Rapid Molecular Discrimination between Infection with Wild-Type Varicella-Zoster Virus and Varicella Vaccine Virus

U. Lässker, T.C. Harder, M. Hufnagel, M. Suttorp

Abstract

Varicella-zoster virus (VZV) infection in immunocompromised patients may cause life-threatening complications. Prevention measures include administration of VZV immunoglobulin, acyclovir and live attenuated varicella vaccine. After vaccination, a mild varicella-like exanthem appears in up to 5% of vaccinees. Morphologically this exanthem cannot be differentiated from wild-type (wt) varicella. The risk of virus transmission after varicella vaccination, in contrast to wt varicella, is low, even in immunocompromised patients. We report on a 2-year-old girl with relapse of cerebral anaplastic ependymoma, who received one dose of varicella vaccine. Two weeks later, a maculopapular rash developed while she was an inpatient on the oncology ward. Using VZV-specific PCR and restriction fragment length polymorphism (RFLP) analysis, we were able to diagnose wt varicella infection. Thus, appropriate prevention measures (VZV immunoglobulin and acyclovir) were justified for close contacts to prevent virus transmission. No secondary cases occurred.

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Introduction

In immunocompromised patients, especially in oncology patients, contact with varicella-zoster virus (VZV) without treatment is associated with a 30% risk of life-threatening infection and a mortality rate of 7% [1]. Common prophylactic postexposure measures are administration of hyperimmune globulin and acyclovir. In 1974, a live attenuated varicella vaccine was established by *Takahashi* et al. [2] in Japan after isolating and attenuating the virus from the vesicular fluid of a 3-year-old boy with chickenpox named Oka. Ten years later, the vaccine was licensed for common use [3, 4]. Numerous trials demonstrated a good tolerance of the vaccine; less than 1% of vaccinees develop fever and complaints regarding the injection site (pain, swelling, pruritus) are reported in 13–20%. A mild varicella-like, maculopapular, rarely vesicular rash is seen in 5% of the vaccinees [5].

Contagiousness of vaccine-induced chickenpox has been evaluated in several studies. Virus transmission within a healthy population has only occurred in three single cases [6–8]. Thus, immunocompromised patients are unlikely to be infected by household contacts of those who receive the vaccine virus [9]. Conversely, virus transmission from vaccinated immunocompromised patients to healthy contact persons is seen in up to 17% of cases, depending on the number of vesicular lesions the vaccinee develops [10]. Since 95% of vaccinees develop sufficient antibody titers against VZV, vaccination is considered to provide efficacious protection against wild-type (wt) VZV infection, especially for high-risk oncologic populations [11]. We report on a case that challenged us to differentiate between wt and vaccine-induced chickenpox.

Case Report

Relapse of anaplastic ependymoma was diagnosed during routine follow-up in a 2-year-old girl 15 months after the initial diagnosis and 9 months after the last cycle of chemotherapy [12]. Two days after admission, the girl developed subfebrile temperatures and a maculopapular rash with six single vesicles. Morphologically, the exanthema was compatible with a VZV infection. Two weeks earlier the girl had been vaccinated against VZV (Varilrix®, GlaxoSmithKline, Munich, Germany). According to the parents, there had been no known contact with a case of chickenpox. Thus, the question arose whether this non-immunocompromised patient had vaccine-induced or wt varicella.

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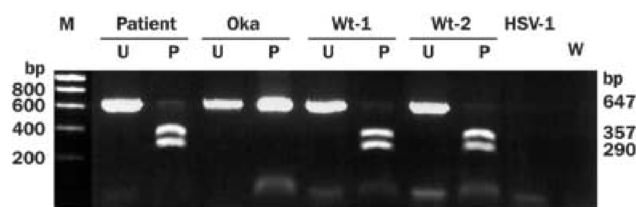

Figure 1. Endonuclease restriction digests of PCR-amplified DNA. The patient sample shows the characteristic pattern of wt VZV DNA. Lane 1: molecular size marker. Lanes 2 and 3: PCR-amplified DNA obtained from the vesicular lesions of the patient by cotton swab. Controls are shown in lanes 4–11. Lanes 4 and 5: VZV vaccine strain (OKA). Lanes 6–9: VZV wt isolates (WT). Lane 10: herpes simplex virus type-1 (HSV-1). Lane 11: water control (W). Lanes 2, 4, 6 and 8: undigested PCR amplification product (U) with a specific length of 647 bp. Lanes 3, 5, 7 and 9: *Pst*I-restriction digest (P) showing the expected 357 bp and 290 bp fragments in wt VZV DNA but no digestion in the OKA vaccine strain.

Since wt infection could not be discriminated at this point, we immediately introduced preventive measures appropriate for contacts of VZV on the oncology ward, despite the fact that vaccine-induced chickenpox was expected. All eight patients exposed (age 1–12 years, with no history of chickenpox and seronegative for VZV) received hyperimmune globulin and acyclovir. To obtain a definite diagnosis, two separate vesicular lesions were unroofed to take samples for virologic examination. These specimens were evaluated by VZV-specific PCR and restriction fragment length polymorphism (RFLP) analysis.

Materials and Methods

Samples

25 μl of fluid from the base of two vesicular skin lesions of our patient were obtained using a cotton swab and stored at room temperature in a sterile tube.

