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Low Specific T-Cell Immunity Against Mpox Elicited in People With HIV-1 and PrEP Users After Subcutaneous Vaccination Compared to Natural Infection

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ABSTRACT

In August 2024, the World Health Organization declared mpox infection a Public Health Emergency of International Concern for second time since 2022 and recommended vaccinating all people at-risk, such as people with HIV-1 (PWH) and prophylaxis pre-exposition (PrEP) users. We recruited PWH and PrEP users who received one or two doses of subcutaneous Imvanex® or Jynneos® and compared specific T-cell immunity with participants who passed mpox natural infection or were Nonexposed to mpox. CD4 + T cells from people who had mpox showed the highest capacity to produce IL-2 (1.8-fold, $p = 0.0328$), as well as IFNγ (2.5-fold, $p = 0.0247$), and IL-4 (1.8-fold, $p = 0.0373$) from naïve CD4 + T cells, in response to MHC-II-restricted mpox-related peptides, compared to vaccinated participants. CD8 + T cells from individuals who had mpox also showed the highest capacity to produce IFNγ (1.6-fold, $p = 0.0321$) and TNFα (2.1-fold, $p = 0.0084$) against MHC-I-restricted peptides. Therefore, the most potent and robust T-cell responses were developed after mpox infection, while they were barely detectable after vaccination. These results support the need to explore booster doses or improved vaccines to enhance cellular immunity in at-risk populations. More studies are needed to evaluate the capacity of mpox vaccines to confer long-term protection.

Olivia de la Calle-Jiménez, Guiomar Casado-Fernández, Montserrat Torres, and Mayte Coiras contributed equally.

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1 | Introduction

Mpox, caused by mpox virus (MPXV), emerged in Central (clade I) and West (clade II) Africa in 1970 [1]. Since May 2022, outbreaks of subclade IIb MPXV have spread to non-endemic countries via travel and mainly through sexual contact [2, 3], prompting the World Health Organization (WHO) to declare it a Public Health Emergency of International Concern (PHEIC) on July 23, 2022 [4]. Although these outbreaks were rapidly controlled, vaccination for people with HIV-1 (PWH) and pre-exposure prophylaxis (PrEP) users was recommended because men who have sex with men (MSM) and PWH were most affected during the 2022–2023 outbreak [5]. Cases in previously vaccinated individuals were also reported [6]. On August 14, 2024, WHO again declared mpox a PHEIC due to a new, more lethal clade I lineage that emerged in the Democratic Republic of the Congo in September 2023 [7], now rapidly spreading in Central Africa and globally disseminated [8]. In response, WHO is expediting the Emergency Use Listing (EUL) for mpox vaccines to improve access in lower-income countries [9].

Mpox symptoms develop over 2–4 weeks and include headache, fever, a rash that can form scabs, and lymphadenopathy [10]. Although clinical diagnosis is aided by these symptoms, a positive PCR for MPXV is the most reliable test [11]. Severe complications, such as encephalitis, pneumonitis, and secondary bacterial infections, occur more often in immunocompromised individuals, and co-infection with HIV-1 can worsen outcomes due to reduced CD4 counts [12, 13]. Moreover, variable immune responses, including asymptomatic cases, contribute to viral spread.

Natural infection triggers both B- and T-cell responses [14]. MPXV primarily infects CD14+ monocytes, evading T-cell responses and inducing immune escape [15]. Infection causes increased levels of monocytes, granulocytes, and antibodies, along with CD4 lymphopenia and elevated C-reactive protein (CRP) levels that impair Natural Killer (NK) cell function [16]. Consequently, both CD4+ and CD8 + T-cell responses and cytokine production are reduced [17], and type I interferon responses are inhibited [10].

There are no FDA-approved treatments for mpox [11], although antivirals like brincidofovir and tecovirimat are available through clinical trials or compassionate use [16, 18]. Thus, vaccination is essential to prevent infection and control viral spread. In 2019, the FDA approved a replication-deficient MVA-based vaccine (Jynneos®) for smallpox and mpox prevention in high-risk adults, administered as two doses 4 weeks apart [19]. The EMA approved the same vaccine as Imvanex® in July 2022 [20]. Real-world vaccine effectiveness for Jynneos® is estimated at 66–86% after full vaccination [19, 21, 22], yet its long-term immune impact remains nuclear [23]. Robust T-cell responses are observed 2 months after full vaccination [14], but most studies have focused on the humoral response soon after receiving one or two doses of vaccine [21, 22, 24, 25]. While antibody titers decline rapidly after natural infection, especially in PWH, T-cell responses may persist for at least 6 months [26]. Nevertheless, the persistence of mpox-specific T-cell immunity following vaccination has not been fully determined, especially in comparison to the immunity elicited by natural infection.

