Laboratory Acquired Infection with Recombinant Vaccinia Virus Containing an Immunomodulating Construct

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Handling of Vaccinia virus represents a risk for laboratory-acquired infections, especially in individuals without completed vaccination. We report the case of a Vaccinia infection in a previously vaccinated researcher working with various genetically modified strains. We could confirm the infection by electron microscopy, positive cell culture, virus-specific PCR, sequence analysis, and viral neutralization test. The isolated virus contained a construct that had been used to decrease leukocyte adhesion by interacting with the LFA-1/ICAM-1 axis. The immunomodulating nature of the inserted construct might thus have added to the infectivity of the virus.

We emphasize on the necessity of Vaccinia vaccination in the field of orthopoxvirus and molecular and cellular biology using recombinant Vaccinia viruses (Williams and Cooper, 1993). In contrast, mandatory guidelines are lacking in Europe and North America. The immunomodulating nature of the inserted construct might thus have added to the infectivity of the virus. The 40-year-old Caucasian male had been working at a research department for over 10 years with genetically modified Vaccinia virus strains carrying various inserts that had been introduced into the virus specific thymidine kinase gene. He had been vaccinated with Vaccinia at the age of 1 and 12 years in the upper arm by standard scarification procedures. After an interruption of about 12 months he restarted his laboratory work and handled several times infected cell cultures with high concentrations of recombinant Vaccinia virus (~10⁷ plaque forming units (PFU)/ml).

Three days prior to presentation at our department he had noticed the growth of a vesicular lesion at the distal phalanx of the 2nd finger of his right hand, which slowly progressed to a 15-mm sized infiltrated inflammatory nodule with central hemorrhagic necrosis (Fig 1A,B). A second inflamed vesicular lesion developed at the 3rd finger of the left hand two days later. After an unsuccessful surgical incision he presented at our department for diagnosis and treatment.

The clinical appearance of a dark red raised firm nodule with central necrosis was suggestive for ortho- or parapoxvirus infection, e.g., cow-poxvirus or Orf virus. The patient denied recent contact to cats, cows, or sheep but reported regular professional contact to Vaccinia virus. General medical examination revealed no abnormal results and the patient showed no signs of cellulitis or lymphadenopathy. Only the palms of both hands showed signs of mild skin barrier disturbance (e.g., small erosions) from working with unprotected hands in cold temperature over a prolonged period. After informed consent was obtained, a skin biopsy and serum samples were taken for microbiological, electron microscopy, and immunological analysis.
The lesions were treated with topical disinfectants (such as polyvidone iodine ointments) leading to complete healing of skin lesions within two weeks.

METHODS

The biopsy was split for electron microscopy (EM) and viral culture for orthopoxvirus using the permanent African green monkey kidney cell line MA 104 cultured in minimal essential medium supplemented with 5% fetal calf serum followed by molecular biology analysis. In addition, serum samples were taken for antibody determination using the virus neutralization assay. The test was performed following the method described by Czerny et al, using 100 plaque-forming units of orthopoxvirus strain Vaccinia Virus Munich 1 and MA 104 cells in 24-well Linbro plates (Czerny et al, 1996). Plaques were visualized with crystal violet after 24 h of inoculation at 37°C and 5% CO2.

Electron microscopy of infected cells was performed as previously described (Pfeifer et al, 1996). The isolated virus was further examined by PCR. Briefly, DNA was extracted from the infected cell culture as described elsewhere (Meyer et al, 1994). DNA of the patients’ isolate, Vaccinia virus reference strain Western Reserve (WR) and cowpoxvirus Brighton (BR) was screened using a PCR targeting the A-type inclusion (ATI) protein gene (Meyer et al, 1994). The resultant fragments were digested with BglII and separated on an agarose gel. The ATI-PCR assay is based on sequences flanking a region that exhibits various deletions in variola, vaccinia, mousepox, monkeypox and camelpox virus as compared to cowpox virus. Thus, depending on which of the six species is being examined, the ATI-PCR provides a DNA fragment of distinct size. In a second PCR (VTK-PCR), an amplification of the thymidine kinase gene was carried out as previously described (Thomas et al, 1990). Sequencing of the inserted DNA was performed using an ABI Prism 310 sequencer (Applied Biosystems GmbH, PE Biosystems Weiterstadt, Germany), first with a primer that allows identification of the inserted gene and second with a primer located within the human cytohesin-1 gene which allows to uncover the E157K mutation (see legends to Fig 2).
EM analysis of the biopsy showed a single orthopoxvirus particle, a result further confirmed by EM of the infected cell culture, which revealed multiple orthopoxvirus particles after the first passage.

