Detection of Molluscum Contagiosum Virus (MCV) DNA in the Plasma of an Immunocompromised Patient and Possible Reduction of MCV DNA With CMX-001

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Molluscum contagiosum virus (MCV) is a poxvirus that causes localized papules in healthy persons. We evaluated a woman with severe immunodeficiency and disseminated MCV. During treatment with CMX-001, an antiviral with activity against other poxviruses, MCV DNA was detected in 20% of plasma samples. When the patient was not receiving CMX-001, MCV DNA was detected in 50% of samples. We also noted improvement in warts on her fingers during CMX-001 therapy. Although MCV is caused by direct inoculation of virus into skin in healthy persons, in a severely immunocompromised person MCV DNA was present in blood and may spread by viremia.

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Molluscum contagiosum virus (MCV) is a molluscipoxvirus that causes papules with umbilicated centers [1]. Lesions are often located on the chest or proximal extremities; however, they can be found anywhere on the body except the palms and soles. In healthy persons, skin lesions are usually a few millimeters in diameter, are present in groups of <100 lesions, and typically resolve without therapy after several months.

In patients with impaired T-cell immunity, MCV can affect large portions of the body, and large lesions, up to 1 centimeter or larger, may be present. Treatment of these patients is often challenging and includes curettage, podophyllotoxin, topical imiquimod, topical cidofovir, and systemic interferon-α [1, 2]. MCV infection is thought to spread by inoculation of virus into breaks in the skin by person-to-person transmission, by fomites, or by autoinoculation from scratching. MCV is not thought to spread in the blood, and detection of viral DNA in blood has not been reported.

We evaluated a patient with severe T-cell immunodeficiency due to dedicator of cytokinesis 8 protein (DOCK8) deficiency [3] and widespread cutaneous involvement with MCV. We found MCV DNA in the patient’s plasma when she was not receiving CMX-001, a lipid–conjugated form of cidofovir that has activity against other poxviruses (reviewed in [4]). MCV DNA was found in 4 of 11 plasma samples but in only 1 of 10 samples of peripheral blood mononuclear cells (PBMCs).

MATERIALS AND METHODS

Informed consent was obtained from 4 patients with MCV and 14 healthy controls at the National Institutes of Health Clinical Center on protocols approved by the institutional review boards of the National Institute of Allergy and Infectious Diseases (NIAID) and the National Cancer Institute. Citrated blood samples were collected, and PBMCs and plasma were separated by Ficoll-Hypaque gradient centrifugation and stored in vapor-phase liquid nitrogen.

Virus present in the 1.5-mL plasma or 1.0-mL PMBC samples (containing 1–5 million cells) was isolated by centrifugation at 14 540 g for 2 hours at 4°C. The pellet was resuspended in phosphate-buffered saline; carrier RNA was added at a concentration 10 μg/mL; and DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen). Quantitative real-time polymerase chain reaction (qPCR) was performed using primers and probes to the MCV p43K gene, as reported elsewhere [5]. Reaction mixtures contained 1X TaqMan Universal Master Mix (Applied Biosystems), 5'- and 3'-p43K primers at a concentration of 0.4 μmol/L, p43K-probe at a concentration of 0.2 μmol/L, and 12 μL of template DNA in a total volume of 25 μL. All amplifications were performed in duplicate. Reactions were done using an ABI 7500 real-time PCR system (Applied Biosystems) with the following conditions: 50°C for 2 minutes, 95°C for
10 minutes, 40 cycles at 95°C for 20 seconds, and 60°C for 1 minute. A standard curve consisting of 10-fold serial dilutions of MCV BamHI-J plasmid (a gift from Bernard Moss, NIAID) from 5 to 50 000 copies along with 100 ng of carrier RNA (Qiagen) was included for each set of qPCR assays to quantify the MCV DNA copy number present in patient plasma or PBMCs. The assay detects a minimum of 5 copies of MCV DNA in each 25-μL reaction in ≥1 replicate. All samples with ≥1 positive value by qPCR were reported as positive.