In this cohort study, we analyzed the efficacy and durability of T-cell responses against MPXV in PWH and PrEP users 8 months after one or two doses of MVA-based vaccines, compared with responses from natural infection, to assess vaccine protection for future outbreaks.

2 | Methods

2.1 | Study Subjects

All participants of this study were recruited at Hospital Clínico San Carlos and Centro Sanitario Sandoval (Madrid, Spain) ($n = 93$). Sociodemographic and clinical data of all participants are described in the Results section. Fifty-five individuals were previously in contact with MPXV as follows: People who received one dose of Jynneos®/Imvanex® ($n = 13$) (henceforth, 1-dose Vaccinated cohort); people who received two doses of Imvanex® or Jynneos® ($n = 11$) (henceforth, 2-doses Vaccinated cohort); and people with previous mpox infection ($n = 31$) (henceforth, MPXV- infection cohort). A fourth cohort of people who had no previous contact with MPXV ($n = 38$) was recruited as basal control (henceforth, Nonexposed cohort). All participants were over 18 years old.

2.2 | Ethical Statement

All individuals who participated in this study gave informed written consent to participate. Current Spanish and European Data Protection Acts ensured confidentiality and anonymity of all participants. Protocol for this study (CEI PI 70_2022) was prepared in accordance with the Helsinki Declaration and previously reviewed and approved by the Ethics Committees of Instituto de Salud Carlos III (IRB IORG0006384).

2.3 | Blood Samples Processing

Blood samples were collected in Vacutainer EDTA Collection Tubes (Becton Dickinson, Madrid, Spain) and immediately centrifuged in a Ficoll-Hypaque density gradient (Corning, NY, USA) to isolate peripheral blood mononuclear cells (PBMCs) that were cryopreserved until analysis.

2.4 | T-Cell Stimulation With MPXV-Related Peptides

PBMCs were thawed and cultured for 1 h in RPMI medium supplemented with 10% (v/v) fetal bovine serum, 2mM L-glutamine, 100UI/ml penicillin, and 100μg/ml streptomycin. Cells were incubated for 4 h at 37°C with 0.4 μg/ml of two different vaccinia peptide pools (PepPools) purchased from Mabtech (Nacka Strand, Sweden) in the presence of CD28/CD49d co-stimulator (1μg/ml) and brefeldin A (BD Bioscience), so the production of T-cell intracellular cytokines could be quantified. PepPool 1 contained 56 MHC II-restricted defined peptides from 34 vaccinia virus proteins; 91% of these peptides shared > 90% homology with MPXV (Mabtech reference

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3635-1). PepPool 2 contained 102 MHC I-restricted defined peptides from 59 vaccinia virus proteins; 79% of these peptides shared > 90% homology with MPXV (Mabtech reference 3670-1).

2.5 | Analysis of Surface Activation Markers By Flow Cytometry

Upon activation as described above, the conjugated antibodies CD3-BV510 and CD8-APC-H7 (BD Biosciences, San Jose, CA) were used to determine the levels of CD4+ (CD3 + CD8-) and CD8+ (CD3 + CD8 +) T cells. CD3 + CD8- were assumed to be CD4 + T cells, which included those cells with downregulated CD4 expression caused by HIV-1 infection [27]. CD4 + T cells were also stained with CD25-FITC (Immunostep, Salamanca, Spain). CD4 + T cell memory subpopulations were analyzed using CCR7-FITC and CD45RA-PE-Cy7 (BD Biosciences) as follows: naive (TN) ($CD45RA^{+}CCR7^{+}$), central memory (TCM) ($CD45RA^{-}CCR7^{+}$), effector memory (TEM) ($CD45RA^{-}CCR7^{-}$), and terminally differentiated effector memory (TEMRA) ($CD45RA^{+}CCR7^{-}$). CD8 + T cells were also stained with CD107a-PE Cy7 to evaluate their degranulation capacity [28]. Isotype controls were used to determine the background signal. Data acquisition was performed with BD LSRFortessa X-20 flow cytometer (BD Biosciences) and data analysis with FlowJo software v10.8.1 (Tree Star Inc., Ashland, OR). Gating strategy is shown in Supporting Figure 1.

