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Immunoelectron Microscopy for Rapid Diagnosis of Varicella-Zoster Virus in a Complicated Case of Human T-Cell Lymphotropic Virus Type 1 Infection

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Rapid techniques for the detection of herpes simplex virus (HSV) and varicella-zoster virus (VZV) are needed for optimal therapeutic management. VZV infection poses a serious threat, especially to seriously ill patients, for instance, immunocompromised patients. We report a case of human T-cell lymphotropic virus type 1-positive leukemia complicated by atypical multidermatomal herpes zoster. Viral culture and standard serological tests failed to prove VZV infection. Herpesvirus infection was confirmed by cytodiagnosis (Tzanck test). The final diagnosis of VZV was made by immunoelectron microscopy (IEM), which can differentiate between HSV and VZV. Immunoglobulin M antibodies in serum directed against VZV were detected by IEM but not by immunofluorescence. Because IEM was able to identify virus and analyze sera in only 2 h, it is considered a valuable additional tool for the rapid diagnosis of HSV and VZV infections.

Most varicella-zoster virus (VZV) infections can be readily diagnosed by characteristic clinical appearance. However, because vesiculobullous eruptions of other viral and nonviral skin infections can resemble those of VZV, the infection can be misdiagnosed (6). Clinical diagnosis of herpes zoster in immunocompromised patients is even more difficult, especially in the early stages of the disease, when only a few herpeticlike vesicles may be present, and these may not have the typical dermatomal localization (11). In addition, such patients may have hyperkeratotic nodules and crusted verrucous lesions caused by acyclovir-resistant VZV (1, 8, 9, 14). In immunocompromised patients, antiviral treatment of varicella and herpes zoster reduces serious complications of the infection (15). The rapidity of obtaining a conclusive diagnosis is important for adequate antiviral therapy, because treatment delay diminishes the effectiveness of the antiviral drugs (2, 3, 15). It is important to differentiate between VZV and herpes simplex virus (HSV) at an early stage of the infection to determine the appropriate dose of the antiviral drug. Laboratory tests are used to confirm the clinical diagnosis. Because the classical viral culture usually requires several days, cytologic methods such as the Tzanck test are used to support the clinical diagnosis in an early stage. The Tzanck test cannot distinguish between VZV and HSV infections. Serologic tests can be used to detect rises in VZV antibody titers, but they can only confirm a diagnosis retrospectively.

Virus detection methods, such as the enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction, are becoming more sensitive and rapid and can even replace classical diagnostic tests. These methods detect antigen (viral proteins or intact viral particles), immunoglobulin M (IgM)-class antibodies, or nucleic acids. Some tests are unable to give information rapidly (viral culture); others may not be sensitive or specific enough (ELISA) or must be used under very strict laboratory conditions (polymerase chain reaction). We demonstrate in the study described here that immunoelectron microscopy (IEM) can give a rapid (2-h) diagnosis that includes virus detection, virus typing, and study of IgG and IgM antibody levels.

Case report. A 33-year-old Moroccan female immigrant presented with a large necrotic skin ulcer on the left thigh that was surrounded by herpetic pustules (Fig. 1). Zosteriform-localized herpetic pustules were observed on the left upper leg, the right arm, and cranially on the right half of the back, while isolated herpetic vesiculopustules were found scattered all over the trunk. The herpetic eruptions persisted for about 6 weeks, at which time she was admitted to our clinic. The Tzanck test was carried out on smears from pustular lesions on the left thigh to confirm the clinical diagnosis of herpes. VZV infection was diagnosed clinically and was later confirmed by IEM. Acyclovir was administered intravenously for the next 8 days. During therapy the herpes lesions became crusted. Five days after acyclovir medication was stopped, however, the patient developed new papular and, later, vesicular lesions in the area of the older crusted lesions. A relapse of the herpesvirus infection was demonstrated by the Tzanck test, and VZV was again confirmed by IEM. Acyclovir medication was resumed. During acyclovir medication, vesicular and pustular lesions remained positive for herpesvirus infection, as shown by the Tzanck test. Finally, in about 3 weeks, the lesions became crusted and healed. Virus isolation from the skin ulcer and herpetic lesions was repeatedly unsuccessful. The patient's condition worsened steadily. She developed adult T-cell leukemia and clinical symptoms of AIDS. Serologic examinations (human T-cell lymphotropic virus type 1 enzyme immunoassay kit 307724; Abbott) revealed elevated antibodies against human T-cell lymphotropic virus type 1, but tests

