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Prolonged mpox disease in people with advanced HIV: characterization of mpox skin lesions

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Abstract

We report three complicated and prolonged cases of mpox in people with advanced HIV not on antiretroviral therapy (ART) at mpox diagnosis. Multiple medical countermeasures were used, including prolonged tecovirimat treatment and immune optimization with ART initiation. Immunofluorescence of skin biopsies demonstrated a dense immune infiltrate of predominantly myeloid and CD8+ T-cells, with a strong type-I interferon local response. RNAscope detected abundant replication of monkeypox virus (MPXV) in epithelial cells and dendritic cells. These data suggest that prolonged mpox in people with advanced HIV may be due to ongoing MPXV replication, warranting aggressive medical countermeasures and immune optimization.

Keywords

Mpox; Monkeypox Virus; HIV; AIDS; Orthopoxvirus

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BACKGROUND

Monkeypox virus (MPXV) is in the genus *Orthopoxvirus* and is related to *Variola virus* (the virus that causes smallpox), and the causative agent of the multinational outbreak during 2022–2023. Although most mpox cases are self-limited and recover with supportive care, severe and fatal cases occur in persons with significant immunosuppression, most commonly advanced HIV infection. [1–5] Case descriptions include disseminated, coalescing, necrotizing lesions; involvement of multiple organ systems; and severe sepsis physiology. [1, 2] Disease can be protracted and paradoxical worsening after antiretroviral therapy (ART) initiation has raised concern for possible immune reconstitution inflammatory syndrome (IRIS), a dysregulated T-cell driven phenomenon. [2]

Management of severe, prolonged illness among patients with mpox and advanced HIV is challenging as it is unclear to what extent the disease is driven by virologic or inflammatory processes. Steroids, which are used to treat IRIS, are associated with severe illness and even death in animal models. [5, 6] Current recommendations for treatment of severe mpox emphasize early immune function optimization (e.g., ART for people with HIV) and early treatment with medical countermeasures active against orthopoxviruses (e.g., tecovirimat, brincidofovir, vaccinia immune globulin intravenous [VIGIV]). [5] To better understand disease pathogenesis in patients with advanced HIV, we evaluated tissues from skin biopsies and performed immunofluorescence and in situ hybridization (ISH) for MPXV mRNA to evaluate the distribution of virus in tissues and the local immune response.

METHODS

Study participants

Formalin-fixed, paraffin-embedded tissue samples were collected from six patients with HIV not on ART: three with PCR-confirmed MPXV infection (CD4 counts of 127, 7, and <20 cells/μL) and three without mpox (CD4 counts of 7, 44, and 248 cells/μL) participating in unrelated longitudinal observational cohort studies of IRIS in persons with advanced HIV (NCT00286767 and NCT02147405). These controls presented with bacterial, molluscum contagiosum, or Kaposi's sarcoma-associated herpesvirus (KHSV) co-infections and tested negative for MPXV; biopsies were obtained at similar intervals (46–126 days) post-ART initiation as case samples. All patients signed informed consent before tissue biopsy, which were performed per the judgment of treating clinicians, and informed of publication of results as case reports. All samples were collected for routine clinical care. Case reporting activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy.§

In situ hybridization (ISH): rnascope

Next-generation ISH was performed on skin biopsy sections as previously described. [7] Briefly, following antigen retrieval and protease treatment, the slides were incubated for two hours at 40°C with MPXV probe (Targeted region: 2–1292-ACD-534678). [8, 9]

§ See e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq

Amplification reagents from the RNAscope 2.5 HD Brown Detection Kits were used for Tyramide Signal Amplification Plus Cy3.5 immunofluorescence detection. To identify the cells harboring viral messenger RNA (vmRNA), we combined RNAscope detection (TSA Plus Cy3.5) with immunofluorescence to detect different cell markers: CD3, DC-SIGN, Pan-Cytokeratin, CD20, CD1a, CD68/163, CD14, CD11b. Those cell markers allowed us to identify B, myeloid, natural killer, Langerhans and epithelial cells, and potential targets for MPXV. Slides were washed, incubated with Alexa secondary antibodies (ThermoFisher Scientific) for one hour at room temperature, and washed in TBS-Tween (0.05% v/v). Slides were counterstained with DAPI, washed, and cover slipped. Representative pictures were taken using confocal microscope FluoView FV10i.

