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Short communication

Viremia in human Cowpox virus infection

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Abstract

Background: Several poxviruses can infect humans and cause diseases of varying severity. Besides the eradicated Variola virus that induced high mortality rates, numerous further human pathogenic orthopoxviruses are potentially fatal but generally cause less severe infections. While infection-related viremia was described for Variola virus and seems to be rare for Monkeypox virus, it is still debated for Vaccinia virus. So far, viremia in Cowpox virus-infected humans has not been reported.

Objectives: To estimate the potential risk of Cowpox virus to disseminate and develop severe infections, two Cowpox virus patients were examined for viremia.

Study design: Whole blood, serum and fluid from virus-induced lesions were analyzed by serology or quantitative real-time PCR.

Results: Real-time PCR and sequence analysis of the hemagglutinin gene confirmed Cowpox virus in the lesions of both patients. Serology performed on serum obtained at the same time as the lesion specimens demonstrated orthopoxvirus-specific IgG and IgM antibodies, indicating a recent orthopoxvirus infection. In addition, Cowpox virus DNA was detectable in whole blood, but not in serum, as late as week 4 post-infection.

Conclusions: In contrast to observations following vaccination with Vaccinia virus, DNAemia in patients with localized symptoms of a Cowpox virus infection does not seem to be a rare event. However, its relevance for Cowpox virus pathogenicity has to be elucidated.

Keywords: Orthopoxvirus; Cowpox virus; Viremia

1. Introduction

The orthopoxvirus genus comprises several species that are of different pathogenicity to humans. Variola virus (VARV), probably the most prominent orthopoxvirus (OPV), has exclusively infected humans inducing mortality rates of up to 40% (Moore et al., 2006). Fortunately, VARV could be eradicated by vaccination with the closely related Vaccinia virus (VACV) (Henderson, 1976), which, however, caused mild to severe adverse effects in a relatively high percentage of vaccinees, leading to cessation of vaccination after the global eradication of VARV (Kretzschmar et al., 2006). Transmission of VACV to close-contact persons has been shown for spouses and children (Garde et al., 2004; Marris, 2007). Recently, natural human VACV infections occurred that were not linked to vaccination but were transmitted by infected cattle (Damaso et al., 2000; Leite et al., 2005; Nagasse-Sugahara et al., 2004). Human Monkeypox virus (MPXV) infection in Africa is clinically very similar to VARV infection, causing mortality rates of up to 10% when becoming systemic (Arita et al., 1972). In 2003 a milder MPXV variant was imported to the USA by infected rodents and caused non-fatal MPXV infections (Likos et al., 2005). Compared to African MPXV, these infections mostly induced localized lesions (Centers for Disease Control and Prevention, 2003). Humans can be infected by Cowpox virus (CPXV) via direct contact to diseased cats (Coras et al., 2005) or, as recently described, to diseased rodents (Wolfs et al., 2002). Human cowpox presents mostly as localized self-limiting infection (Honlinger et al., 2005) but can become fatal in immunosuppressed patients (Czerny et al., 1997).

Abbreviations: VARV, Variola virus; VACV, Vaccinia virus; MPXV, Monkeypox virus; CPXV, Cowpox virus; OPV, orthopoxvirus; FITC, fluoresceine isothiocyanate.

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Investigation of the VARV pathogenesis showed two viremic phases that enabled virus dissemination in the infected individual (Damon, 2007). VARV viremia was shown by virus isolation from whole blood. In contrast, viremia in VACV vaccinees seems to be an extremely rare event and seems to be detectable by PCR only between days 3 and 7 after vaccination (Cummings et al., 2004). However, vaccination adverse effects can be linked to viremia or DNAemia in many cases (Fulginiti et al., 2003). For human infections with MPXV it is discussed that there is potentially a viremia. Most of the recent MPXV cases in the USA showed localized lesions and only 3/12 patients investigated were viremic as shown by PCR (Likos et al., 2005). So far, human CPXV infections have not been correlated with the occurrence of viremia. However, the given facts indicate that viremia can be directly correlated to dissemination of human orthopoxvirus infection and to the increased risk of severe complications.

2. Methods

During the past year two human CPXV infections were diagnosed of which whole blood was available. Both cases were emerging from different areas of Northern Germany and both patients were non-vaccinated immunocompetent women of 21 and 25 years, respectively. Infection was transmitted by cats suffering from cowpox, as diagnosed by the local veterinarian. Human infection was localized with one typical vesicle in the face or at the shoulder, respectively. Since there are no approved poxvirus-specific therapies, broad-spectrum antibiotics were administered to reduce the risk of bacterial super-infection. About 10–12 days after vesicle formation, whole blood, serum and a swab soaked with lesion fluid from both patients were submitted for diagnosis.

