Novel Seroassays Detect Mpox-Specific and Vaccine-Induced Orthopoxvirus Immunity

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BACKGROUND

Mpox (previously known as monkeypox) is a disease caused by the mpox virus (MPXV) belonging to the Orthopoxvirus (OPXV) genus which includes smallpox and vaccinia virus (VACV). The 2022 global mpox outbreak has produced more than 85,000 confirmed cases, predominantly in locations that have not historically reported mpox. The current outbreak has disproportionately affected people living with HIV (PLWH), who represent 40% of confirmed mpox cases. VACV-based vaccines provide strong protection against mpox disease but complicate differentiation of infection-specific from vaccine-induced immunity. We tested the reactivity of serum from recently vaccinated or mpox-infected individuals and controls to a dozen MPXV/VACV proteins and developed and validated a novel immunoassay to measure vaccine response and distinguish mpox infection from vaccination.

METHODS

Vaccine and control sera were obtained from ongoing studies and specimen repositories with IRB approval. Mpox-vaccinated individuals (N=3) received Modified Vaccinia Ankara – Bavarian Nordic (MVA-BN) vaccine ≥ 2 weeks prior to serum collection and had no history of mpox infection. OPXV-naïve control sera (N=71) were collected prior to the global mpox outbreak from both PLWH and HIV-negative individuals < 40 years of age (born after routine smallpox vaccination ended). Mpox sera (N=5) were from individuals who recovered from PCR-confirmed mpox. Seroprevalence was measured in samples from 214 individuals (55% PLWH) collected between June 2022 and January 2023 who denied a history of mpox vaccination or infection. Indirect IgG ELISAS were performed using conserved OPXV antigens: A27L, A29L, A30L, A33R, A35R, B16R, C19L, D6L, E8L, H3L, I1L. For the MPXV-specific assay we used the complete, 1879 amino acid B21R protein which is present in MPXV but absent in VACV. Statistical analyses were performed on background-reduced optical densities (OD) with two-tailed Mann-Whitney tests for comparisons.

E8L and B21R protein-based ELISAs detect mpox vaccine-induced and infection-specific serum IgG antibodies, respectively.