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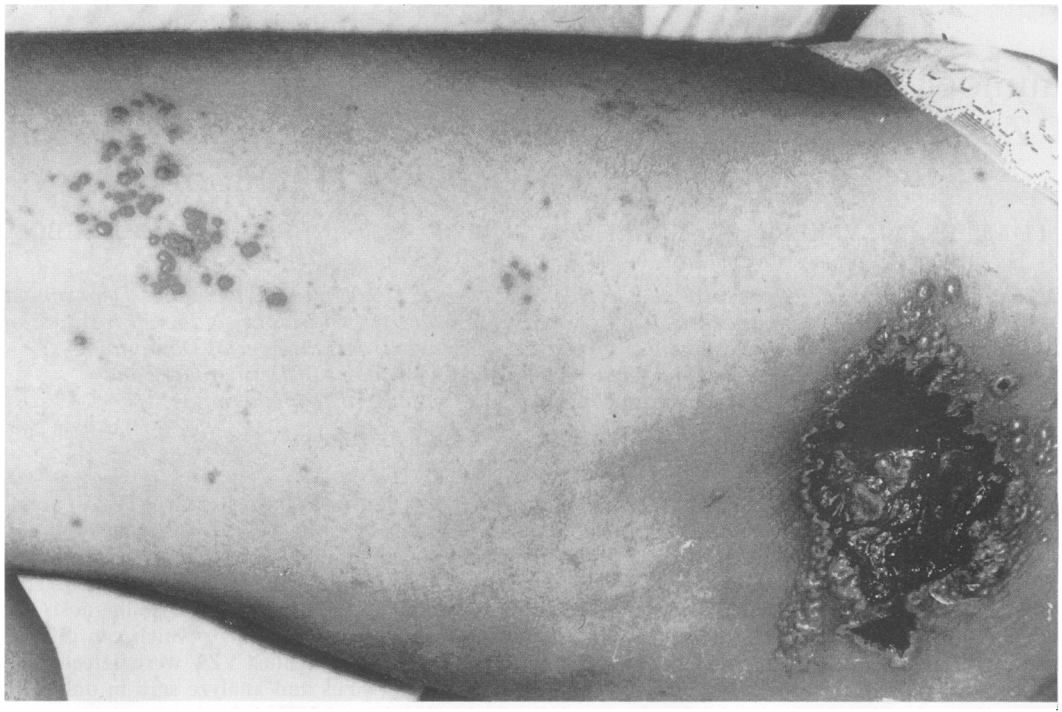

FIG. 1. Necrotic skin ulcer and herpetic pustules on the left leg of the case patient on the day of admission.

for human immunodeficiency virus (HIV) were negative. Despite intensive antileukemia treatment, the patient died within 2 months after the onset of the leukemia.

Disseminated herpes zoster is frequently associated with the presence of a malignancy or underlying immune deficiency (1). Herpes zoster occurs seven times more frequently in HIV-infected individuals than in noninfected people and appears early in HIV-induced immunodeficiency (7). In our patient, the first sign that suggested acquired immune deficiency was a multidermatomal herpes zoster infection with a large necrotic ulcer on the left thigh. This ulcer finally healed after prolonged acyclovir medication.

Standard virological methods. The Tzanck test was the first laboratory test that diagnosed a clinical herpesvirus infection in our patient and was applied as described before (4–6, 10). Immunofluorescence tests (IFTs) performed on smears of lesions can distinguish between VZV and HSV, but studies that make use of IFT are scarce. Some investigators (11, 12) have used VZV culture as a reference test for determining the sensitivity of these IFTs. The isolation of VZV usually takes days, if the virus multiplies at all, and therefore is inappropriate for sensitivity studies and rapid diagnosis. Although cytodiagnosis by immunofluorescence can be very specific, sensitivity probably is influenced by unpredictable factors caused by the stage of the lesion. Therefore, IFT was not used to detect virus in smears in our study. Herpesvirus isolation and virus typing by immunofluorescence were carried out by standard virological procedures. Recent VZV and HSV infection could not be proven serologically by complement fixation tests (Table 1). IgG and IgM antibody titers were assessed by IFT performed on acetone-methanol-fixed HSV- and VZV-infected monolayers. No specific IgM antibodies against VZV were detected by IFT (Table 1).