To prove orthopoxvirus-specific antibodies in the patients' sera, immunofluorescence staining of CPXV-infected human cells was performed according to standard procedures. Briefly, CPXV-infected Hep2 cells (MOI 0.1) were propagated on glass slides for 24 h at 37 °C. Cells were fixed in 4% formalin and stained with serial dilutions of the patients' serum, followed by a FITC-conjugated goat anti-human IgG (1:50, Caltag Laboratories/Invitrogen, Carlsbad, CA, USA), counterstained with Evans Blue and evaluated by fluorescence microscopy.

DNA from the lesion fluid, serum and whole blood was prepared using a Qiagen Blood kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Quantitative real-time PCR amplification was applied to detect orthopoxvirus DNA (Nitsche et al., 2004). Briefly, stretches of the genes rpo18, VETF and A13L were quantitatively analyzed with a LightCycler by correlation to calibration curves as described previously. For exact virus typing the complete hemagglutinin (HA) gene locus with 936 bp in length was amplified and sequenced with the OPV-generic flanking primers OPV HA S TgTTACCACRYAATTATATAATgTATAAATgCg and OPV HA AS AgATTTTACTATYCCAgACATTTATgTAAgTC.

3. Results

As shown by serology, both patients showed orthopoxvirus-specific antibody titers of >1:160 (IgM) and >1:2560 (IgG), indicating a recent orthopoxvirus infection. PCR analysis of the vesicle fluid and sequencing of the HA gene confirmed that the patients were infected by similar but different strains of CPXV.

Interestingly, the additional analysis of whole blood by quantitative real-time PCR revealed a CPXV DNAemia with ~1000 copies/ml of whole blood. Assuming an incubation period of 12–14 days, this DNAemia was detectable in both patients 10–12 days after the appearance of CPXV-specific lesions as late as in week 4 post-infection. No DNA was found in the corresponding serum, indicating cell-associated viremia which has also been postulated for VARV viremia (Jahrling et al., 2004). In comparison, using the same PCR methods, no DNA was detected in the blood of 15 smallpox vaccinees at several time points after vaccination (data not shown), which is in accordance with recent publications (Cummings et al., 2004).

4. Discussion

Although the infection of healthy humans with CPXV is generally localized, CPXV is potentially fatal for immunosuppressed individuals (Eis-Hubinger et al., 1990). Today it is not clear whether the increased number of diagnosed human CPXV infections reflects an increased awareness of physicians or the fact that the part of the population vaccinated against smallpox decreases with time. In fact, recent cases of human cowpox diagnosed in the German Consultant Laboratory for Poxviruses were always observed in young non-vaccinated people (own observations and Essbauer et al., 2002).

Virus spread through blood is a prerequisite for virus dissemination and can be linked to the severity of a poxvirus infection. While viremia could be demonstrated for VARV (Downie et al., 1950), it seems to be less frequent for MPXV (Likos et al., 2005) and is even controversially discussed for VACV (Bray, 2003; Fenner et al., 1988; Savona et al., 2006). While some studies failed to demonstrate VACV viremia after vaccination (Cummings et al., 2004), others were able to prove VACV DNA shortly after vaccination but only in a small proportion of the investigated vaccinees (Savona et al., 2006). Infectious VACV or virus proteins could not be demonstrated so far (Srinivasan et al., 2006). In contrast, in cases of adverse vaccination complications, viremia was common (Fulginiti et al., 2003).

Here, the DNAemia of CPXV as late as approximately 4 weeks post-infection was shown in two independent patients.

Table 1
Infection characteristics of Variola virus, Monkeypox virus, Vaccinia virus and Cowpox virus

	Infection local/disseminated	Viremia	Risk
Variola virus	−/+	+	+++
Monkeypox virus	+/+	−/+	++
Vaccinia virus	+/(+)[a]	−/(+)[b]	+
Cowpox virus	+/(+)[a]	+	?

(−) None; (+) low; (++) moderate; (+++) high; (?) unknown.
[a] Associated with complications.
[b] Controversially discussed.

Although only two patients were investigated, CPXV DNA was clearly detectable in both patients but this was not possible in VACV vaccinees during a follow-up course of 4 weeks post-vaccination using the same methods. The sole presence of viral DNA is generally no definite indication for infectious virus particles; however, the circulation of virus-free DNA at a concentration of 1000 genome equivalents per ml is not likely and would also have allowed DNA detection in serum, which was not the case. Isolation of CPXV from whole blood was not performed because complete blood specimens were subjected to DNA preparation. To prove a viremic phase in CPXV infection, attempts to isolate infectious virus are required.

Considering the high genetic similarity of CPXV to VARV (Meyer et al., 2002), the pronounced DNAemia found in both investigated cases of localized human cowpox over weeks post-infection suggests a potentially high risk of virus dissemination with severe complications (Table 1). Therefore, increased awareness by dermatologists regarding human cowpox is advocated, along with the development of new, effective antiviral substances to treat human orthopoxvirus infections.

Conflict of interest

The authors declare that there is no conflict of interest.

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