Immunofluorescence staining and image analysis

To assess the local immune response, we performed multi-plex immunofluorescence (IFA) staining targeting the following cell types and immune markers: CD8, CD68/CD163 combo, CD4, CD207, MPO (myeloperoxidase), IL-17, IL-18, Myxovirus resistance protein A (MxA), and interferon-gamma (IFNγ). Alexa secondary antibodies were used to visualize the different markers. All slides were scanned using the Fusion (AKOYA) microscope. Image analysis was performed on markers of interest to detect differences between cases and controls.

Scanned immunofluorescence slides were annotated and analyzed with QuPath software. [10] In representative regions showing dermis and epidermis from skin biopsies, we measured the positive pixel percentage of five markers: CD8, MxA, CD4, and CD68/CD163.

RESULTS

Mpox case presentations

Patient 1—A mid-60's year old male with HIV (CD4 127 cells/μL) presented with a diffuse rash 10 days after a sexual encounter (Supplementary Figure 1). He was diagnosed with mpox and received a 14-day course of oral tecovirimat and re-initiated ART (Table 1). He returned 42 days post-diagnosis with new foot and leg lesions and worsening facial lesions. Knee lesion cultures grew group A *Streptococcus* and *Staphylococcus aureus* and swab tested PCR positive for MPXV. Biopsy of this lesion demonstrated histopathologic features consistent with mpox. He received a second 14-day course of oral tecovirimat. At 92 days post-diagnosis, the patient received a third 14-day course of oral tecovirimat for new lesions on his legs and persistent scrotal lesions. After a brief loss to follow-up, the patient returned 224 days post-diagnosis with all lesions resolved.

Patient 2—A late-20's year old male with HIV (CD4 7 cells/μL) presented with diffuse necrotic skin and oropharyngeal lesions and difficulty swallowing. He had been diagnosed with mpox 84 days prior to admission and started on oral tecovirimat but was lost to follow-up. During hospitalization, he underwent biopsies of the right foot and left arm to rule out malignancy. He received ART, a 110-day course of oral tecovirimat, three doses of VIGIV, and one 14-day course of brincidofovir. His lesions showed minimal improvement and remained PCR positive for MPXV throughout his hospitalization. He

developed complications including renal failure requiring dialysis and multiple episodes of sepsis due to bacteremia and fungemia. He passed away on hospital day 202. Post-mortem autopsy results showed MPXV antigen by IHC in lung and skin tissue.

Patient 3—A late-30s year old male with HIV (CD4 <20 cells/μL) presented with diffuse rash and penile lesions within 10 days of receiving his first dose of JYNNEOS vaccine. He was diagnosed with mpox and initiated on tecovirimat and ART. His clinical course through two hospitalizations was complicated by urinary retention necessitating a suprapubic catheter, mpox-associated abscesses, and digital osteomyelitis. A shoulder lesion biopsy performed 84 days after initial evaluation was PCR positive for MPX. He was discharged from the hospital four months into his illness; in total, therapy included 6 months oral/intravenous tecovirimat, 3 months of intravenous cidofovir, 2.5 months topical cidifovir, and four doses of VIGIV. Lesions fully resolved 170 days after symptom onset.

