Zoonotic Vaccinia Virus: Clinical and Immunological Characteristics in a Naturally Infected Patient


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Vaccinia virus was used as vaccine to eradicate smallpox. We report a zoonotic case of vaccinia virus infection in a 30-year-old patient who became infected after handling sick dairy cattle. The patient had inflamed lesions and systemic symptoms. Laboratory findings were indicative of down-modulated immune responses to the virus.

Orthopoxvirus infections are, once again, a concern of physicians and researchers worldwide. The possible use of smallpox as a bioterrorist weapon and the emergence of monkeypox virus in Africa and the United States have made the scientific community aware of the threat of Orthopoxvirus infection outbreaks. The real magnitude of the occurrence of Orthopoxvirus infections in humans is further stressed by factors such as the maintenance of vaccinia virus (VACV) infection in milking buffaloes in India [1], the increasing number of cowpox virus infections in Europe [2, 3], and the emergence of VACV infection in Brazil and, likely, other parts of South America [4–7].

From June through November 2005, outbreaks of exanthemtic disease among humans and cattle were reported in poor and undeveloped populations living in the rural countryside of Minas Gerais in southeastern Brazil. The characteristics of the outbreaks were similar to those of outbreaks of VACV infection that were described in the country during previous years [5–8]. An investigation was conducted in many of the affected rural communities to gather epidemiological and clinical data and to promote community awareness about the disease. Individuals affected and not affected by the outbreaks were enrolled in the study after they signed an informed consent document. The study was approved by the Centro de Pesquisas René Rachou Ethics Review Committee.

A structured questionnaire was used to collect data on infection signs, symptoms, and risk factors; exposures; and demographic characteristics. In most locations, physicians and public health authorities were unfamiliar with the course of the disease; this frequently led to poor diagnosis and administration of incorrect treatment. The epidemiological data retrieved from the study revealed that all but one of the patients were male, and 94% were milkers; this characterized the disease as an occupational zoonosis (F.G.d.F., unpublished data). The progression of the disease was essentially identical for most infected patients. In this report, we focus on a patient whom we were able to observe during the complete development of the illness.

Our study patient was a 30-year-old man living in a rural area near Serro, Minas Gerais, Brazil. The patient, who had not been vaccinated against smallpox (vaccination was terminated before he was born, and he lacked a vaccination scar), worked as a farmer and reported occupational contact with cows that had lesions on their teats and udders. After repeated contact with sick animals, the patient noticed the development of skin lesions on his hands that appeared as nodular swellings and were itchy; the next day, he became febrile. A few days later, the nodular lesions became papules, and local edema appeared (figure 1). A total of 8 skin lesions on both of the patient’s hands were characterized as umbilicated pustules surrounded by inflamed tissue. Approximately 12 days after the initial appearance of the lesions, they turned into necrotic and painful ulcers; after a short time, all of the lesions developed scabs, most of which sloughed. 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the disease took ~4 weeks, and the patient receded from work during this time. Although he did not seek medical assistance, we provided treatment for his fever and pain. If the patient had developed secondary bacterial infection, he would have received topical or oral antibiotic treatment (other patients in the study required this treatment). We also applied occlusive bandages to the patient's lesions to avoid autoinoculation [2].

Immunological characteristics of the patient were also analyzed. He did not acknowledge a history of immunological disorders or any recent major infection or disease. Blood samples were obtained 5 days after the patient noticed the lesions (~10 days after onset of infection). Samples from healthy subjects also enrolled in the study were analyzed for comparison. Although the patient's hematocrit was apparently normal, there was a slight decrease in his level of antigen-presenting cell, including decreases in the numbers of B lymphocytes and macrophages. PBMCs were obtained by Ficoll diatrizoate density gradient centrifugation and were cultured in RPMI 1640 medium supplemented with 1.6% L-glutamine and 5% of AB Rh+ heat-inactivated normal human serum. As a viability control, cells were stimulated with 2.5 μg/mL of the PHA mitogen, and all samples proliferated similarly. Compared with PBMCs from healthy individuals, PBMCs from the infected patient showed low proliferative responses and low production of cytokines after stimulation with VACV antigens in vitro. The levels of IFN-γ production were surprisingly lower in the infected patient than in the uninfected control subjects (393 pg/mL vs. 1500 pg/mL) (figure 2); this was particularly notable, because high production of IFN-γ by CD4+ and CD8+ T lymphocytes is a hallmark of Orthopoxvirus infection [9].

Cytokine detection in supernatant of cultured PBMCs was evaluated by cytometric bead array immunoassay (Becton Dickinson), according to the manufacturer's protocols. The T, B, and natural killer cells and monocytes, as well as activation status, were quantified after in vitro antigenic stimulation of cultured PBMCs with use of mouse anti-human monoclonal

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**Figure 1.** Lesion patterns and evolution of a primary zoonotic vaccinia virus infection. A, Initial papule lesion at the base of the middle finger. B, Lesions a few days later. Papules evolved into pustules and ulcers with focal necrotic tissue. C, Detail of panel A. Areas of inflammation can be seen surrounding the lesions. D, Detail of panel B. E, Pustules and ulcers, which coincided with the appearance of peripheral lymphangitis on the patient's left arm. The whole progression of the disease, from initial papule to cicatrization, took 21–25 days.

