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Detection of Vaccinia Virus DNA, but not Infectious Virus, in the Blood of Smallpox Vaccine Recipients

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Abstract

The authors of a recent study suggested that the duration of deferral for blood donations by smallpox vaccinees should be extended, based on detection of vaccinia virus DNA in 5 blood samples by PCR and the potential for viremia. We found that 4 of 202 blood specimens (from 3 of 27 smallpox vaccinees) were positive for vaccinia virus DNA by PCR; none were positive for virus by culture. Throat swabs were negative by PCR and culture. Thus, while some blood specimens contained vaccinia virus DNA, infectious virus was not detected. Current guidelines for deferral of blood donation in vaccinees seem appropriate.

Keywords

smallpox vaccine; vaccinia virus; poxvirus

1. Introduction

While the United States discontinued routine childhood vaccination against smallpox in 1972, vaccination resumed in 2002 due to concerns about bioterrorism. Uncertainty about the infectiousness of vaccinia in vaccinees, and about when vaccine recipients can safely donate blood, has emphasized the importance of determining whether recipients can shed virus from the oropharynx and if blood contains infectious virus. Studies in the 1930's and 1950's using smallpox vaccine strains that were likely less attenuated than the current New York City Board of Health (NYCBOH) strain reported viremia (i.e. positive culture for virus in the blood) between the third and tenth day after vaccination [1]. Studies in the 1960's using the NYCBOH strain of vaccinia indicated that viremia is rare, and can occur in immunocompromised persons or in those with multiple cutaneous lesions, but not in those with only localized lesions [2,3]. Cummings et al studied vaccinees receiving the NYCBOH strain vaccinia (Dryvax) and in 2004 they reported no evidence of virus in the blood of serial samples from 28 volunteers (total of 220 specimens) by polymerase chain reaction (PCR), culture, or antigen detection [4]. Srinivasan and colleagues reported negative cultures from plasma and whole blood for vaccinia in 665 specimens from 95 vaccinees [5]. In 2006 Savona et al. detected about 1,000 copies of vaccinia genome DNA per milliliter in the blood by PCR in 5 of 77 vaccinees (5 of 125

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specimens) receiving Dryvax vaccinia; vaccinia cultures were not performed [6]. Vaccinia DNA was detected by PCR in the blood in 4 patients at 6 to 8 days after vaccination and in 1 patient at 20 to 22 days after vaccination. Based on their findings, the authors concluded that “our results suggest that until more data are available, extending the duration of deferral for blood donation would be appropriate.”

Early studies from Germany and Russia using smallpox vaccines that likely were more virulent than the NYCBOH strain reported detection of vaccinia in tonsillar swabs in some persons 3 to 5 days after vaccination [1]. Vaccinia was isolated from throat swabs in 7% of children with uncomplicated vaccinia without pharyngitis, and from the oropharynx of 49% of children with tonsillitis after vaccination. Klote et al. in 2005 evaluated throat swabs from 144 Dryvax vaccinees (801 specimens) and found no evidence of virus by PCR, culture, or antigen detection [7]. In contrast, Savona et al. detected virus DNA in throat swabs in 11 of 77 vaccinees (12 of 125 specimens) by PCR [6].

While vaccinia virus DNA has been detected by PCR in both blood and throat swabs in one study, cultures for the virus were not performed [6]. Since detection of viral DNA by PCR does not indicate that infectious virus is present, we tested blood and throat swabs from vaccinees by both PCR and shell vial culture for vaccinia virus.

2. Methods

2.1. Study population

We obtained serial blood and throat swabs from 28 consecutive employees at the National Institutes of Health vaccinated with the Dryvax smallpox vaccine (16 primary vaccinees and 12 revaccinees) from March 2003 to January 2007. Healthy employees at least 18 years old were vaccinated as a routine part of their employment either because they were laboratory workers who would be exposed to vaccinia virus, or health care workers who might be first responders in the event of a smallpox outbreak. The protocol was approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases and all vaccinees signed a written consent. Samples were obtained before and every other day after vaccination for the first two weeks and then at 1 month after vaccination for a total of 7-8 specimens per patient.

