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Varicella in Chimpanzees

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Two chimpanzees were inoculated subcutaneously with the wild-type Oka strain of varicella-zoster virus (VZV). Viral DNA was detected in peripheral blood mononuclear cells of both animals using the polymerase chain reaction (PCR) shortly after inoculation. Ten days after inoculation both animals developed an erythematous, papular rash near the site of inoculation that extended into the adjacent dermatome. Viral DNA was found by PCR in a skin biopsy from one of the animals at the time of the rash. While only two animals were studied, the development of a mild form of varicella in chimpanzees indicates that these animals might be useful for molecular studies of viral genes involved in virulence or attenuation of VZV. © 1996 Wiley-Liss, Inc.*

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INTRODUCTION

A live attenuated vaccine for varicella-zoster virus (VZV) has been used to vaccinate children in Asia and Europe and was recently approved for use in the United States. Wild-type Oka strain virus was isolated from a boy with typical chicken pox, passaged in cell culture, and subsequently shown to be attenuated in humans [Takahashi et al., 1975]. Most healthy children inoculated subcutaneously with the Oka vaccine virus do not have a rash but do develop humoral and cellular immunity to the virus. The biological basis for attenuation of the Oka vaccine strain is unknown. The development of an animal model which reproduces the signs and symptoms of varicella would be useful for studying attenuation of the live Oka vaccine virus.

Research on the pathogenesis of VZV has been limited due to the lack of animal models that reproduce the pattern of disease seen in humans. Inoculation of small animals, such as guinea pigs [Lowry et al., 1993] or rats [Debrus et al., 1995], with VZV results in viremia with spread of the virus to the central nervous system. While hairless guinea pigs have been reported to develop a papular rash [Myers et al., 1991], the animals do not develop vesicles, and reactivation of latent virus has never been reported. Recently, inoculation of human fetal skin implanted subcutaneously in severe combined immunodeficiency disease (SCID)-hu mice resulted in lesions in the epidermis that contained viral DNA [Moffat et al., 1995]. Infection of marmosets with VZV results in viral replication in the lungs with a mild transient pneumonitis and induction of VZV antibody, but the animals do not develop symptomatic disease [Provost et al., 1987]. Gorillas have been reported to develop vesicular lesions that contain VZV [Myers et al., 1987]. While other nonhuman primates can develop severe disease after infection with simian VZV-like viruses, these simian viruses are distinct from human VZV [Fletcher and Gray, 1993; Gray et al., 1992; Harbour and Caunt 1979]. Thus, there remains a need to identify an animal host in which VZV pathogenesis can be studied.

A number of observations suggest that the chimpanzee may be a suitable host for certain types of research study of VZV. Most chimpanzees raised in the wild have naturally acquired antibodies to VZV or to a virus that highly cross-reacts with VZV [Myers and Connelly, 1992]. Chimpanzees raised in captivity have developed vesicular lesions typical of chicken pox, and a herpesvirus has been detected in the lesions by electron microscopy; however, VZV-specific antibodies or nucleic acid has not been demonstrated [Heuschele, 1960; McClure and Keeling, 1971].

A chimpanzee herpesvirus (CZHV) was isolated from an animal with a varicella-like vesicular rash [McClure and Keeling, 1971]. While studies of the nucleic acid of CZHV have not been reported, gel precipitation tests indicate that CZHV shares at least one antigen with VZV but that several other antigens from VZV are not present in CZHV. CZHV is distinct from other primate VZV-like viruses and from herpes simplex virus types 1 and 2 [Harbour and Caunt, 1979].

One early attempt to infect a single chimpanzee with VZV did not result in disease [Felsenfeld and Schmidt, 1979]. Moreover, zoster (shingles) has never been ob-

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served in any animal infected with VZV. Nonetheless, new methods of studying and identifying VZV infection have been developed, making it reasonable to reexamine the potential of VZV to infect chimpanzees. As an initial step in studying pathogenesis, we inoculated two chimpanzees subcutaneously with the wild-type Oka strain of VZV and studied the animals for development of skin lesions, viremia, and VZV-specific antibodies.

MATERIALS AND METHODS

Wild-type Oka VZV (passage 6 in human embryonic lung cells, kindly provided by M. Takahashi [Takahashi, 1988]) was passaged twice in MRC-5 cells. Primates were individually housed under BL-2 biohazard containment. Chimpanzees 1442 and 88A04 were inoculated subcutaneously with MRC-5 cells containing 4×10^5 plaque-forming units (PFU) of wild-type Oka VZV. The left breast of each animal was shaved and the inoculum injected below the nipple (T4 dermatome). At serial time points (every 1–3 days) animals were anesthetized, temperatures were recorded, blood was obtained, and animals were inspected for lesions. A biopsy of a rash was performed in one animal.