2.6 | Analysis of Intracellular Production of Cytokines By Flow Cytometry

After surface staining, cells were fixated and permeabilizated with IntraPrep Permeabilization Reagent (Beckman Coulter, Spain) for intracellular staining and evaluation of cytokine production. CD4 + T cells were stained with IL-2_BV605, IL-4_APC (BD Biosciences), and IFNγ-PE (Beckman Coulter, Brea, CA). CD8 + T cells were stained with IFNγ-PE, TNF-α-PE (Beckman Coulter), and Granzyme B (GZB)-FITC (BD Biosciences). Isotype controls were used to determine the background signal. Data acquisition and analysis by flow cytometry was performed as described above. Gating strategy is shown in Supporting Figure 2.

For clarity, a summary of all analyses performed for this study is provided in Supplementary Table 1.

2.7 | Statistical Analysis

All statistical analyses were conducted in GraphPad Prism v10.2.1 (GraphPad Software, San Diego, CA). Continuous variables such as cell phenotyping and intracellular production of cytokines, were first assessed for normality using the Shapiro–Wilk test to confirm Gaussian distribution. Data meeting normality assumptions were then compared across the four cohorts (Nonexposed, 1-dose vaccinated, 2-dose vaccinated, and MPXV-infection) by ordinary one-way ANOVA to test for an overall group effect; significant ANOVA results ($\alpha = 0.05$) were followed by Tukey's multiple-comparison post hoc test to identify which specific pairs of cohorts differed. Categorical outcomes such as gender, HIV-1 infection status, PrEP use, previous mpox infection, vaccination status against Orthopoxvirus, and the presence versus absence of a detectable T-cell response to each peptide pool were tabulated in contingency tables (Table 1) and analyzed by the chi-square test to evaluate associations between cohorts and categorical outcomes. P-values < 0.05 were considered statistically significant.

3 | Results

3.1 | Cohorts' Description

Observational, multi-center, cross-sectional, cohort study that recruited 93 participants. Recruitment began in December 2022 and ended in August 2023. The main sociodemographic and clinical characteristics of all participants are summarized in Table 1 and detailed in Supplementary Table 2. All participants were male. Median age was 34 years old (Interquartile range (IQR) 27–46) for MPXV-Nonexposed cohort, 34 years old (IQR 32–40) for 1-dose Vaccinated cohort, 38 years old (IQR 35–41) for 2-doses Vaccinated cohort, and 37 years old (IQR 34–47) for MPXV-infection cohort. Forty-one participants (44%) were positive for HIV-1 for a median of 8 years (IQR 5–10), and they were distributed in all cohorts, as indicated in Table 1. PWH were on standard antiretroviral therapy and had undetectable viral load. No significant differences in the CD4/CD8 ratio were found between PWH from the different cohorts. The rest of the participants (56%) were PrEP users.

Individuals of MPXV-infection cohort ($n = 31$) had MPXV infection for a median of 258 days (IQR 254–285) (8.6 months) before blood sample collection. Infection with MPXV was diagnosed by clinical signs and symptoms and confirmed by qPCR according to WHO recommendation [29]. Nonexposed participants were also screened for negative MPXV qPCR. Participants who were vaccinated with one or two doses of Imvanex®/Jynneos® ($n = 25$) received standard subcutaneous vaccination a median of 232 days (IQR 180–239) (7.7 months) before blood collection sample: specifically, a median of 338.5 days (IQR 288–349) (11.3 months) for 1-dose Vaccinated cohort and 126 days (IQR 72–169) (4.2 months) for 2-doses Vaccinated cohort. Time between vaccine doses was at least 28 days. Vaccination was safe and serious adverse events were not reported for any participant. Two individuals (15.4%) of 1-dose Vaccinated cohort had received previous vaccination against smallpox in the 1970s.

3.2 | Stronger CD4 + T Cell Response to MHC II-Restricted MPXV-Related Peptides in Individuals Who Had Mpox

Individuals from MPXV-infection cohort showed CD4 + T-cell lymphopenia compared to 1-dose Vaccinated cohort (−1.5-fold, $p = 0.0114$), as well as to MPXV-nonexposed

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TABLE 1 | Sociodemographic and clinical data of all participants in the study. Statistical significance between cohorts was assessed using chi-square test; significant p-values are shown in bold.