Nested PCR was performed using primers that correspond to a different portion of the MCV genome than that used for qPCR. The forward primer for the first reaction (5’-CCGATCTTTGC GAGGGTGTT-3’) was derived from Thompson [6], and the reverse primer for the first reaction (5’-CGTGAACTGTGCT GCATTG-3’) was designed to yield a 195–base pair PCR product. The first reaction mixture consisted of 5–10 μL of viral DNA template isolated from the patient or control plasma or PBMCs, 0.4 μmol/L primers, 2.5 mmol/L MgCl2, 1 mmol/L dNTPs, and 1 μL of Taq DNA polymerase (Invitrogen) in a total of 25 μL. The PCR conditions included denaturation at 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, 56°C for 1 minute, 72°C for 1 minute, a final extension of 72°C for 7 minutes, and then cooling to 4°C. The second reaction was carried out using 2.5 μL of the first-round PCR product as the template, along with primers 5’-CCTCGCAGTAGGGGCTCCTC-3’ and 5’-CGTGTTCTCGAAAGACCTC-3’. The remainder of the reaction mixture and amplification conditions were the same as those for the first round of PCR. MCV DNA isolated from a skin lesion was used as a positive control in the PCR amplification. The amplified DNA was run on a 2% low-melting-point agarose gel, and DNA was isolated from the gel and sequenced. The DNA sequence was aligned to GenBank sequences using the National Center for Biotechnology Information BLAST (Basic Local Alignment Search Tool) program.

RESULTS

A 21-year-old woman presented to the National Institutes of Health Clinical Center with DOCK8 deficiency, human papillomavirus (HPV) verrucous lesions on the fingers, and disseminated MCV involving the face, neck, axillae, abdomen, and extremities (Figure 1). She had an immunodeficiency syndrome, diagnosed in infancy, and had a history of eczematous dermatitis, recurrent herpes zoster, recurrent pneumonia, sinusitis, and skin and soft-tissue infections. At the time of her evaluation for treatment of disseminated MCV, she also had extensive HPV lesions on her fingers, chronic sinusitis, eosinophilic esophagitis, and bronchitis due to Pseudomonas aeruginosa. Her medications included intravenous immunoglobulin, acyclovir, atovaquone,

Figure 1. Molluscum contagiosum virus (MCV) papules on the forehead (A), neck (B), dorsum of the hands and proximal fingers (C) (with human papillomavirus verrucous lesions on the distal fingers), and abdomen (D) in a patient with DOCK8 deficiency and MCV DNA in the blood.
levofloxacin, and prednisone (20 mg/d). Interferon-α (2 million units 3 times weekly) had been given for her disseminated MCV and warts but was discontinued because of intolerance.

Owing to the severity of the patient’s MCV and HPV infections, compassionate-use CMX-001 treatment was begun. Although CMX has activity against several poxviruses (reviewed in [4]), it is unknown whether CMX-001 also inhibits MCV, because MCV cannot be grown in cell culture. CMX-001 was given orally at 2 mg/kg for 1 dose and then 1 mg/kg each week thereafter (Figure 2). At week 3 the patient was found to have pneumonia due to *Histoplasmosis capsulatum*, and posaconazole treatment was begun.

After 4 weeks, CMX-001 was withheld because of elevated serum transaminase levels. At this time, several MCV lesions on the patient’s face appeared to have improved, whereas other MCV lesions appeared slightly worse. In addition, the warts on the patient’s fingers were clearly improved, and she could remove a ring from her finger for the first time in many months. Her serum transaminase levels returned to near normal, and CMX-001 treatment was restarted at 1 mg/kg at week 6, increased to 2 mg/kg at week 7, and then withheld again at week 8 because of elevated serum transaminase levels. The drug was given again at 1 mg/kg during weeks 9–11, after these levels had improved. It was then discontinued at week 12 because of recurrent serum transaminase elevations; at this time, the MCV and the warts had not improved further.

The patient’s serum transaminase levels returned to normal, and she subsequently underwent nonmyeloablative double umbilical cord blood transplantation for treatment of her immunodeficiency. Although her MCV and HPV lesions markedly improved after transplantation, engraftment failed, several infections developed, including a fungal pneumonia, and the patient died during induction chemotherapy for a second transplant. At autopsy, MCV was detected in the skin but not in other tissues.

PBMCs and plasma were obtained from the patient, and from 3 other patients with MCV infection. Two of these patients had DOCK8 deficiency and eczematous dermatitis; one had disseminated MCV, and the other had localized MCV and a few warts on the toes. The third patient had common variable immunodeficiency, eczematous dermatitis, and localized MCV. PBMCs and plasma samples from the patients and from 14 healthy persons were assayed for MCV DNA by qPCR.