Peripheral blood mononuclear cells (PBMC), obtained from 5 ml of whole blood, were separated using Ficoll-Hypaque. One aliquot of PBMC was frozen at −20°C for polymerase chain reaction (PCR), and one aliquot (about 10^6 cells) was immediately used to infect duplicate wells containing whole human fibroblast cells (WHF; BioWhittaker, Walkersville, MD). WHF cells were incubated with PBMC overnight; the next morning, WHF cells were washed with phosphate-buffered saline and fresh media was added. One week later, infected cells were passaged onto fresh WHF cells, and the cells were observed for 6 weeks for cytopathic effects.

For PCR, PBMC or skin biopsies were resuspended in lysis buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 0.001% Triton X-100, 0.0001% SDS) and incubated with proteinase K (600 μg/ml) at 56°C, and nucleic acids were extracted with phenol and precipitated in ethanol. PCR was performed using VZV primers TK4 and TK5, which amplify a region of VZV ORF31 [Lowry et al., 1993]. Amplified products were electrophoresed on a 3:1 Nusieve gel (FMC Bioproducts, Rockland, ME), transferred to a nylon membrane, and hybridized to a radioactive probe corresponding to the relevant portion of VZV ORF31.

Serum was assayed for antibody to VZV using an indirect immunofluorescent assay. Serial dilutions of serum were applied to VZV-infected cells on glass slides, washed, incubated with goat anti-human polyvalent immunoglobulin, and examined by fluorescence microscopy. Serum was assayed for CZHV using a dot-blot assay [Heberling and Kalter, 1986]. White blood cell counts and serum alanine aminotransferase and gamma-glutamyltransferase were determined.

RESULTS

PBMC obtained 1, 6, and 11 days after inoculation of chimpanzee 1442 contained VZV DNA by PCR, and cells obtained 2 days after inoculation in chimpanzee 88A04 were positive for VZV DNA by PCR. Seven days after inoculation, both animals developed a leukocytosis, and 9 days after inoculation chimpanzee 1442 developed a fever of 100.3°F. Ten days after inoculation, both animals developed a rash bilaterally over the breasts (involving dermatomes T4 and T5). The rash was erythematous and papular but not vesicular or pustular, and no mucosal lesions were noted (Fig. 1). A careful examination of the skin did not show lesions at any other site. The rash completely resolved in chimpanzee 88A04 by day 11 and in chimpanzee 1442 by day 12. On day 11, PCR of a skin biopsy from chimpanzee 1442 was positive for VZV DNA. Liver function tests were unchanged during the first 3 weeks after inoculation. VZV serology in chimpanzees rose from <1:4 preinoculation to 1:16 (chimpanzee 1442) and 1:32 (chimpanzee 88A04) at 8 weeks postinoculation. Titers to CZHV rose from <1:10 preinoculation to 1:10 9 weeks postinoculation for chimpanzee 1442 and were 1:80 both preinoculation and postinoculation for chimpanzee 88A04. All cultures of PBMC and skin biopsies were negative.

DISCUSSION

After subcutaneous inoculation with wild-type VZV, two chimpanzees became viremic and developed a papular rash. The rash was located near the site of inoculation and extended into an adjacent dermatome but did not disseminate farther. This verifies that the animals were infected but that the infection was mild and not characteristic of classic varicella.

The infection observed in the chimpanzees after injection with the wild-type Oka virus shares some features with the mild eruption seen in healthy children after inoculation with the attenuated Oka vaccine virus. About 4% of vaccinated children develop a papular rash that is frequently located at the site of inoculation [Weible et al., 1985]. In nonimmunocompromised hosts, it has been exceedingly difficult to culture the virus from these lesions. It is uncertain as to whether the papular nature of the rash and its localization near the site of inoculation in the animals were due to passage of the virus in cell culture, the route of inoculation, or some feature peculiar to chimpanzees. The location of the rash (present in two dermatomes) and the site that was biopsied for PCR (located several centimeters from the inoculation site) suggest that the viral DNA detected by PCR was due to hematogenous spread to the skin and not to residual DNA from the inoculation.

In a prior study, a chimpanzee inoculated with cell-free VZV by both the intratracheal and subcutaneous routes failed to develop a rash [Felsenfeld and Schmidt, 1979]. The strain used in the study (CaQu) was different from that used in our study and the virus had been passaged more times in cell culture than our virus. The transient and very localized nature of the rash in our animals (lasting 24–48 hr) indicates that animals must be closely examined on a daily basis after inoculation.

Due to the mild disease associated with varicella infection in the chimpanzees, sera from both animals were tested retrospectively for antibodies to CZHV. One animal that was seronegative before inoculation had a rise