Immunostaining

In samples from people with mpox, we observed a higher density of CD8+ cells and myeloid cells within dermal infiltrates than among control samples (Figure 1A-D). In case lesion samples, CD4+ cell numbers were low. Langerhans cells were only found in the healthy epithelium of the lesion and zero to few B cells were detected. To characterize the type of immune response in the lesions, we assessed the expression of MxA (regulated by type I interferon) and IFNγ (type II interferon response marker). We observed a high expression of MxA indicating that type I interferon response was the main antiviral response present in the skin lesions (Figure 1C). Expression of IL-17 and IL-18 were detected at low levels in all biopsies (data not shown). Neutrophil infiltration, detected by surrogate marker MPO, was detected at lesion sites.

Assessment with combined ISH (RNAscope) and immunofluorescence revealed high levels of active replicative MPXV within lesion biopsies mainly localized to the epidermis, upper dermis, and radicular sheaths of hair follicles. No MPXV was observed in the sebaceous glands or the hypodermis stroma (Figure 1E). Acute necrosis and ulceration of the epidermis was observed at sites of viral replication. We identified the MPXV-infected cells as epithelial cells by their location, shape, and cytokeratin staining, and as dendritic cells by their expression of DC-SIGN, CD8, and CD1a (Figure 1E-H). HIV-B genome was not detected via RNAscope; however, it is important to note the low number of CD4+ T-cells present in the skin lesions.

DISCUSSION

In tissue biopsy samples from three patients with prolonged mpox illness and advanced HIV, high concentrations of MPXV were found in skin lesions and hair follicles. No MPXV-infected B cells were detected. These data suggest that prolonged mpox disease in people with advanced HIV is likely due to direct MPXV infection and ongoing replication, which may progress despite the use of currently available medical countermeasures.

Evidence suggests that B and T lymphocytes are critical to controlling MPXV and other orthopoxvirus infections. [11, 12] In orthopoxvirus infections, cytolytic CD4+ and

CD8+ T-cells eradicate virus-infected monocytes and can minimize viral dissemination. [11] Animal model data indicate that CD4+ T-cell depletion before MPXV challenge reduces the development of protective B-cell and antibody responses and is associated with increased disease severity. [11, 13] HIV infection can mimic this effect through CD4+ T-cell deficiency with dysfunctional follicular helper T-cell support for B-cell maturation and antibody production and impairment of cytotoxic T-cells. [14]

In situ analysis of the skin lesions allowed us to establish localization, abundance, and type of immune cells present in the lesion material. Within the dermis and epidermis, infiltration of neutrophils (MPO+) in clusters and low numbers of CD4+ T-cells were observed. Within the upper dermis, right under infected epithelial cells, we detected a high density of myeloid and CD8+ cells (mainly dendritic cells and CD8+ T-cells). At sites of infection, recruitment, infiltration, and localization of these cell types may restrict lesion size by preventing viral spread. Additionally, within the mpox lesions, exclusively type 1 (MxA) interferon markers were expressed, suggesting a strong local innate immune response. [15] Despite the large number of CD8+ cells, neutrophil and myeloid infiltration, and an important type 1 IFN immune response, the level of MPXV-infected cells was high. Few B cells were present, and Langerhans cells were low or absent in lesion areas but were present within normal epidermis. Although we did not assess the functionality (killing and phagocytosing capabilities) of the immune cells present in lesions, findings indicate that the local immune response was insufficient to control MPXV replication.

Using RNAscope, we detected a remarkable number of cells harboring MPXV mRNA within the dermis and epidermis, as well as free virion within healthy area of the epithelium, suggesting that MPXV can be found outside of the lesions, as previously demonstrated during MPXV animal studies and in fatal mpox cases. [11, 16] In addition, two types of cells infected by MPXV were characterized: one identified as dendritic cells by DC-SIGN and CD1a expression, and the other identified as epithelial cells based on their localization and expression of the Pan-cytokeratin marker. MPXV-infected B cells were not detected. A high concentration of MPXV mRNA was found in the hair follicles, specifically in the external radicular sheath. These findings suggest the need for more aggressive antiviral approaches targeting viral replication and enhancing immune control mechanisms.