The persons performing the PCR analysis were blinded to the identity of the samples. MCV DNA was detected in 1 of 5 plasma samples (20%) obtained from the index patient with DOCK8 deficiency during CMX-001 therapy and in 3 of 6 (50%) obtained when she was not receiving CMX-001 (Figure 2). The MCV DNA levels ranged from 15 to 58 copies/mL of plasma.

To verify that the qPCR actually detected MCV DNA, we performed nested PCR on plasma with primers that differed from those used for qPCR. Sequencing of the PCR product obtained from nested PCR showed 100% identity to nucleotides 5279–5375 of MCV subtype 1 [7].

PBMCs were tested for all but 1 of the 11 time points from the index patient and were positive for MCV DNA only in the sample at week 15. Results of PCR for MCV DNA from plasma and PBMCs from the other 3 patients with MCV infection were negative, as were results for plasma from 14 and PBMCs from 5 healthy controls.

**DISCUSSION**

We found MCV DNA in several plasma samples collected on different days from a patient with disseminated MCV infection. Because MCV cannot be grown in cell culture [8], we could not ascertain whether infectious virus was present in the samples.

Viremia, with detection of infectious virus in blood, has been reported in patients with smallpox [9] and in early studies with smallpox vaccine that was less attenuated than the current New York City Board of Health strain [10]. Vaccinia DNA, but not infectious virus, was detected in the blood of vaccine recipients receiving the Dryvax formulation of vaccinia [11]. Monkeypox and cowpox DNA, but not infectious virus, has been detected in the blood of persons infected with these viruses [12, 13].

Prior investigators have not reported MCV (a molluscipoxvirus) or MCV DNA in the blood. However, orthopoxviruses circulate in PBMCs, rather than in plasma in nonhuman primates [14]. Because we detected MCV in 4 of 11 plasma specimens (36%) but in only 1 of 10 PBMC specimens (10%),

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Time course of treatment with CMX-001 and detection of molluscum contagiosum virus in plasma and peripheral blood mononuclear cells (PBMCs) in a patient with DOCK8 deficiency. Numbers next to plasma and PBMCs indicate molluscum contagiosum virus (MCV) DNA levels (copies per milliliter); dashes, undetectable MCV DNA. Abbreviation: ND, not done.
MCV may circulate preferentially in the plasma in severely immunocompromised patients with disseminated MCV lesions. Unlike orthopoxviruses that infect multiple tissues, MCV has only been detected in the skin; the observation that MCV DNA was found preferentially in the plasma may be related to release of virus directly from the skin into the bloodstream. Thus, the pathogenesis and spread of molluscipoxviruses are probably different from those of orthopoxviruses.

Our patient was not treated long enough to determine whether CMX-001 had efficacy against her MCV infection; however, her HPV infection clearly improved clinically while she was taking the drug. Interestingly, MCV DNA was detected in only 1 of 5 samples obtained while the patient was receiving CMX-001 but was detected in 3 consecutive samples after CMX-001 was discontinued. CMX-001 is a lipid conjugate of cidofovir and has antiviral activity against a large number of double-stranded DNA viruses, including variola, cowpox, and vaccinia [4]. The lipid moiety improves the bioavailability of CMX-001 such that it is administered orally and is taken up rapidly by cells, where the lipid molecule is cleaved off and intracellular cidofovir is converted to cidofovir diphosphate by cellular kinases. The latter is incorporated into viral DNA, and viral DNA synthesis is impaired. Although CMX-001 has been used to treat a patient with disseminated vaccinia who recovered from the infection, the concurrent use of other antivirals and the recovery of his lymphocyte count made it uncertain that CMX-001 was responsible for the improvement [15].

In summary, for the first time we have detected MCV DNA in the blood of a patient with widely disseminated MCV disease. MCV DNA was detected in 4 plasma samples from different days but in only 1 PBMC sample (obtained the same day as a positive plasma sample). Viral DNA was detected in 20% of plasma samples obtained while the patient was receiving CMX-001 and in 50% of samples obtained while she was not receiving the drug. Thus, CMX-001 may have activity against MCV.

**Notes**

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