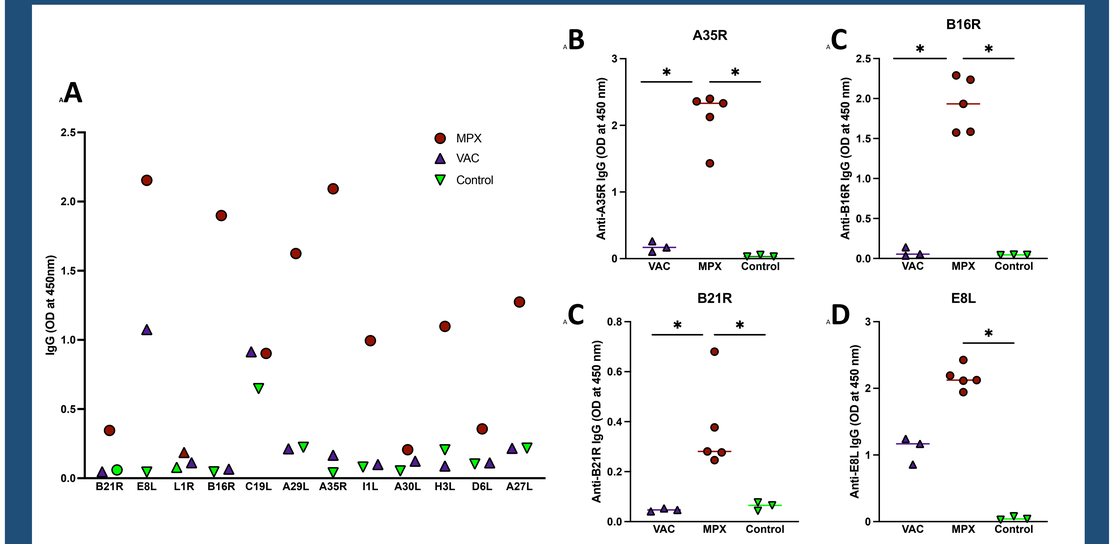

Figure 1. Reactivity of convalescent mpox and MVA-BN vaccine sera to Orthopoxvirus antigens. **A.** Geometric mean IgG (OD value) of mpox (N=5), vaccine (N=3), and control (N=3) sera against 12 Orthopoxvirus antigens. **B-E.** Individual IgG response of mpox, vaccine, and control sera against A35R, B16R, B21R, and E8L, respectively.
VAC = MVA-BN vaccinated; MPX = convalescent mpox patient.
*P < 0.05

RESULTS

Reactivity of vaccine serum was low or nonspecific for most antigens (A35R, L1R, B16R, A29L, A30L, A27L, I1L, D6L, A33R), while convalescent sera reacted strongly to several antigens (Fig 1 A-E). B21R IgG was detectable in all mpox sera but low in vaccine sera (OD 0.27 vs. 0.07, P < 0.05) (Fig 1D). E8L showed consistently high levels of reactivity in vaccine and convalescent sera with low background (Fig 1E). We re-ran the E8L assay with vaccine sera against a larger pool of controls and again found high levels of IgG in vaccinated but not in control sera (OD = 0.62 vs. 0.056, P < 0.0001) (Fig 2A). At an OD cutoff of 0.76, sensitivity and specificity of the E8L assay for mpox infection were 100%; in our pool of 214 recently collected sera, 14 (6.5%) were E8L IgG-positive at this threshold (Fig 2B). On average, E8L-positive individuals were significantly older than E8L-negative individuals (55y vs. 46y, P < 0.05).

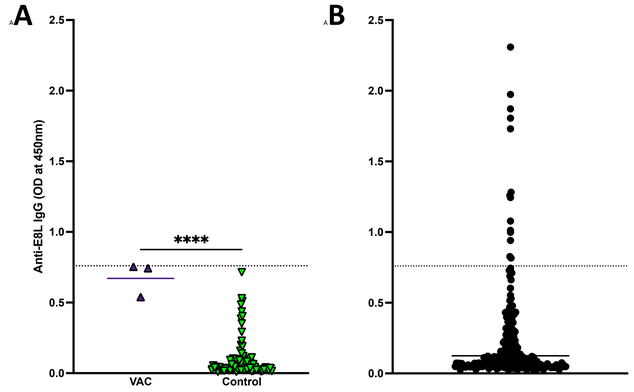

Figure 2. E8L IgG seroprevalence **A.** Anti-E8L IgG levels in recently vaccinated individuals (N=3) and pre-outbreak controls (N=71). **B.** Anti-E8L IgG levels post-outbreak (N=214). Dotted lines represent an OD cutoff of 0.76 for mpox positivity.
****P < 0.0001

CONCLUSIONS

We developed and validated a mpox-specific seroassay that uses the complete B21R peptide, which can distinguish recent infection from vaccination, which in turn was associated with a robust E8L antibody response. We found a 6.5% E8L IgG positivity in serum from high-risk (eg, PLWH) individuals with no reported history of mpox vaccination or infection. Collectively, our assays provide tools for conducting vaccine response and immunosurveillance studies to longitudinally detect immunity to MPXV, determine the true prevalence of MPXV infection and identify asymptomatic community spread. Future directions include confirming E8L seroprevalence results using a multiple OPXV antigen approach and evaluating antigen-specific vaccine response in prospectively collected samples from diverse cohorts.

ADDITIONAL KEY INFORMATION

This work was supported by a Miami Center for AIDS Research (CFAR) Pilot Grant to Suresh Pallikkuth. We acknowledge support from the Miami CFAR at the University of Miami Miller School of Medicine which is funded by a grant (P30AI073961) from the National Institutes of Health.