The fragment size obtained for the patients' isolate and Vaccinia virus WR in the AT-assay were identical and slightly smaller as compared to cowpox virus BR. BglIII digest electrophoresis was used to further enhance fragment size motility and the resulting patterns were identical for the obtained isolate and Vaccinia virus WR but different from cowpox virus BR (data not shown). The VTK-PCR utilizes primers flanking the insertion locus of the thymidine kinase gene. A fragment of about 530 bp was amplified from Vaccinia virus WR which corresponds well to the size (528 bp) described in the literature (Thomas et al, 1990). A fragment of about 1800 bp (see Fig 2) was amplified from the obtained patients' sample indicating that the thymidine kinase gene contains an insertion of approximately 1.3 kb. Further sequence analysis revealed that the inserted DNA fragment encoded a fusion protein of the human cytogenes-1 cDNA with a transmembrane Ig sequence (Kolanus et al, 1996). The glutamic acid residue at pos. 157 of cytogenes-1 was mutated to a lysine residue. E157 has been shown to be critical for catalytic activity and the mutation to K leads to a complete loss of GEF (guanine exchange factor) activity. This mutant had been shown to impair leukocyte adherence functions in cellular assays in a previous report (Geiger et al, 2000).

Anti-Vaccinia antibodies evaluated in consecutive serum samples were elevated, showing a titer of 1:64, increasing to 1:256 three weeks later, an increase in antibodies which is usually not observed 30 years after vaccination. Routine blood count parameters were found within normal limits.

DISCUSSION

We have documented a case of human infection by the genetically modified Vaccinia strain Western Reserve used for research studies. Up to now there has been no report of clinical symptoms in individuals exposed to genetically modified laboratory strains without previous sting injury. Our patient developed two clinically typical pox lesions although he had been vaccinated with the highly similar Vaccinia strain twice during childhood. The case presented here emphasizes the possible risk of clinical infections by laboratory strains even when they are handled properly and no concomitant disruptions of the epidermal barrier are present as seen in our patient. The duration of a protective immunity after vaccination is a subject of current debate (Cohen, 2001). The induced T-cell reactivity towards the Vaccinia virus seems to remain for decades (Demkowicz et al, 1996; Ennis et al, 2002). Since the course of the infection in our patient was relatively mild without no generalization or signs of systemic disease it is likely that he had incomplete protective immunity from the vaccination 28 years ago. This might, however, not be the case for individuals without Vaccinia-specific immunity.

There is now an increasing number of young researchers and students within the field of biomedical sciences who have not been routinely vaccinated against smallpox, as most countries stopped their vaccination programs in the 1970s. At present, no general recommendation exists for Vaccinia vaccination of laboratory workers.

The CDC gives a clear guideline by suggesting that "all persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cow pox viruses are being conducted should have documented evidence of satisfactory vaccination within the preceding 10 years." (http://bmlbd.odd.nih.gov/viral.htm#pox). The situation is less clear in Europe. General recommendations do not exist and there is currently no licensed vaccination product available. It is questionable whether the advantages of a general vaccination strategy outweigh the risk of vaccination complications that have been observed repeatedly (for a review see Isaacs, 2002). The case reported here indicates that infections with genetically modified strains can occur even when handled properly and argues in favor of vaccination programs for laboratory staff.

However, the infectivity of the vaccinia construct may depend on the insert. The VTK-PCR revealed that a cytogenes-1 fusion protein was inserted into the Vaccinia vector. Cytogenes-1 is a regulatory interaction partner of the β2 integrin αLβ2 (LFA-1) and a guanine exchange factor (GEF) for ADP ribosylation factor (ARF)-GTPases (Kolanus et al, 1996). The identified E157K mutation leads to a dominant negative construct that has been shown to suppress the adhesion of human peripheral blood lymphocytes to ICAM-1 (Geiger et al, 2000). Taking into account that cytogenes-1 plays a crucial role as a regulator of LFA-1 activation and LFA-1 dependent spreading of lymphoid cells, it is tempting to speculate that the virus construct contributed to a locally diminished immune response. A similar phenomenon has not been reported so far. However, one should remain alert whether or not the infectivity of laboratory strains of Vaccinia virus is affected by the inserts.

Fortunately, the course of the disease in our patient was self-limited. Yet, in more severe infections systemic treatment has to be considered for patients with laboratory acquired Vaccinia virus infection. Currently the CDC recommends the use of immunoglobulins (VIG) for severe cases (ACIP, 2001). However, these immunoglobulins are not ubiquitously available and therefore the use of cidofovir might be considered (De Clercq, 2002), as animal studies have shown promising results.

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REFERENCES