	Nonexposed ($n = 38$)	**1-dose Vaccinated ($n = 13$)**	**2-doses Vaccinated ($n = 11$)**	**MPXV-infection ($n = 31$)**	***p* value**
Age, median (IQR)	34 (27–46)	34 (32–40)	38 (35–41)	37 (34–47)	0.8653
Male gender, *n* (%)	38 (100)	13 (100)	11 (100)	31 (100)	1
PWH, *n* (%)	11 (28.9)	3 (23.1)	1 (9.1)	26 (83.9)	**< 0.0001**
Time of HIV-1 infection (years), median (IQR)	5 (3–7)	8.5 (8–9)	Unk.	10 (6–11)	**0.0274**
%CD4, median (IQR)	31.8 (22.7–34.3)	43.9 (41.7–44.6)	22.3 (22.3)	23.1 (15.6–28.4)	0.1774
%CD8, median (IQR)	26.5 (18.8–32.3)	39 (31.5–46.9)	17.5 (17.5)	22.3 (17.7–30.9)	0.8526
Ratio CD4/CD8, median (IQR)	1 (0.7–1.9)	1.1 (1–1.4)	1.3 (1.3)	0.9 (0.7–1.6)	0.2514
PrEP users	27 (71.1)	10 (76.9)	10 (90.9)	5 (16.1)	**< 0.0001**
%CD4, median (IQR)	30.4 (25.8–39)	34.4 (32.7–42.7)	32.9 (27.1–38.1)	29 (25.6–36.9)	0.6126
%CD8, median (IQR)	21.8 (17.6–30.2)	22.9 (20.3–26.4)	35.8 (27.0–40.5)	23.2 (18–29.3)	0.0874
Ratio CD4/CD8, median (IQR)	1.3 (0.9–2.2)	1.8 (1.6–1.9)	0.9 (0.8–1)	1.2 (1–1.6)	0.1070
Previous mpox infection, *n* (%)	0 (0)	0 (0)	0 (0)	31 (100)	**< 0.0001**
Time from mpox infection to sample (days, median (IQR)	NA	NA	NA	258 (254–285)	—
Vaccination against Orthopoxvirus, n (%)	0 (0)	13 (100)	11 (100)	1 (3.2)	**< 0.0001**
Only mpox vaccination	0 (0)	11 (84.6)	11 (100)	1 (3.2)	**< 0.0001**
Only smallpox vaccination	0 (0)	0 (0)	0 (0)	0 (0)	1
Mpox and smallpox vaccination	0 (0)	2 (15.4)	0 (0)	0 (0)	**0.0016**
Time from mpox vaccination to sample (days, median (IQR)	0 (0)	338.5 (288–349)	126 (72–169)	147.5 (107–188)	0.2854

Abbreviations: HIV-1, Human immunodeficiency virus type 1; IQR, Interquartile range; MPXV, Monkeypox virus; NA, Not Applicable; Unk., Unknown.