DNA Extraction and PCR

Viral DNA was extracted from the cotton swab by means of the QIAamp DNA blood kit® (Qiagen, Hilden, Germany) according to the manufacturer´s recommendations. For PCR amplification of VZV DNA, two specific primers (sense: 5'-AAGTTTCAGCCAACGTGCCAATAAA-3'; anti-sense: 5'-AGACGCGCTTAACGGAAGTAACG-3') were used. The resulting PCR product, a fragment of the open reading frame 38, migrates as a 647 bp fragment in a 1% agarose gel [13].

The reaction mixture for the PCR consisted of 50 pmol of each primer, 1.5 U HotStarTaq® (Qiagen, Hilden, Germany), 200 μM (each) deoxynucleoside triphosphates and 100 ng of template DNA in 50 μl HotStarBuffer® (Qiagen, Hilden, Germany). PCR amplification was carried out with an initial denaturation for 15 min. Each cycle consisted of denaturation at 95 °C for 20 sec, annealing at 56 °C for 20 sec and extension at 72 °C for 30 sec (35 cycles), followed by a final extension step at 72 °C for 10 min.

RFLP Analysis of PCR Products

In contrast to OKA vaccine strain DNA, wt VZV DNA harbors a specific *Pst*I restriction site (5'-CTGCAG-3'/3'-GACGTC-5'). *Pst*I digestion of the amplified 647 bp PCR product results in two fragments of 357 bp and 290 bp size in wt VZV DNA, whereas the OKA vaccine strain shows the undigested 647 bp fragment. In contrast to some clinical wt VZV isolates in Japan, the vaccine RFLP pattern (absence of *Pst*I restriction site) has not been seen in wt VZV isolates from USA, Australia and Europe [14]. PCR-amplified products (5 μl) were digested with 10 U *Pst*I at 37 °C for 1 h in universal buffer (Stratagene, Heidelberg, Germany). The digests were analyzed directly by agarose-gel electrophoresis (1%) [7].

Discussion

Unexpectedly specimens isolated from the two lesions were both identified as wt VZV DNA, showing the typical restriction digest pattern (Figure 1). Thus, the patient must have been exposed to wt VZV prior to (or shortly after) the day of vaccination, and the vaccine had been administered during (or just prior to) the incubation period. Vaccination may have eased the course of the disease but could not completely prevent its course. Therefore, wt infection with increased risk for immunocompromised patients on the oncology ward had to be considered.

Since wt VZV infection in immunocompromised children (e.g. due to cancer or transplantation) is still a life-threatening condition, prophylactic vaccination with live attenuated vaccine is generally recommended to prevent severe disease in this high-risk population [3, 15].

The degree of contagiousness of vaccine-induced chickenpox is of major importance. In 1984, *Weibel* et al. [4] vaccinated 468 healthy children against VZV and studied their healthy siblings (n = 446) who received placebo. In 94% of vaccinees seroconversion was evident by detection of relevant antibody titers to varicella. Seroconversion or clinical disease did not occur in any of the evaluated siblings. The authors concluded that the risk of viral spread from healthy vaccinees is not clinically relevant.

In contrast, three publications report transmission of vaccine virus from single cases within a healthy population. *La Russa* et al. [7] documented the case of vaccine virus transmission from a healthy 38-year-old mother to her two healthy children and proved it by specific PCR and RFLP analysis. *Salzmann* et al. [8] reported a 12-month-old toddler developing more than 30 vesicular lesions after vaccination against VZV. Two weeks later his seronegative pregnant mother showed about 100 typical VZV skin lesions. RFLP analysis documented the vaccine-type infection of the mother. Evaluation of the fetal tissue after elective abortion did not detect virus transmission to the fetus. Topically administered steroids may have suppressed the immune response of the toddler's skin and supported the viral spread. The pregnancy of the infected mother might have induced immunosuppression, as indicated by her significant skin disease, which is more commonly found in immunocompromised vaccinated persons. A comparable case was reported by *Huang* et al. [6]. A 32-year-old mother at 39 weeks of gestation presented with a generalized papulovesicular rash. Varicella infection was confirmed by serologic studies and it was concluded that there had been

viral spread from her recently vaccinated healthy children. However, viral differentiation by RFLP analysis was not performed and, therefore, wt VZV infection could not definitely be excluded.

To investigate the risk of virus transmission by vaccinees, *Diaz* et al. [9] vaccinated 37 healthy children whose siblings (n = 30) were suffering from malignancies. Serologic findings demonstrated lack of vaccine virus transmission to the immunocompromised children. The results implicate that vaccination of household contacts is a useful protection against wt VZV infection for immunocompromised patients.

Tsolia et al. [10] vaccinated 482 patients with leukemia in remission and detected vaccine virus transmission to healthy siblings in up to 17%, depending on the numbers of skin lesions the vaccinees developed. The clinical course of the disease in the siblings was much milder than in wt VZV infection.

In conclusion, the type of VZV infection (wt vs OKA vaccine) and the immune status of the vaccinee are critical for the risk of transmission, especially in immunocompromised contacts. Immunocompetent vaccinees rarely shed vaccine virus and pose hardly any risk to immunocompromised patients, whereas immunocompromised vaccinees have an increased risk of transmitting the vaccine virus and of causing disease. The case report stresses once more that wt infection should always be considered even without obvious contacts. VZV-specific PCR with RFLP analysis are very useful tools to discriminate between the two viral strains [9]. Results can be obtained within 24 h. The documented wt chickenpox in our case made preventive measures (administration of hyperimmune globulin and acyclovir) reasonable. Thus, PCR and RFLP analysis can help balance the risks and costs and support physicians in their decisions regarding postexposure prophylaxis.

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