2.2. Vaccinia virus cultures and PCR

Whole blood was treated with ACK lysis buffer (Biosource, Rockville, MD) to lyse red blood cells; the remaining white blood cells were centrifuged, washed, sonicated, and applied to shell vial cultures containing RhMK cells. The specimens were centrifuged onto the cell monolayers followed by incubation for 18-24 or 48 hr and staining with fluorescein isothiocyanate conjugated rabbit antibody to vaccinia virus (Biodesign International, Saco, ME). The equivalent of 0.2 ml of whole blood was applied to each of two shell vial cultures. PCR from throat swabs and whole blood were performed as described previously [8]. One-fifth of the DNA extracted (equivalent to 0.04 ml of whole blood) was used for each PCR reaction. The lower limit of positive controls used to generate the standard curve was 125 copies/ml; the lower limit of detection of the PCR assay was 25 copies per ml. Samples were assayed as they were received.

3. Results

3.1. Vaccinia culture and PCR from blood

Shell vial culture of the blood was negative for vaccinia in serial cultures from 27 vaccinees (206 specimens). Three of the 27 vaccinees (11%) had low positive (<250 copies per ml) PCR

signals (4 of 202 blood specimens) (Table 1). Each of the PCR-positive blood samples had a shell vial culture that was negative from the same patient on the same day. The 4 positive PCRs were obtained on day 4 after vaccination in a revaccinee, day 9 in a primary vaccinee, and on days 9 and 11 in a revaccinee. Subsequent blood samples for PCR testing in each of these persons were negative. The time between the prior smallpox vaccination and revaccination was longer in the two revaccinees that had a vaccinia PCR-positive blood sample (mean 41 yrs, median age 41 yrs. between vaccinations) than in the 10 revaccinees that did not have positive blood tests (mean 30 yrs, median age 29 yrs).

3.2. Vaccinia culture and PCR from throat swabs

Shell vial cultures of throat swabs were negative for vaccinia in serial cultures of 28 vaccinees (213 specimens); all 28 vaccinees had throat swabs that were negative for vaccinia PCR (190 specimens).

4. Discussion

PCR was positive in 4 blood samples at low levels, and PCR was negative in throat swabs from all vaccinees. No smallpox vaccinees had detectable infectious virus based on cultures from the blood or throat, including blood at the same time points from the same patients which were PCR-positive. One recent study reported negative results for detection of vaccinia in the blood or throat using conventional culture and PCR [4], while another study that used PCR alone detected vaccinia virus DNA at both of these sites [6] (Table 2).

Our study differed from the other studies in the amount of material used for PCR. We used DNA equivalent to 0.04 ml of whole blood in each PCR reaction. We used fresh blood that was assayed at the same facility in which it was drawn. In contrast, in a study in which PCR from whole blood samples of all vaccinees was negative for vaccinia virus [4], the authors used DNA extracted from 0.001 ml of whole blood divided into each of two PCR reactions (J.F. Cummings and G.V. Ludwig, personal communication), and blood was transported to the testing site within 48 hours and then frozen before testing. Thus, our results may differ from those of the prior study that did not detect vaccinia virus DNA in blood [4], since we used 40-times the amount of blood for PCR when compared to the older study and we used fresh blood that had not been frozen and thawed. In another study that detected viral DNA in 5 blood specimens by PCR [6], the authors extracted 0.1 ml of DNA from 1 ml of plasma and used 0.005 ml of extract for each 20 μ l real-time PCR reaction (M. Savona, personal communication).

