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Chronic Varicella-Zoster Virus Skin Lesions in Patients with Human Immunodeficiency Virus Are Related to Decreased Expression of gE and gB

Arjen F. Nikkels, Bernard Rentier, and Gérald E. Piérard

Department of Dermatopathology and Laboratory of Fundamental Virology, Institute of Pathology, University of Liège, Liège, Belgium

The pathogenesis of chronic, verrucous varicella-zoster virus (VZV) cutaneous lesions in human immunodeficiency virus (HIV)–infected persons is unknown. It has been hypothesized that these lesions are due to an altered pattern of virus gene expression. Immediate early and late (L) gene expression in five chronic verrucous VZV lesions, four full-blown herpes zoster vesicular lesions in HIV-infected persons, and eight vesicular herpes zoster lesions in immunocompetent individuals was semiquantitatively assessed immunohistochemically using specific antibodies to the IE63, gE (L), and gB (L) proteins. All patients had evidence of IE63 expression in keratinocytes; however, gE expression was either weak or absent in keratinocytes of three verrucous lesions, and gB was either weak or absent in two. These results suggest that chronic VZV skin lesions are associated with diminished gE and gB expression. It is inferred that the VZV behavior in keratinocytes may vary from a latency-like state to a fully developed, productive infection.

Varicella-zoster virus (VZV) is responsible for chickenpox and shingles. It also produces verrucous, longstanding cutaneous lesions in human immunodeficiency virus (HIV)–infected persons [1–9]. Such lesions are frequently associated with acyclovir resistance, which may be due to multiple, non-uniform mutations in the thymidine kinase gene [7]. The cause of epidermal hyperplasia and chronicity of the lesions is unknown; however, it has been hypothesized that the VZV gene expression could be altered [8].

On the basis of analogy to the prototypic virus herpes simplex virus (HSV), the VZV replicative cycle is supposed to be regulated in a cascade of three successive steps involving the immediate early, early, and late phases. The IE63 protein is present in the nuclear and perinuclear area of keratinocytes in full-blown VZV skin lesions of immunocompetent and HIV-infected individuals [10]. The ORF 68– and ORF 33–encoded major glycoproteins gE and gB have also been demonstrated in the cell membranes and cytoplasm of keratinocytes. During viral latency, IE63 expression has been detected in infected ganglionic neurons in rats [11, 12] and humans [13]; however, gE expression was lacking in the animal model [11].

Using light microscopy, immunohistochemistry, and in situ hybridization, we focussed on the histologic presentation, the IE63, gE, and gB expression patterns, and the presence of viral nucleic acids in chronic verrucous VZV skin lesions of 5 HIV-infected patients. The findings are compared with fully devel-

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Reprints or correspondence: Dr. A.F. Nikkels, Dept. of Dermatopathology, CHU Sart Tilman, B-4000 Liège, Belgium.

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Table 1. In situ hybridization of VZV nucleic acids and immunohistochemical detection of protein IE63 and glycoproteins gE and gB of VZV in biopsy samples from HIV-infected patients and controls.

Samples	Antibodies							Probes *Eco*RI-A, *Eco*RI-B, pVZV
	IE63	VL8 (anti-gE)	VL2 (anti-gB)	VL8 1/10	VL2 1/10	VL8 1/20,1/40	VL2 1/20,1/40	
Chronic verrucous VZV skin lesions, by patient no., patient age/sex								
1, 52/F	++	−	−	−	−	−	−	+
2, 39/M	++	−	−	−	−	−	−	+
3, 30/M	++	+	−	−	−	−	−	+
4, 32/F	++	+	+	+	−	−	−	+
5, 22/M	++	+	−	−	−	−	−	+
Positive control samples of vesicular herpes zoster lesions from								
HIV-infected persons (n = 4)	++	++	+	++	+	++	+	+
Immunocompetent persons (n = 8)	++	++	+	++	+	++	+	+
Other control samples								
Normal skin (n = 15)	−	−	−	−	−	−	−	−
Herpes simplex lesions (n = 7)	−	−	−	−	−	−	−	−

oped vesicular herpes zoster skin lesions from HIV-infected and immunocompetent persons.

Material and Methods

Tissue samples. Biopsy samples from chronic verrucous lesions were obtained from 5 HIV-infected patients, who were 22–52 years old. The samples were formalin-fixed and paraffin-embedded for routine processing. Punch biopsies of vesicular herpes zoster lesions from 4 HIV-infected and 8 immunocompetent persons were included as positive controls. Additional controls included normal skin (n = 15) and HSV skin lesions (n = 7).

Histology. Skin biopsies were routinely dewaxed, stained for 20 s with hemalun and 2% eosin and mounted in DePeX mounting medium (Gurr, Poole, UK).