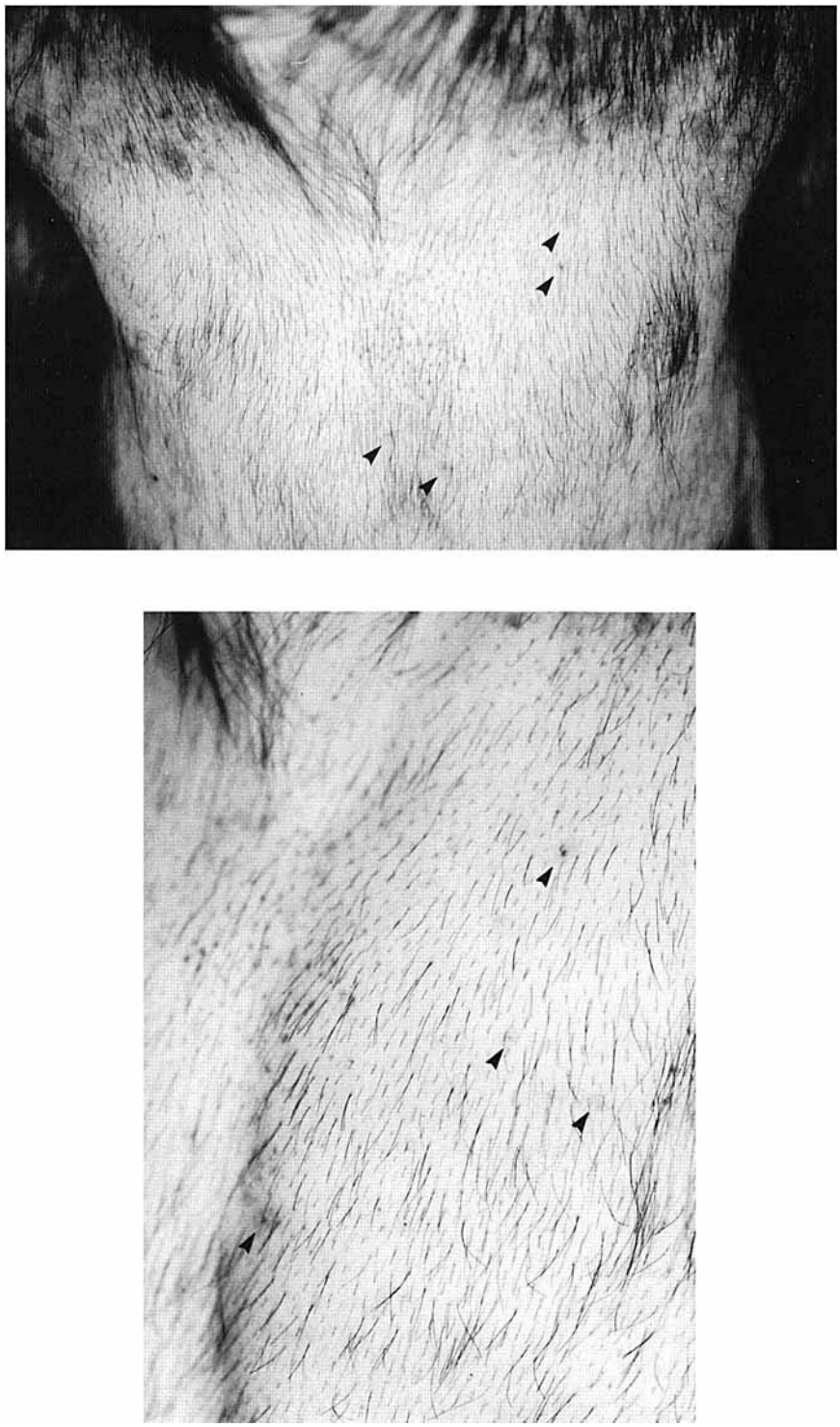

Fig. 1. Rash in chimpanzee 1442 11 days after inoculation with VZV. Arrows indicate lesions.

in antibody titer of one tube dilution. While few data are available for serologic responses to CZHV, the change in titer was less than the four-fold increase generally associated with seroconversion for most viral infections. A second animal, seropositive for CZHV prior to inoculation, had no change in titer after inoculation. Since the disease in both chimpanzees was similar, it is unlikely that preexisting antibody to CZHV in the second animal protected this animal from severe disease with VZV.

This study shows that chimpanzees develop a mild form of varicella after inoculation with VZV. The chimpanzee model would not be useful for studying herpes

zoster due to the inability of obtaining tissue from the central nervous system to study latency. At present, the only other animal models that reproduce some of the features of varicella with a rash are the SCID-hu mouse and hairless guinea pig. While the SCID-hu mouse does develop lesions resembling chicken pox after inoculation of human fetal skin implants with VZV, the model is limited since the human implants are directly injected with virus rather than infected during viremia [Moffat et al., 1995]. The hairless guinea pig model can be used to study pathogenesis; however, the nonspecific dermatitis that develops in some sham-infected animals and the varying frequency of rash in different experiments have detracted from the usefulness of the model [Myers and Connelly, 1992]. While chimpanzees are limited and represent an expensive model, the development of a rash in both animals described here suggests that small numbers of animals might be used for very specific studies.

The molecular basis for attenuation of the Oka vaccine strain of VZV is unknown. Development of the attenuated Oka vaccine required initial testing in humans, due to the lack of an animal model for varicella [Takahashi et al., 1975]. The development of a mild form of varicella in chimpanzees after inoculation with wild-type virus may be useful for testing future VZV vaccines. In addition, the recent development of molecular techniques to manipulate the VZV genome [Cohen and Seidel, 1993] enables one to construct VZV mutants that might be useful for studying viral genes involved in virulence or attenuation of VZV.

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