Chapter 11  Polymerase Chain Reaction Diagnosis of Varicella Zoster Virus*

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Summary

Primary infection of humans by varicella zoster virus (VZV) results in chickenpox (varicella). Virus establishes latency in sensory ganglia and reactivates decades later to produce shingles (zoster). Disseminated zoster and postherpetic neuralgia are the two major complications of VZV reactivation in the elderly. There is increasing evidence that VZV can reactivate subclinically (zoster without skin rash), particularly in immunocompromised patients. Thus, the clinical import of rapid diagnosis and use of antiviral drugs in these patients is clear. Although most clinical assays are restricted by their dependence on an adequate host immune response, and by their limited sensitivity and specificity, PCR technology enables the detection of minute amounts of a specific viral DNA sequence in a large excess of background DNA. The availability of the complete DNA sequence of the viral genome has facilitated the diagnosis of VZV by PCR during acute and latent infections. VZV DNA has already been detected by PCR in DNA isolated from human blood MNCs, throat swabs, vesicles and crusts obtained at different times during viral infection, and in latently infected human ganglia. In addition, PCR has also been used to study the early site of viral replication and the mode of transmission of VZV infection. Lastly, the use of nested-primers and quantitative PCR will enhance the sensitivity of detection and permit determination of virus burden in biological specimens. These findings will aid in studies of VZV pathogenesis.

Introduction

Varicella zoster virus (VZV) causes childhood chickenpox (varicella), becomes latent in dorsal root ganglia, and reactivates decades later to produce shingles

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(zoster). Rash is characterized by vesicles on an erythematous base. Skin and mucosal lesions of varicella are generalized, while those of zoster are restricted to 1–3 dermatomes. Both disorders are acquired by household or direct contact with skin lesions or respiratory secretions of infected humans (Ross 1962; Gordon and Meader 1929; Evans 1940; LeClair et al. 1980; Gustafson et al. 1982; Brunell 1989).

Zoster, a common problem in elderly and immunocompromised individuals (Gallagher and Merigan 1979), is often complicated by postherpetic neuralgia (pain which persists for months to years after rash), by disseminated zoster or encephalitis or both, and less often by granulomatous arteritis. Rapid clinical diagnosis of VZV infection and any attendant complications is essential since antiviral treatment does exist.

Because conventional approaches to the diagnosis of VZV are time-consuming and not always precise, they are of limited value to the clinician. Nevertheless, before discussing the polymerase chain reaction (PCR), we briefly review the standard diagnostic techniques to offer some perspective on their usefulness and some speculations regarding their potential application in conjunction with PCR in the diagnosis and study of the pathogenesis of VZV-induced disease. These techniques include histologic and ultrastructural examination, attempts to isolate virus from infected tissue, and serologic assays that measure the humoral or cell-mediated immune response to VZV.

Histopathological diagnosis of VZV infection uses Giemsa-stained smears of vesicle scrapings to detect multinucleated giant cells and Cowdry A intranuclear inclusions characteristic of herpesviruses (Taylor-Robinson and Caunt 1972). Oral mucosa smears and cells in sputum from VZV-infected individuals have also been shown to display intranuclear inclusions (Cooke 1960, 1963; Williams and Capers 1959).

Electron microscopy (EM) study may reveal herpes virions in infected tissue. Monocytes obtained during the early stages of zoster have been shown to contain herpesvirus particles (Twomey et al. 1974). However, EM is cumbersome and does not distinguish the different herpesviruses; its diagnostic usefulness today is to complement viral and immunocytochemical analyses of herpesvirus-infected tissue.

VZV can occasionally be isolated by cocultivation of infected tissue with human or primate cells. It has been isolated from blood mononuclear cells (MNCs) of 10 of 12 (83%) otherwise healthy immunocompetent children 4–5 days before the onset of varicella, and 100% of children 1–2 days before rash, as well as on the first day of rash (Asano et al. 1985; Ozaki et al. 1986). These findings indicate that the greatest magnitude of blood-borne virus dissemination occurs just before the onset of disease. In those studies, the cytopathic effect characteristic of VZV was seen only after a second subculture in human embryonic lung cells. VZV has also been isolated from the blood of immunosuppressed adult zoster patients (Gold 1966; Feldman and Epp 1976; Gershon et al. 1978; Myers 1979), but not from the blood of healthy individuals with zoster or immunosuppressed patients without zoster. Isolation of VZV from the pharynx, a putative site of virus infection and replication shortly after