While we did detect vaccinia virus DNA in the blood by PCR in 4 specimens, all cultures of blood were negative using the shell vial assay. The amount of whole blood we used for culture (0.4 ml) was 10 times the amount of whole blood (0.04 ml) that was used in each PCR reaction. We cultured white blood cells, rather than plasma, since orthopoxviruses circulate in primates primarily within leukocytes in the blood [9]. Cell culture of vaccinia is more sensitive than infection of mice; in a study of severely immunodeficient mice, animals developed illness after inoculation with 10^6 plaque forming units (PFU) of vaccinia, but were asymptomatic with 10^5 PFU of virus [10]. Therefore culture of vaccinia is 100,000 times more sensitive than inoculation into immunodeficient mice. PCR is clearly a more sensitive test than culture for detection of vaccinia virus both for clinical specimens [8] and for cell culture specimens [11]. The latter study showed that PCR is about 100-fold more sensitive than culture for detection of orthopoxviruses. However, detection of poxvirus DNA by PCR does not indicate that infectious virus is present. A large proportion of vaccinia virus particles in cell culture apparently are not infectious. The ratio of vaccinia virus particles (observed by electron microscopy) to PFU (determined by infection in cell culture) for vaccinia virus is 1,500 to 1,900 particles per PFU [12].

Vaccinia virus can be cultured from the vaccination scabs and from the vaccine site until the scab spontaneously separates from the skin. The NYCBOH strain of vaccinia has been cultured from the blood of persons with moderate or severe complications of vaccinia [2,3]. Based on these findings, current recommendations for deferral of blood donors in recipients of smallpox vaccine state that persons without vaccine complications should be deferred from donating blood until after the vaccine scab has spontaneously separated or for 21 days after vaccination, whichever is longer [13]. Persons with complications of vaccination, such as generalized vaccinia, eczema vaccinatum, or progressive vaccinia, should defer from donating blood until 14 days after all vaccine complications have resolved completely. None of our patients had vaccine complications, and we did not detect vaccinia in blood by PCR beyond 11 days after vaccination. Therefore while our sample size was not large, deferring blood donation for a minimum of 21 days after vaccination in persons without vaccine complications seems adequate.

Our findings indicate that while some blood samples are weakly positive by PCR for vaccinia, they do not contain detectable infectious virus based on shell vial culture assays. We cannot exclude the possibility that a more sensitive culture assay might detect infectious virus in the PCR-positive samples. We conclude that infectious virus is rarely shed from the throat or present in the blood of healthy recipients of the Dryvax smallpox vaccine. These findings suggest that the risk of transmission of infectious vaccinia virus by blood transfusion is low and that current guidelines for blood donation in vaccinees [13] are likely to be adequate.

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Table 1
Results of vaccinia virus culture and PCR from smallpox vaccine recipients

Site	Test	No. Positive Specimens/Total No. Tested (%)	No. Positive Vaccinees/Total No. Tested (%)
Blood	Shell vial culture	0/206 (0)	0/27 (0) ¹
Blood	PCR	4/202 (2.0) ²	3/27 (11) ¹
Throat	Shell vial culture	0/213 (0)	0/28 (0)
Throat	PCR	0/190 (0) ³	0/28 (0)

¹One patient did not have blood tested for culture or PCR because sufficient blood was not available.

²All four blood PCR samples were positive at <125 copies/ml, and all 4 had concurrent blood cultures that were negative for vaccinia. While the sample clearly gave a positive signal, the lower limit of the standard curve in the assay was 125 copies.

³The first four consecutive vaccinees did not have some samples tested for PCR because they were not processed properly.

Table 2

Summary of prior studies since 2002 to detect vaccinia virus or viral DNA in the blood or throat

Site	Test	No. Specimens Positive/ No. Tested (%)	No. Vaccinees Positive/ No. Tested (%)	Reference
Blood ¹	PCR	0/220 (0%)	0/28 (0%)	[4]
Blood ²	PCR	5/125 (4%)	5/77 (6.5%)	[6]
Blood ¹	Culture	0/220 (0%)	0/28 (0%)	[4]
Blood, ^{1,2}	Culture	0/665 (0%)	0/95 (0%)	[5]
Blood, ¹	Antigen ³	0/220 (0%)	0/28 (0%)	[4]
Throat	PCR	12/125 (9.6%)	11/77 (14%)	[6]
Throat	PCR	0/801 (0%)	0/144 (0%)	[7]
Throat	Culture	0/801 (0%)	0/144 (0%)	[7]
Throat	Antigen ³	0/801 (0%)	0/144 (0%)	[7]

¹ whole blood² plasma³ antigen detected by electrochemiluminescence