Mpox-Specific T Cell Responses in Recovered and Vaccinated Individuals

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BACKGROUND

- Human monkeypox virus (MPXV) is a large, double-stranded DNA virus belonging to the ***Orthopoxvirus*** genus and causes Mpox disease[1].
- Infection with MPXV may cause a pustular rash, fever, myalgia, lymphadenopathy, myocarditis, and is sometimes lethal[1,2].
- In May 2022, effective human-to-human transmission of MPXV (clade IIb) was seen for the first time outside of endemic countries[2] and, as of February 2025, 124,831 MPXV infections have been confirmed globally[3]. About 40% of Mpox cases in 2024 occurred in persons who are living with HIV (PLWH)[7].
- Currently, there is no MPXV-specific vaccine, and we are reliant upon vaccinia virus-based vaccines such as Imvamune® which has shown limited effectiveness against Mpox[5].
- As more lethal clade Ib MPXV infections are reported outside of endemic regions, there is an urgent need to develop robust Mpox prophylaxis which will require rigorous characterization of cell-mediated immunity against MPXV, especially in PLWH. This work seeks to contribute to that effort.

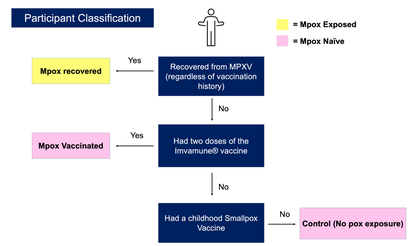
Hypothesis: Mpox-specific T cell responses will be observed upon stimulation with 46 peptides derived from proteins expressed during the "early" phase of viral replication in people who have recovered from Mpox, and people who are vaccinated against Mpox, regardless of HIV status.

KEY FINDINGS

- Using predicted peptide epitopes, we identified strong IFN-γ, but not granzyme B, responses in MPXV vaccinated and convalescent individuals.
- PLWH tended to show higher cytokine responses compared to people not living with HIV in the recovered group, but this was not statistically significant.
- Most of our predicted peptides showed IFN-γ responses in at least 50% of participants, suggesting our peptides contain promising epitopes for new MPXV vaccines.

STUDY COHORT

A



B

Groups	Mpox-Recovered	Mpox-Vaccinated	Mpox-Naïve	Cohort Totals
No. of Participants	10	9	9	28
Age, Mean ± SD (years)	42 ± 13	40 ± 16	41 ± 15	41 ± 14
Childhood Smallpox Vaccine (Y, N or U)	4/6	2/7	0/9	-
Two doses of Imvamune® Vaccine (Y/N)	1/9	9/0	-	10/18
Median number of days post symptom onset or last vaccine	376 days	150 days	-	-
HIV Status (PLWH and on ART/SN)	6/4	4/5	5/4	15/13

Fig 3: (A) Schematic of cohort classification system. **(B)** Summary of patient data (Y, Yes; N, No; U, Undisclosed; ART, antiretroviral treatment; SN, HIV seronegative.)

METHODS

1. Select Candidate T Cell Epitopes From MPXV Proteins Expressed During "Early" Phase of Viral Replication

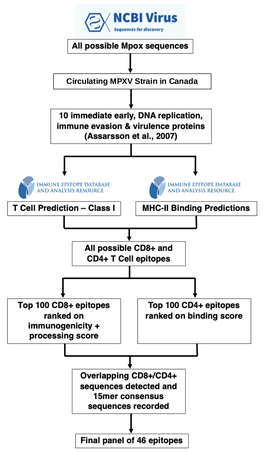


Fig 1: We used *in silico* prediction methods provided through the Immune Epitope Database (IEDB) to predict T cell epitopes from a circulating MPXV strain. We focused on epitopes found in 10 "early" phase proteins as these are highly expressed within 2 hours post infection for Vaccinia virus[6], as we predicted T cell responses against these peptides would be important for Mpox resolution.

2. Test Immunogenicity of Epitopes in Recoverees, Vaccinees, and Controls using Enzyme-Linked Immunosorbent Spot Assay (ELISpot)

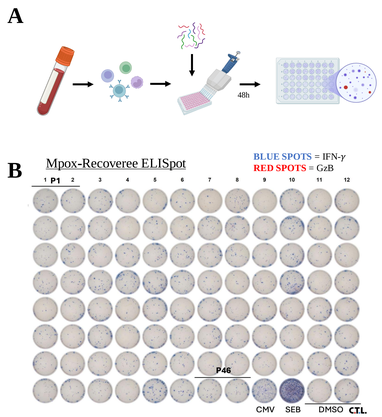


Fig 2: (A) Peripheral blood mononuclear cells (PBMCs) were isolated from blood, seeded in ELISpot plates, and stimulated with a single peptide for 48h before the plates were developed to quantify the number of cytokine-releasing cells. **(B)** An ELISpot assay from an MPXV-recovered person is shown. We quantified the number cells producing interferon-gamma (IFN-γ) and granzyme B (GzB). Peptides were tested in duplicates, and SEB and CMV peptides were used as positive controls. DMSO acted as a negative control. The average number of spot forming cells per million PBMCs (SFC/10^6 PBMCs) was calculated, and negative control averages were deducted to account for background cytokine release.