Immunohistochemistry. Immunohistochemistry was done using the rabbit polyclonal antibody anti-IE63 [11] and the murine monoclonal antibodies VL8 and VL2, which were directed to the VZV glycoproteins gE and gB, respectively, as previously reported [14, 15]. A semiquantitative approach using various primary antibody dilutions (VL8 and VL2 antibodies undiluted, 1/10, 1/20, and 1/40) was used to evaluate differences in gE and gB signal strength. Immunohistochemical signals were rated as strong (++), weak (+), or nondetectable (−). Serial sections were prepared for immunohistochemistry and in situ hybridization methods to study the signal localization of the VZV proteins and nucleic acids, respectively. Control slides were identically treated.

In situ hybridization. Skin sections were assessed by in situ hybridization for the presence of VZV nucleic acids. The assays were done according to a previously published protocol [14] using the digoxigenin-labeled *Eco*RI-A and *Eco*RI-B restriction endonuclease fragments of VZV DNA, which code for gE and gB, respectively, and the biotin-conjugated anti-VZV probe pVZV (region unknown; Kreatech, Amsterdam). The in situ hybridization signals were coded as present (+) or absent (−). Controls were treated identically.

Results

Histology. One verrucous lesion showed marked hyperkeratosis, parakeratosis, and acanthosis, with some swollen keratinocytes containing enlarged eosinophilic nuclei. Cytolysis was not observed. The four other verrucous lesions also exhibited a variable degree of acanthosis with hyper- and parakeratosis. Intraepidermal vesicles bordered by plump keratinocytes exhibiting any sign of cytolysis were present.

The acute vesicular lesions from the other patients displayed the characteristic histopathologic features of VZV skin infection (i.e., the presence of uni- or multilocular vesicles in the epidermis, ballooning degeneration of keratinocytes, multinucleated syncytial giant cells with ground glass–appearing nuclei, and cytolysis of infected keratinocytes). No differences were noted between vesicular lesions in HIV-infected and immunocompetent persons.

Immunohistochemistry. The results of the semiquantitative assessment of the gE and gB glycoproteins and of the IE63 protein are summarized in table 1. The pattern of IE63 immunostaining was predominantly nuclear and occasionally cytoplasmic (figure 1). When present, the gE and gB signals were most often seen in membranes of infected keratinocytes and more sporadically in the cytoplasm. Immunolabeling using the anti-IE63, VL8, and VL2 antibodies or normal rabbit serum instead of primary antibodies on normal skin samples and HSV skin sections was consistently negative.

In situ hybridization. Table 1 summarizes the results of the in situ hybridization assays using the *Eco*RI-A, *Eco*RI-B, and pVZV probes. The signal localization was predominantly nuclear and sometimes perinuclear in keratinocytes. Serial sections demonstrated that the immunohistochemistry and in situ hybridization signals were present in the same keratinocytes.

