelling of mitochondria were also noted in cells with viral particles. The distribution of viral particles correlated well with the immunohistological findings of the skin lesions in the vesicular stage.

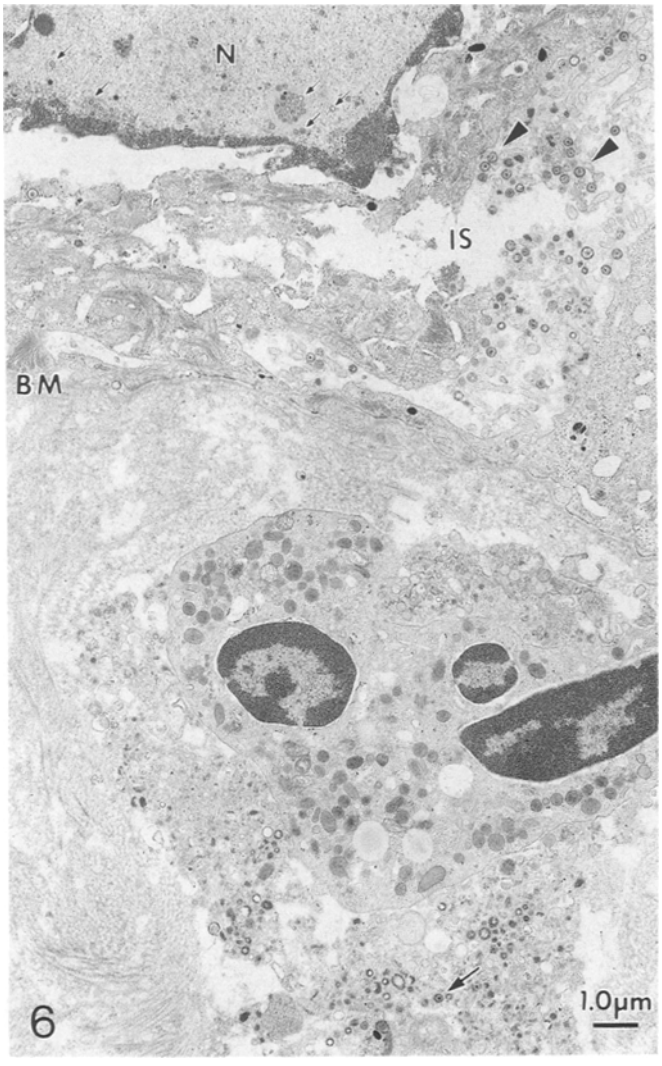


Fig. 6. Ultrastructure of VZV-infected cells in the vesicular stage. Many viral particles of Herpes virus type (*arrowheads*), measuring 180 nm in diameter, were found in the intercellular space (*IS*) in the epidermis. The nucleus (*N*) of the keratinocyte contained nucleocapsids (*small arrows*), 100 nm in size. Beneath the basement membrane (*BM*) of the epidermis, viral particles (*large arrows*) were also observed in the cytoplasm of degenerated cells. In addition, marked intercellular oedema was observed. Electron microscopy, ×6300

Discussion

Clinical classification of the skin lesions of herpes zoster into four stages was well correlated with the immunohistochemical findings. In the initial stage (erythematous stage including papules), the viral antigens were not found in the skin of 5 out of 7 cases; however, inflammatory changes were found in the upper part of the dermis. In the subsequent vesicular stage, viral antigens were found not only in the epidermis, but also in the upper dermis. In the pustular stage, the antigens were distributed only in the epidermis around and within pustules, and occasionally in the dermis. In the ulcerative stage, viral antigens were almost completely absent in the skin lesions.

Herpes zoster is considered to occur by reactivation of latent VZV in sensory ganglia (Gelb 1990; Straus et al. 1988). On reactivation, the virus is thought to travel to the skin through the sensory nerves. Viral antigens could not be detected within the cutaneous nerves and surrounding tissue in the dermis of the initial stage (erythematous stage), although inflammatory changes already occurred. Absence of VZV antigens in the early skin lesions could be attributed to insufficient sensitivity of immunohistochemical methods to detect the low amount of antigens present or by the absence of production of VZV structural proteins in the initial stage of infection. According to an immunofluorescent study of VZV infection in vitro using these monoclonal antibodies, VZV antigens become obviously positive at 14 h after VZV inoculation, while viral replication was already observed at 8–12 h post-inoculation (Yamanishi et al. 1980). From these in vitro findings, positive findings in immunohistochemistry using the monoclonal antibodies may be a reflection of the certain of viral antigens in early histological stage in vivo.