RESULTS

Selected Peptide Epitopes Were Immunogenic in MPXV Recoverees and Vaccinees

MPXV-Specific Immune Responses in PLWH Compared to HIV Seronegative People

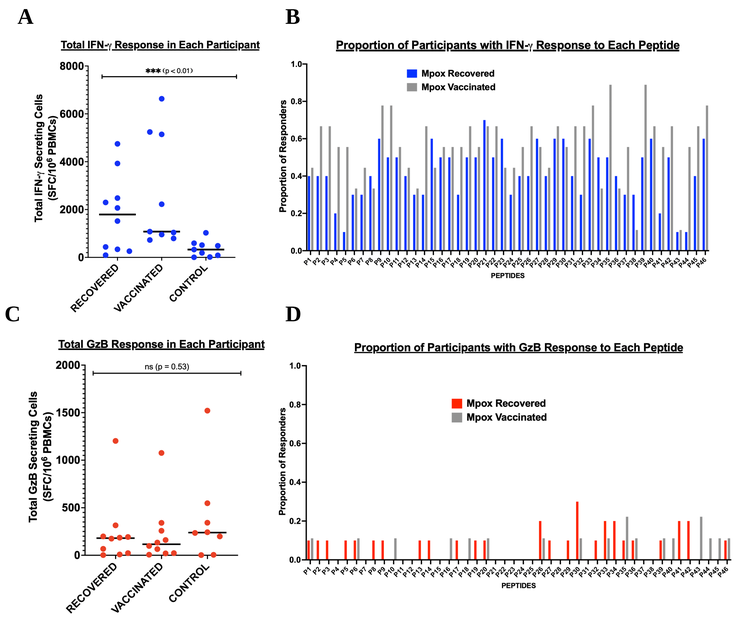


Fig 4: (A and C) Scatter plots displaying the total number of IFN-γ **(A)** and GzB **(C)** SFC/10^6 PBMCs per participant across the 46 peptides for our three study groups. Dots represent an individual participant. Solid black lines indicate the median for each group. Kruskal-Wallis tests assessed significance among groups. **(B and D)** The proportion of participants who showed a response to each peptide, with respect to IFN-γ **(B)** or GzB **(D)** secretion, is shown for recoverees and vaccinees. Positive peptide responses were defined as at least twice the mean in the control group and ≥ 20 SFC/10^6 PBMCs.

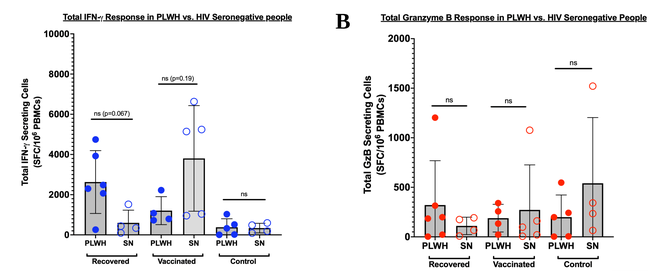


Fig 5: Bar graphs indicating the total IFN-γ **(A)** and GzB **(B)** SFC/10^6 PBMCs per participant, stratified into PLWH and SN people. Dots represent individual responses; bars indicate the means ± SD for the subgroup. Mann-Whitney U tests were used to test for statistically significant differences between PLWH and SN people within our groups. All PLWH were on ART.

DISCUSSION

We conclude that our selected MPXV peptides are immunogenic in Mpox recoverees and vaccinees. MPXV-specific IFN-γ responses varied significantly among the groups, with recoverees having the highest median total IFN-γ response, followed by vaccinees. Strong IFN-γ responses early in infection may be a key immune strategy against MPXV. In contrast, GzB responses were consistently lower than IFN-γ responses and showed no difference in responses among the groups. Low GzB responses could suggest a limited role of T cell-mediated cytotoxicity in MPXV infection, but the etiology of this remains unclear.

Individual peptides induced variable responses in participants. 35 peptides induced an IFN-γ response in at least 50% of recoverees or vaccinees. In contrast, 14 peptides failed to induce a GzB response in any individual. This suggests some peptides contain more epitopes than others, more potent epitopes, or that our peptide selection method failed to include GzB-inducing epitopes. Further exploration is needed to elucidate the capacity of cytotoxic T cells to respond to MPXV peptides produced early in infection.

Stratifying by HIV status, we observed an unexpected trend: among recoverees, PLWH exhibited higher cytokine responses than seronegative individuals, though this was not significant (p=0.067). Conversely, vaccinees showed the opposite trend, though not significantly (p=0.19). This may reflect immune imprinting in PLWH or HLA differences in vaccinees, warranting further investigation and participant recruitment to enhance the reliability of our findings.

In conclusion, our peptides were immunogenic in Mpox recoverees and vaccinees. Peptides which induced responses in over 50% of participants (i.e. P21) will be prioritized for epitope mapping and HLA characterization. Refining MPXV epitopes that induce robust T cell responses, particularly in PLWH, will inform vaccine development for the populations most affected by Mpox outbreaks.

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ABBREVIATIONS: MPXV, human monkeypox virus; MHC, major histocompatibility complex; HLA, human leukocyte antigen; CMV, Cytomegalovirus peptide pool; SEB, Staphylococcus Enterotoxin B; DMSO, Dimethylsulphoxide; P, peptide; IFN-γ, Interferon gamma; GzB, granzyme B; PLWH, persons living with HIV; SN, HIV seronegative).

ACKNOWLEDGEMENTS: We would like to thank our generous participants. All peptides were synthesized by GenScript. Figure 2A was made using BioRender.com.

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