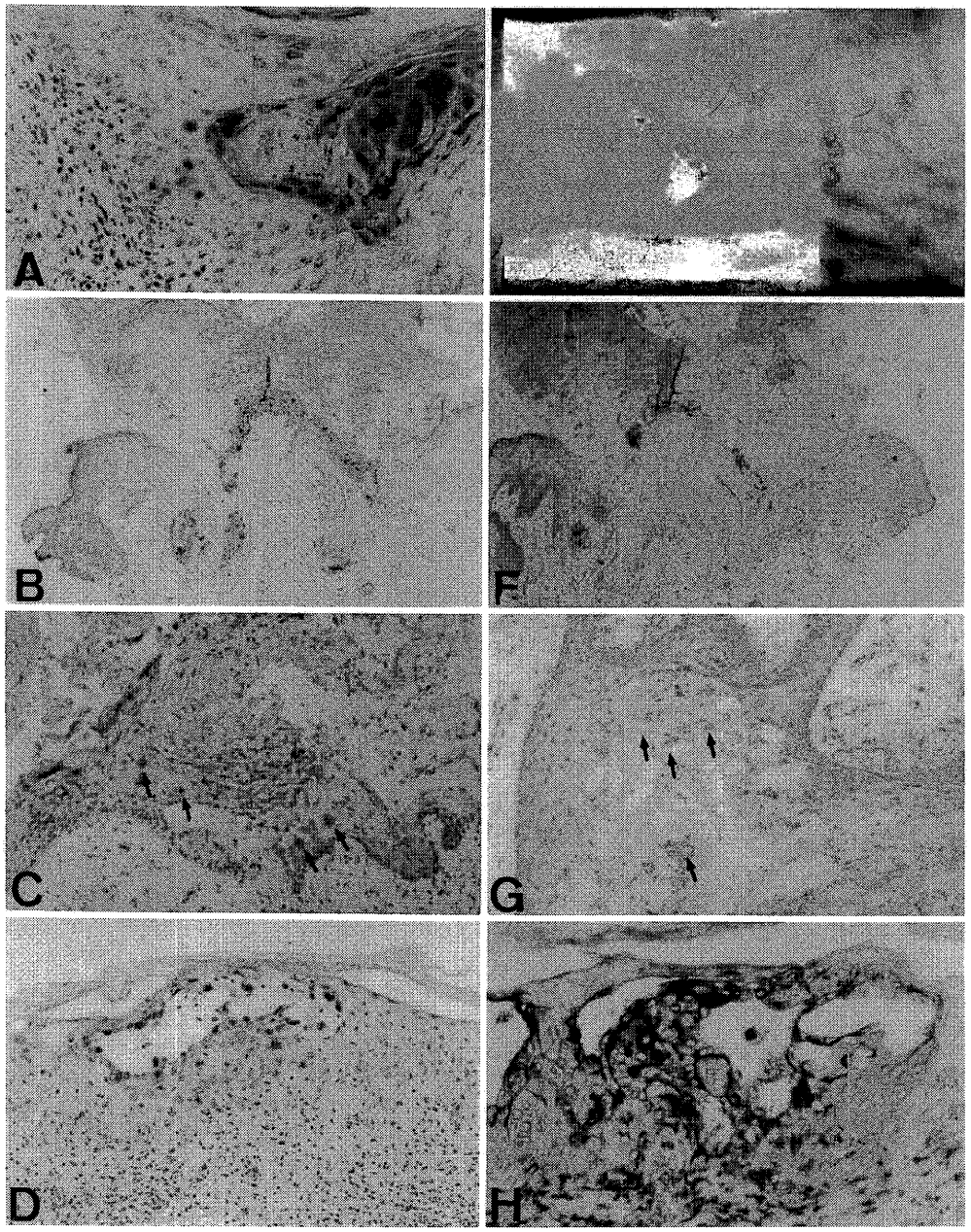

Figure 1. Pattern of IE63 immunostaining in tissue samples from HIV-infected patients presenting with herpes zoster skin lesions, by patient. Patient 1: nuclear and cytoplasmic presence of IE63 protein in infected keratinocytes, magnification ×200 (**A**); positive immunostaining with anti-IE63 polyclonal antibody (PAb) in infected keratinocytes of verrucous lesion, magnification ×25 (**B**); nondetectable gE immunostaining in same lesion (**F**). Patient 3: clinical representation of long-standing verrucous VZV skin lesions on forearm (**E**). Patient 5: positive signal (arrows) with anti-IE63 PAb in verrucous lesion, magnification ×100 (**C**); weak gE expression (arrows) with undiluted VL8 monoclonal antibody (MAb), ×100 (**G**); control IE63 immunostaining, magnification ×100 (**D**); and gE immunostaining with undiluted VL8 MAb, magnification ×200 (**H**).

Probe omission and in situ hybridization on normal skin and HSV skin lesions were negative.

Discussion

Besides having a variable degree of hyperkeratosis, parakeratosis, and acanthosis, warty, long-standing VZV skin lesions in AIDS patients also have a lack of keratinocytes presenting cytolysis [3, 8]. The present histologic findings are in concordance with these observations and underline the heterogeneity of the histopathologic features of these lesions.

The pathogenesis of verrucous VZV lesions remains undetermined. The epidermal hyperplasia is probably stimulated by cytokine release in relation to the long duration of infection rather than a change in the VZV pathogenicity [2]. However, this does not explain the absence of cell lysis and the persistence of verrucous lesions in HIV-infected patients.

Another hypothesis suggests an altered VZV gene expression [8]. In the present study, semiquantitative immunohistochemical assessment showed a nondetectable to weak signal for gE and gB compared with that for controls, although the IE63 was consistently expressed; however, the corresponding genome sequences for gE and gB were present. The immunohistochemical pattern observed for case-patient 1 is reminiscent of that found during dorsal root ganglia VZV latency in the rat, in which IE63 presence was noted without gE expression [11, 13]. The second patient also mimicked such a pattern but also showed considerable histopathologic alterations, including ballooning cells, intraepidermal vesiculation, and moderate acanthosis and hyperkeratosis. The comparatively reduced immunostaining signal in the 3 other cases gave evidence of decreased gE and gB protein synthesis, which probably reflects diminished virion production. The barely detectable production of new virus particles probably allows the keratinocytes to resist the cytolytic effects of massive virion synthesis. In the absence of cytolysis and combined with a low synthesis rate of viruses, the antigenic stimulation of the host defense mechanisms, which is already impaired in HIV-infected persons, remains low. Such a hypothesis could explain the long-standing infection and the epidermal hyperplasia found in some human papillomavirus infections. The precise relationship between the local cutaneous immune system and the virus-host cell relationship remains to be clarified.

In conclusion, chronic verrucous VZV skin lesions are associated with nondetectable to minimally detectable synthesis of the major VZV envelope glycoproteins gE and gB, although their corresponding genome sequences are detected. Such a previously unreported type of VZV infection in keratinocytes is reminiscent of the latency expression patterns in rats [11, 12] and humans [13]. Therefore, VZV infection in keratinocytes appears to encompass a wide spectrum of biologic and clinical presentations. The expression of this spectrum seems, at least in part, to be dependent on the production rate of VZV late proteins, which ranges from nondetectable to high, as observed in the productive stage of infection.

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