The histological changes caused by VZV infection clearly occur in the epidermis initially with perivascular inflammatory infiltrate in the dermis. These changes in-

clude ballooning and formation of intranuclear inclusion bodies and multinucleated giant cells. Ultrastructurally, the herpes virus-type virions have been observed in the spinous keratinocytes in the involved epidermis (Orfanos and Runne 1975). Our study showed the presence of the VZV antigens in the lower epidermis and follicular epithelium in the initial stage. These findings suggest that viral replication of VZV in herpes zoster spreads from the lower part of epidermis. In the vesicular stage, histiocytes, fibroblasts, vascular endothelial cells, and the perineurium of cutaneous nerves in the dermis were also infected by VZV. Electron microscopic studies also suggest viral replication in these cells, because of the presence of viral particles in the nuclei. These findings indicate infectivity of VZV in the connective tissue cells, including endothelial cells. Infection of VZV in the dermis is thought to come from keratinocytes in the epidermis, because VZV antigens in the dermis are only found beneath the VZV-involved epidermis. Usually the viral particles are plentiful in the epidermis compared with the dermis (Orfanos and Runne 1975).

Viral antigens disappear in the pustular stage with progression of necrotic changes within and around small vessels, with neutrophil infiltration and fibrin deposition in the upper dermis. Those vascular changes are consistent with leukocytoclastic vasculitis (Cohen and Trapuckd 1984; Feyrter 1954). Leukocytoclastic vasculitis may cause regional circulatory disturbance in the skin lesions and may play some role in ulceration.

The late lesions described by Ackerman and Ragaz (1984) could be immunohistochemically subdivided into two stages (pustular and ulcerative), because of marked differences of distribution of VZV antigens in the skin lesions. The distinction between the pustular and ulcerative stages is clinically important in the infectivity of skin lesions. It is apparent that healing of skin lesions by VZV is associated with viral elimination from the involved skin; however, other skin eruptions in the pustular stage in the same patients still had viral antigens.

Follicular epithelium is frequently involved even in the early stage. A relatively large amount of viral antigen was observed within the follicle in the vesicular stage; however, vesicular changes were only observed in the superficial part. The follicular epithelium became totally necrotic in the vesicular stage. The reactions of follicular epithelium differ from those of keratinocytes. Eccrine ducts were involved by VZV in one of the immunocompromised patients with non-Hodgkin's lymphoma. Rinder and Murphy (1984) also reported such eccrine duct involvement in a fatal case with malignancy. In these cases, extensive epidermal necrosis was observed clinically and the patients died within 1–2 weeks after the appearance of vesicles (without antiviral therapy). Histological detection of eccrine duct involvement by VZV suggests an increase in the number and size of skin lesions and a poor prognosis for the patient.

Acknowledgements. We gratefully acknowledge Drs. Koichi Yamanishi and Toshiomi Okuno, Department of Virology, Research Institute for Microbial Diseases of Osaka University, in providing the monoclonal antibodies of VZV; Mrs. Yuko Sato for excellent technical assistance; and Mr John Meissner for critical reading of this manuscript.

References

Ackerman AB, Ragaz A (1984) Herpesvirus infection. In: Ackerman AB, Ragaz A (eds) The lives of lesions: chronology in dermatopathology. Masson, New York, pp 85–93

Aoyama Y, Kurata T, Kurata K, Hondo R, Ogiwara H (1974) Demonstration of viral antigens in herpes simplex and varicella-zoster infections. Recent Adv Reticuloendothel Syst Res 14:90–104

Cohen C, Trapuckd S (1984) Leukocytoclastic vasculitis associated with cutaneous infection by herpesvirus. Am J Dermatopathol 6:561–565

Feyrter F (1954) Über den Zoster. Hautarzt 5:391–397

Gelb LD (1990) Varicella-zoster virus. In: Fields BN, Knipe DM (eds) Virology, 2nd edn. Raven Press, New York, pp 2011–2054

Gilden DH, Vafai A, Shtram Y, Becker Y, Devlin M, Wellish M (1983) Varicella-zoster virus DNA in human sensory ganglia. Nature 306:478–480

Hasegawa T (1971) Further electron microscopic observations of herpes zoster virus. Arch Dermatol 103:45–49

Kurata T, Hondo R, Sato S, Oda A, Aoyama Y, McCormick JB (1983) Detection of viral antigens in formalin-fixed specimens by enzyme treatment. Ann NY Acad Sci 420:192–207

Okuno T, Yamanishi K, Shiraki K, Takahashi M (1983) Synthesis and processing of glycoproteins of varicella-zoster virus (VZV) studied with monoclonal antibodies to VZV antigens. Virology 129:357–368

Orfanos CE, Runne U (1975) Virus-Ausbreitung, Virus-Replikation, und Virus-Elimination in der menschlichen Haut beim Zoster. Hautarzt 26:181–190

Rinder HM, Murphy GF (1984) Eccrine duct involvement by herpes zoster. Arch Dermatol 120:261–262

Straus SE, Ostrove JM, Inchauspe G, Felser JM, Freifeld A, Croen KD, Sawyer MH (1988) NIH Conference. Varicella-zoster virus infections: biology, natural history, treatment, and prevention. Ann Intern Med 108:221–237

Yamanishi K, Matsunaga Y, Ogino T, Takahashi M, Takamizawa A (1980) Virus replication and localization of varicella-zoster virus antigens in human embryonic fibroblast cells infected with cell-free virus. Infect Immun 28:536–541