Electron microscopy. The use of colloidal gold IEM techniques for the diagnosis of VZV and HSV infections has previously been described in detail (5, 6, 16). Virus particles were incubated with highly specific gold-tagged human antibodies directed against VZV. Monoclonal antibodies against HSV and gold-tagged rabbit antibodies directed against mouse Ig were used to detect HSV and to distinguish between HSV types 1 and 2. The patient's antibodies, which were bound in vivo to the virus particles that were present in the lesions, were detected with gold-tagged rabbit IgG antibodies directed against human Igs. The antibody class was further characterized with monoclonal antibodies directed against IgG and IgM. These monoclonal antibodies were also used to assess the VZV and HSV antibody titer in the serum of the patient. The reference virus strains, test sera, monoclonal antibodies, and colloidal gold conjugates used were those described previously (16). Two reference serum samples were included in this study (Table 1); one was from a patient with herpes zoster, and one was from a patient with varicella. Serum samples from two healthy people (two of the authors of this report) with no clinical signs of VZV or HSV infection during a period of at least 20 years before testing were used as negative control sera. Pseudorabies virus, a herpesvirus that causes Aujeszky's disease in pigs, was tested by IEM in order to exclude nonspecific reactions.

The complications observed in our patient required an immediate diagnosis of herpes infection and differentiation between HSV and VZV in order to give the proper acyclovir dosage. The dosage administered for VZV infection is two times the dosage administered for HSV infection. Virus particles obtained from skin lesions (about 6 weeks after the first clinical signs of VZV infection) were all trapped in virus-antibody complexes (Fig. 2a). Immunogold labeling identified the antibodies as IgG. Virus typing, which was still possible on antibody-coated virus particles (16), revealed the presence of VZV.

In a previous study on HSV we detected viral immune complexes mainly in pustular lesions (6). We suspected that the presence of viral immune complexes was related to a

TABLE 1. Serodiagnosis of herpes virus infection in two serum samples from the patient and two reference serum samples

Patient or viral infection (patient age)	Time (wk) after onset of disease	Test virus[a]	Serum Antibody titer in serum determined by[b]:						
			CFT	IFT (log_{10})		IEM (log_{10})			
				IgM	IgG	IgM		IgG	
						Core	Envelope	Core	Envelope
Case (33 yr)	6	VZV	1:16	–	3.0	3.6	1.8	4.0	3.5
		HSV	1:16	–	3.6	–	–	3.8	3.2
		CMV				2.3	2.0	>4.0	3.5
		EBV				–	–	>2.6	2.0
		EBV-CA		–	2.0				
		EBV-NA			+				
		EBV-EA			–				
		PRV				–	–	–	–
Case (33 yr)	10	VZV	1:32	–	3.6	2.0	–	3.5	3.2
		HSV	1:8	–	3.6	–	–	3.5	3.2
		CMV				–	–	>4.0	3.2
		EBV				–	–	>2.6	2.4
Varicella (50 wk)	2	VZV		>2.1	>4	4.4	3.5	3.5	3.5
		HSV		–	–	–	–	–	–
		CMV				–	–	–	–
Herpes zoster (22 yr)	1	VZV		2.7	4.8	3	–	3	4
		HSV		–	4.2	–	–	4	3.8
		CMV				–	–	–	–
Control 1 (43 yr)		VZV				–	–	–	–
		HSV				–	–	–	–
Control 2 (45 yr)		VZV				–	–	–	1.7
		HSV				–	–	–	–

[a] CMV, cytomegalovirus; EBV, Epstein-Barr virus; EBV-CA, Epstein-Barr virus capsid antigen; EBV-NA, Epstein-Barr virus nuclear antigen; EBV-EA, Epstein-Barr virus early antigen; HSV, herpes simplex virus; VC, viral culture; PRV, pseudorabies virus; VZV, varicella-zoster virus.
[b] CFT, complement fixation test; IEM, immunoelectron microscopy; IFT, immunofluorescence test; –, no titer.

negative viral culture. Viral immune complexes can be detected when anti-VZV antibodies appear in the serum (unpublished IEM data). If we assume that antibodies in serum can neutralize VZV in vivo more efficiently than they can HSV, this may explain the repeatedly negative viral cultures in the present case.