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**Figure 2.** Levels of IFN-γ on the supernatant of cultured PBMCs that had been isolated from patient samples after in vitro stimulation with vaccinia virus antigens. PBMCs cultured in RPMI medium supplemented with 1.6% L-glutamine and 5% of normal human serum were stimulated with 1 × 10⁶ ultraviolet-inactivated vaccinia virus WR particles for 6 days. Supernatants were collected, and the amounts of IFN-γ were tested using a cytometric bead array immunoassay. NIPs, uninfected patients; IP, infected patient.
antibodies conjugated with fluorescein isothiocyanate or phycocerythrin specific for cell-surface markers, including CD3, CD4, CD8, CD14, CD19, CD25, CD28, CD69, CD80, and CD86, as described elsewhere [10]. The levels of CD8+ or CD4+ T lymphocytes in the infected patient and the control subjects were not different. However, the patient’s T lymphocytes showed lower expression of activation markers, such as CD25, after in vitro stimulation with VACV antigens than did T lymphocytes from the control subjects. That could be an indication of virus-induced down-modulation. In addition, humoral response was evaluated by an inhouse ELISA using whole VACV WR antigens and by virus neutralization tests in cell culture using the same VACV WR strain [11]. With both tests, no antibodies were detected; this could have been attributable to the early time of obtainment of a serum sample (~10 days after the onset of infection).

Outbreaks of disease that were similar to the one described here have been reported in Brazil since 1999 [5–8]. The progression of disease and the observed clinical signs in these Brazilian outbreaks were invariably similar to clinical descriptions of cowpox infection in Europe [2, 3]. The etiological agent associated with these outbreaks has been shown to be VACV, the virus used as vaccine to eradicate smallpox. The origin, route of transmission, and mechanisms of circulation of these zoonotic VACV infections are currently unknown.

To characterize the etiological agent that caused VACV infection in our patient, we collected fluid samples from suppurated lesions with use of a sterile syringe and needle; we were then able to isolate the virus in chorioallantoic membranes of embryonated chicken eggs [7]. The isolated virus was propagated in Vero cells, and extracted DNA was used as a template for PCR with primers targeting the hemagglutinin gene. Nucleotide sequencing of the gene was then performed; this method has been used previously for differentiation of VACV isolates obtained under similar circumstances [5–8]. The DNA sequence (GenBank accession number EF063677) was used to generate a consensus phylogenetic tree constructed by the neighbor-joining method with use of the Tamura-Nei model of nucleotide substitutions implemented in MEGA3. The isolate was grouped with VACV isolates from other outbreaks in Brazil and was coincident (100% identical) with Aracatuba virus (not shown), a VACV isolated from sick cows during an outbreak in São Paulo, Brazil, in 1999 [7].

Viruses in general and, specifically, poxviruses are capable of encoding a number of proteins that modulate immunological functions of the host. To correlate the immunological and phylogenetic findings, we searched the genome of the isolated virus for known poxvirus genes that could have been responsible for the apparent immune modulation found in our patient. Using PCR and nucleotide sequencing, we looked for the presence of the following VACV WR orthologous genes: E3L, which binds double-stranded RNA and blocks IFN production activation; K3L, which is an eIF-2α homolog and blocks IFN production; B18R, which is a soluble IFN-α/β receptor homolog; and B8R, which is a soluble IFN-γ receptor homolog [12]. All genes were present and potentially functional in the genome of this particular viral isolate. To date, it is impossible to indicate which gene and/or protein could have been responsible for the immune modulation found, and all searched genes have the potential to interfere with T and/or B cell activation and IFN functions. These genes have been found on the genomes of other VACV isolates from similar outbreaks, and full-scale genome sequencing of some of these samples will help to further evaluate their pathogenic determinants.

Difficulties involving the correct assessment of VACV infection in Brazil are numerous. Despite the fact that notification of exanthematic diseases affecting cattle and humans is mandatory as part of a foot-and-mouth disease surveillance program, cases such as the one described here are rarely reported. The lack of reporting occurs primarily because the disease affects small and poor rural communities in areas with precarious medical assistance that is difficult to access. When a case of VACV infection is reported to health care professionals, it is frequently mistaken for other infectious infirmities, such as leishmaniasis, mycosis, staphylococcal infection, or anthrax poisoning. Misdiagnosis leads to incorrect treatment being indicated by physicians at outbreak sites. Treatment recommended by these physicians frequently includes corticoids, and use of corticoids is usually associated with worsening of a patient’s condition.

The emergence of VACV has coincided with the increasing population of immunosuppressed individuals worldwide who are vulnerable to otherwise easily resolved infections. An example is a recent US case of severe eczema vaccinatum in a 28-month-old boy with a history of atopic dermatitis [13]. Data on the prevalence of atopic dermatitis or atopic eczema in rural areas of Brazil are nonexistent. However, in developing countries, drugs including vaccinia immunoglobulin and antipoxvirus compound, which were successfully used to treat the US patient with eczema vaccinatum, are not available.

In the present study, we report clinical findings in a patient who was naturally infected with VACV. We found that the patient’s specific immune response to the infection appeared to be down-modulated. Similar results have been obtained for many other individuals affected by the outbreaks of VACV infection and in a cohort study that is currently being conducted. The results of full genome sequencing of the virus isolated from the study patient may help to establish how the virus interacts with the host and how it caused the observed signs and symptoms (full genome sequencing of the virus isolated during this study is currently being performed through an international collaboration between Universidade Federal de
Minas Gerais and the Poxvirus and Rabies Branch of the Centers for Disease Control and Prevention). The increased incidence of Orthopoxvirus infections such as the one described in this article seems to be in direct proportion with the worldwide increase in the number of persons who have not been protected against smallpox because of the cessation of the vaccination program in the 1970s. In 2007, the World Health Organization celebrated the 30th anniversary of the successful end of the smallpox eradication campaign, and paradoxically, concerns have recently emerged that immunity against Orthopoxviruses may no longer be protective in the majority of the world population.

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