Despite extensive local MPXV infection and the presence of robust immune infiltrates, biopsy samples exhibited a scarcity of CD4+ T-cells, an observation consistent with depletion of circulating CD4+ T-cells due to advanced HIV. In immunocompetent patients with mpox, early expansion of activated effector CD4+ and CD8+ T-cells and a poxvirus specific Th1 cell response might protect against MPXV spread, persistence, and mutation. [15, 17] Additionally, intra-host MPXV genomic variability has been observed among patients with severe immunodeficiency that exhibited prolonged viral shedding. [17] In the United States, all 26 patients with confirmed tecovirimat resistance had HIV infection; among those with available data, all had low CD4+ T-cell counts. [18] Advanced HIV infection might favor viral persistence, compartmentalization, and the development of MPXV genomic mutations including those that may confer resistance to tecovirimat. [17, 18]

Limitations of our analysis include the small number of people and biopsy sites studied which may skew our findings, as well as the lack of additional controls including immune intact people with mpox who are unlikely to get skin biopsies for clinical purposes. Further evaluations are ongoing in the Virologic and Immunologic Characteristics of Severe Mpox Among Persons with Advanced HIV (VIRISMAP) study. [19]

In summary, we report large amounts of MPXV and type I interferon, with significant skin infiltration by innate immune cells such as dendritic cells, in patients with HIV and persistent mpox skin lesions. The lack of CD4+ T-cells and IFNγ in skin lesions indicates a lack of robust adaptive immune response in these patients. These findings highlight the need for antiviral treatment optimization in people with defective T-cell responses and suggest that IRIS might not be a significant component in persistent skin lesions of protracted mpox in persons with advanced HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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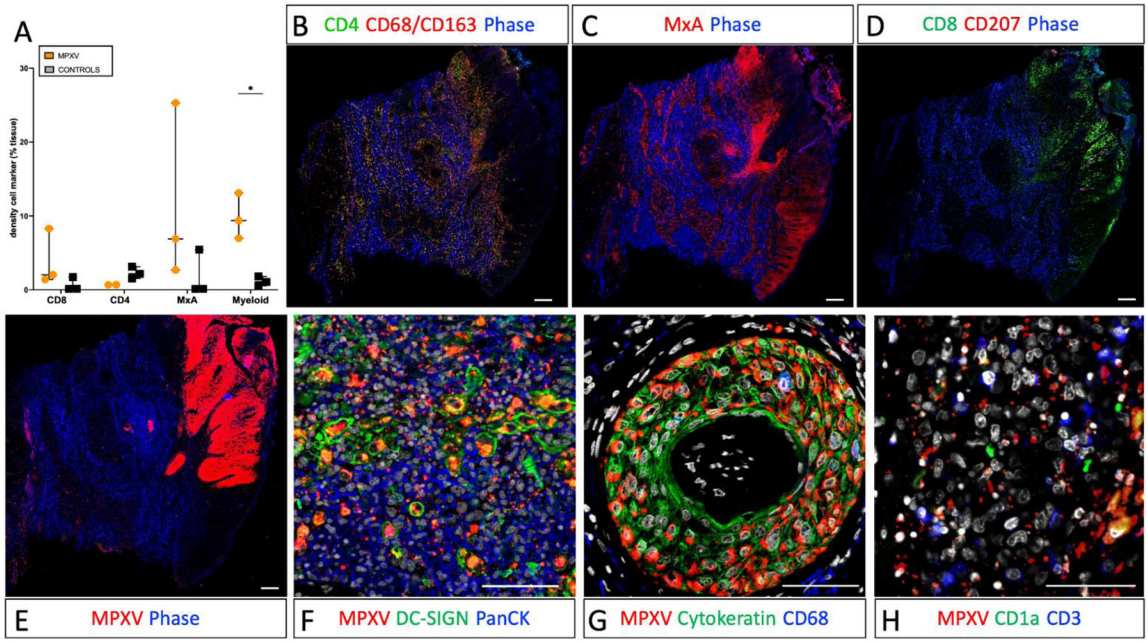

Figure 1:

In situ characterization of the immune response and infection on human skin biopsy at site of lesion.