Colloidal gold IEM detects viral structural proteins and can differentiate between antibodies against viral core and viral envelope antigens in patient serum (Table 1). VZV envelope IgM antibodies were detected in the first serum sample (Fig. 2b). VZV core IgM antibodies were also detected by IEM and remained present in the serum 4 weeks later. HSV IgM antibodies were not detected in either serum sample. VZV and HSV IgG antibodies were detected in both serum samples by IEM. The titers showed little change. Testing for IgM antibodies directed against VZV is the method of choice in the serodiagnosis of varicella. The sensitivity is 27 to 70%, depending on the technique used (13). These IgM antibodies, however, are not always present in cases of herpes zoster. A rise in titer of VZV IgG antibodies can only be observed when the first serum sample is collected at an early stage of the infection.

The antibody titers against VZV of the two reference serum samples determined by IEM corresponded well to the titers of VZV antibodies in the serum of our patient (Table 1). The titers of VZV IgG antibodies in the reference sera and the patient's sera determined by IEM were in the same range as the titers of IgG antibodies against VZV in these sera determined by IFT. IgM antibodies directed against HSV were not detected in any serum sample, including that from our patient. IgM antibodies directed against VZV envelopes were not detected in the herpes zoster reference serum. The varicella and herpes zoster reference sera that were tested for IgM and IgG antibodies against VZV and HSV showed different labeling characteristics by IEM. The varicella serum had high IgM antibody titers against VZV cores and envelopes, while the herpes zoster serum had IgM antibodies only against VZV cores. Moreover, HSV IgG-class antibodies were detected in the herpes zoster serum but not the varicella serum. Neither the patient's serum nor the reference sera contained IgM antibodies to HSV. Therefore, we concluded that IgM-class antibodies directed against the virus core or envelope can be used in IEM to serologically diagnose recent VZV infections. Our patient developed IgM antibodies against both of these viral substructures. These IgM antibodies may have had a much higher titer in the weeks before the first serum sample was collected. The IgM viral envelope antibody titer detected by IEM (1.8 log_{10}) probably was too low for detection by IFT. The IgM viral core antibody titer (3.6 log_{10}) also was not detected by IFT, although acetone-methanol fixation of the test cells allowed the penetration of IgM molecules into the nucleus of the infected cells, where viral cores normally accumulate. The reason for the discrepancy between the