Quantification of the expression of CD8, CD4, MxA and CD68+CD163 (myeloid) markers within the skin lesions of the 3 patients with HIV co-infected with MPXV compared to skin lesions collected in patients with HIV but without MPXV co-infection (controls, n=3) (A). Representative picture of skin lesion overview for CD4, CD68/CD163 (myeloid) and phase contrast (B) MxA and phase contrast (C) and CD8, CD207 (langerin) and phase (D). Representative pictures of the overview localization of MPXV-infected cells in skin lesions (E). Combining RNAscope and IFA we found MPXV vmRNA present in DC-SIGN+ cells in the upper dermis (F) epithelial cells (cytokeratin+) in the hair follicles (G) and CD1a+ cells between the epidermis and the dermis (H) Scale bars:200μm.

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Table 1:

Clinical Summaries of Mpox Patients 1, 2, and 3.

	Patient #1	Patient #2	Patient #3
Sex	Male	Male	Male
Age (years)	Mid 60s	Late 20s	Late 30s
CD4, at mpox diagnosis (cells/μL)	127	7	<20
HIV viral load, at mpox diagnosis (copies/mL)	13,100	597,000	Not available
Most recent CD4, following mpox infection (cells/μL)	127 (at time of mpox diagnosis)	<10	88 (5 months after mpox diagnosis)
Most recent HIV viral load (copies/mL)	<20	405 (6 days prior to death)	<20 (4.5 months after mpox diagnosis)
Time between mpox symptoms and evaluation	60 days	84 days	30 days
Treatments given overall			
Tecovirimat	Three separate 14-day oral courses	4 days IV, 110 days oral	176 days (146 days PO, 30 days IV)
Cidofovir	No	Topical	Yes (94 days of IV, 75 days of topical, two doses of intralesional)
Brincidofovir	No	Yes (14-day course)	No
VIGIV	No	Yes x 3	Yes x 4
Treatments given prior to biopsy			
Tecovirimat	One 14-day course of oral tecovirimat ending 51 days before biopsy	2 days of IV tecovirimat	Yes (day 73)
Cidofovir	No	No	Yes (day 8)
Brincidofovir	No	No	No
VIGIV	No	Yes	Yes (2 doses, last was 42 days prior to biopsy)
Site of lesions	Diffuse	Diffuse	Diffuse
Collected lesions by biopsy	Knee	R foot, L arm	Shoulder
Timing of biopsy after mpox diagnosis	66 days	89 days	84 days
PCR results on biopsy	MPXV +	MPXV +	MPXV +
ISH results on biopsy	MPXV +, HIV −	MPXV +, HIV −	MPXV +, HIV −
ART start	Re-started at diagnosis	Re-started on admission	Re-started 17 days post evaluation
ART regimen	Bictegravir-emtricitabine-tenofovir alafenamide	Bictegravir-emtricitabine-tenofovir alafenamide, Cabotegravir/rilpivirine (IM)	Dolutegravir-lamivudine

	Patient #1	Patient #2	Patient #3
Other pathology/infection associated	*Streptococcus dysgalactiae, Staphylococcus aureus*	Renal failure, Multiple episodes of sepsis due to bacteremia	*Staphylococcus aureus, Klebsiella*, osteomyelitis
Outcome	Fully resolved 224 days after diagnosis	Deceased on day 202 of hospitalization	Fully resolved 205 days after diagnosis

Abbreviations: ART; antiretroviral therapy, HIV; human immunodeficiency virus, ISH; In *situ* hybridization, IM; intramuscular, MPXV; monkeypox virus; PCR; polymerase chain reaction, VIGIV; vaccinia immune globulin intravenous