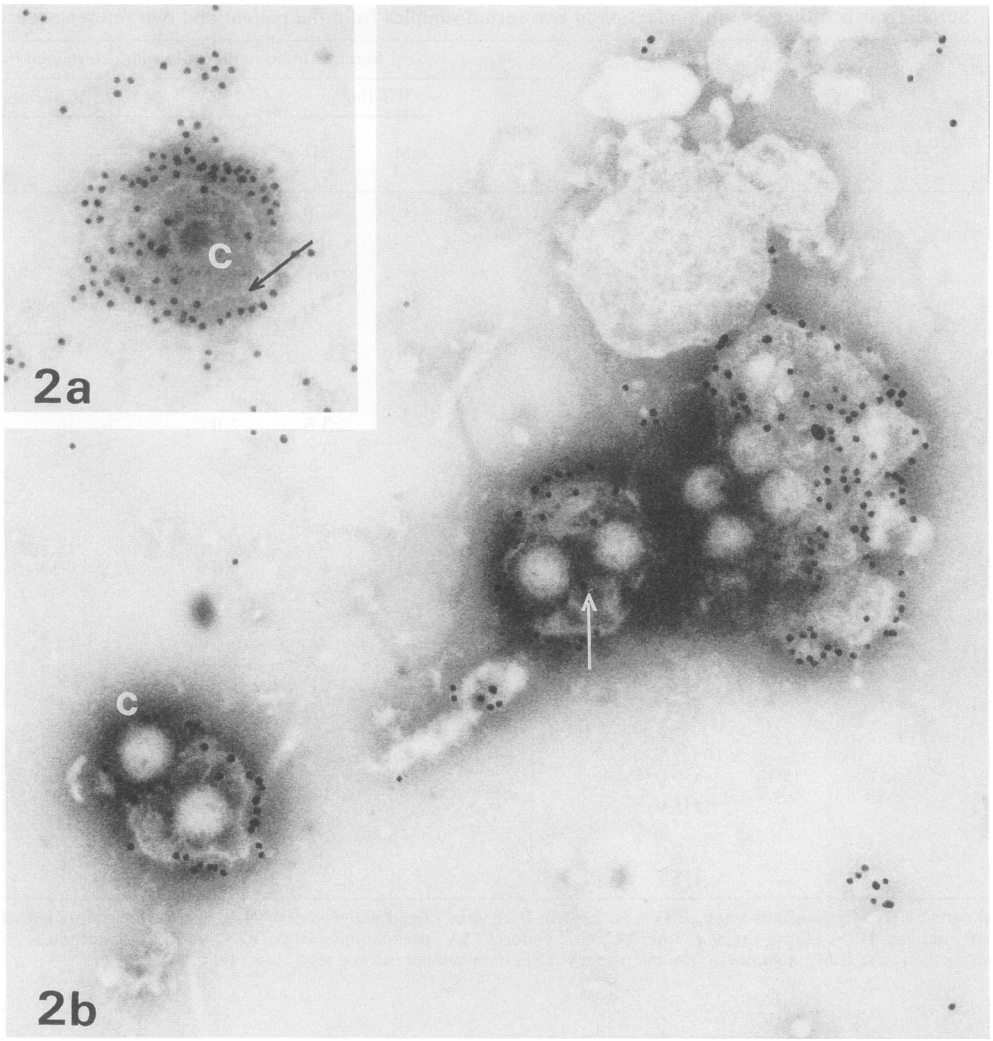

FIG. 2. IEM. (a) VZV particle obtained from the patient's lesion labeled with 18-nm-diameter colloidal gold particles (black dots) coupled to rabbit anti-human antibodies. The gold particles detect patient's antibodies bound to the viral envelope in vivo. c, viral core; arrow, viral envelope with antibody layer. (b) VZV test virus from infected tissue culture cells was first incubated with a dilution of the patient's serum and then with monoclonal antibodies directed against human IgM. The monoclonal antibodies were detected with 18-nm-diameter gold particles coupled to anti-mouse antibodies. c, viral core; arrow, virus particle with two cores. Magnifications, ×65,000.

IEM and IFT results is not clear. It may be that a different subset of IgM-class antibodies which cannot be detected by IFT are detected by IEM, and so we can explain why, in the serum of the patient, an IgM anti-VZV titer was not demonstrated by IFT.

High IgG and IgM antibody levels against cytomegalovirus (CMV) were found by IEM. CMV IgM antibodies were not detected in the second serum sample or in human reference sera of VZV-infected patients. Therefore, we assume that in this case, the IgM antibodies against CMV were induced by a coexisting CMV infection, which is common in immunocompromised patients. Low-level antibody titers against Epstein-Barr virus were detected by IEM. Epstein-Barr virus antibodies were also found by IFT.

Virus typing and diagnosis of viral infection in serum by IEM were completed in about 2 h after sampling and were conclusive for the diagnosis of VZV infection. No other diagnostic technique was as rapid and specific as the IEM. Moreover, the virus was typed and the antibody titer in serum was assessed in one experimental setup. Many hospitals, especially university hospitals, that now use the electron microscope (EM) for pathologic examinations can also use it for rapid virus diagnosis. Because viruses are killed by glutaraldehyde before the EM grids are examined, there is no danger of EM laboratory workers being exposed to infectious viruses.

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