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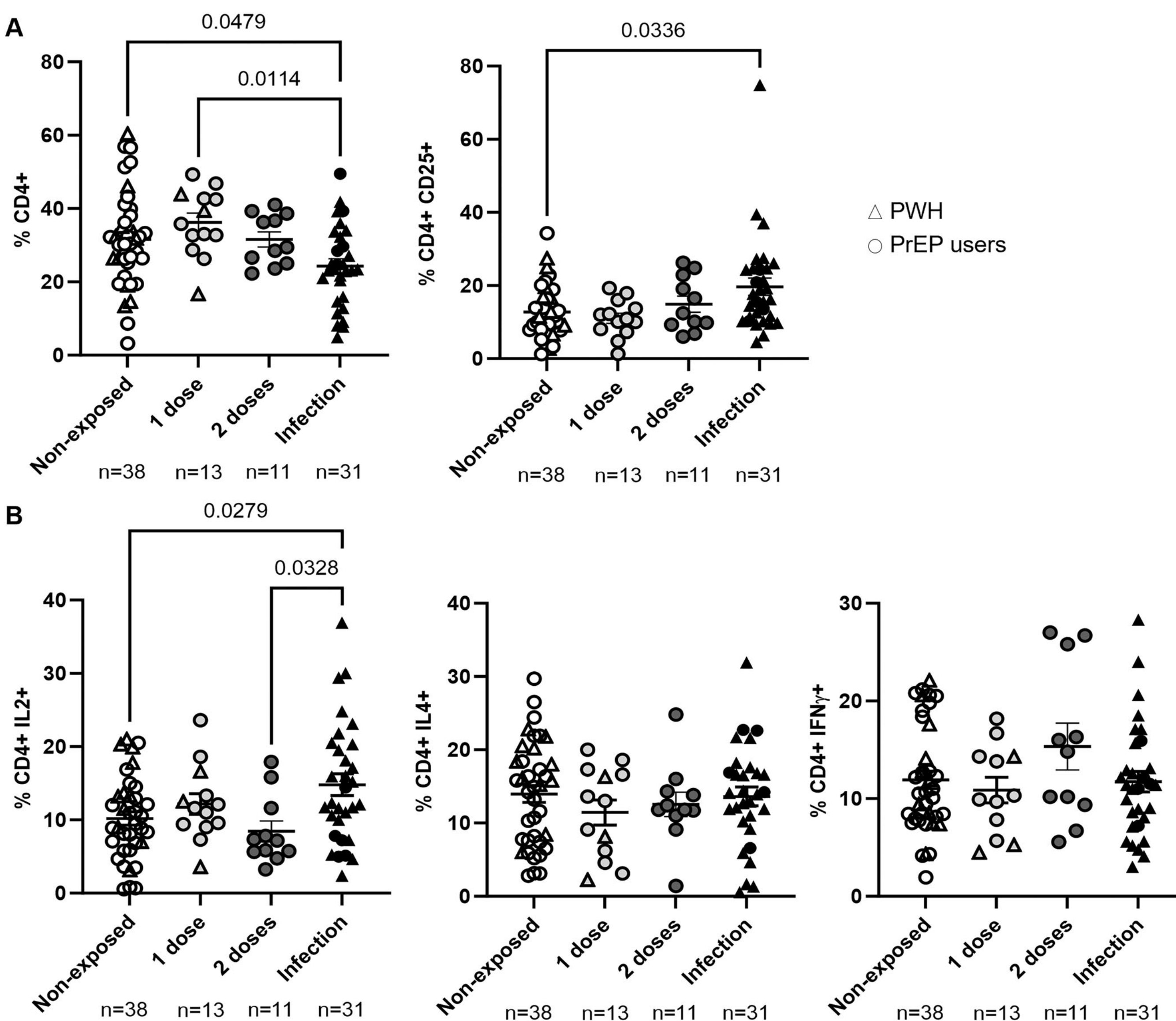

FIGURE 1 | Levels of total CD4 + T cells and production of specific cytokines in response to MHC II-restricted MPXV-related peptides. (A) Analysis by flow cytometry of total CD4 + T cells in participants from the different cohorts (left graph) and surface expression of activation marker CD25 (right graph). (B) Intracellular production of IL-2, IL-4, and IFNγ in total CD4 + T cells in response to MHC II-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent the standard error of the mean (SEM). Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

cohort (−1.3-fold, $p = 0.0479$) (Figure 1A, left graph). Stimulation of CD4 + T cells with MHC II-restricted MPXV-related peptides induced higher surface expression of the activation marker CD25 in these cells from MPXV-infection cohort than from 1-dose Vaccinated cohort (1.7-fold; $p = 0.0336$) (Figure 1A, right graph). Accordingly, stimulated CD4 + T cells from MPXV-infection cohort also showed higher capacity to produce IL-2 in response to MHC II-restricted MPXV-related peptides than 2-doses Vaccinated cohort (1.8-fold; $p = 0.0328$) and MPXV-nonexposed cohort (1.4-fold, $p = 0.0279$) (Figure 1B). The expression levels of IL-4 and IFNγ did not change in total CD4 + T cell population upon stimulation.

The levels of CD4 + T cell memory subpopulations and the specific response of each subset against MHC II-restricted MPXV-related peptides were also analyzed. People from MPXV-infection cohort showed higher levels of CD4 + TN cells than 2-doses Vaccinated cohort (3.1-fold, $p = 0.0352$) and MPXV-nonexposed (2.8-fold, $p = 0.0015$) (Figure 2A, left graph). These cells also showed higher expression of CD25 on the surface upon stimulation (1.9-fold, $p = 0.0479$, compared to MPXV-nonexposed) (Figure 2A, right graph), as well as increased capacity to produce IL-4 (1.8-fold, $p = 0.0373$, compared to 1-dose Vaccinated cohort) and IFNγ (2.5-fold, $p = 0.0247$, compared to 2-doses Vaccinated) than CD4 + TN cells from other cohorts (Figure 2B).

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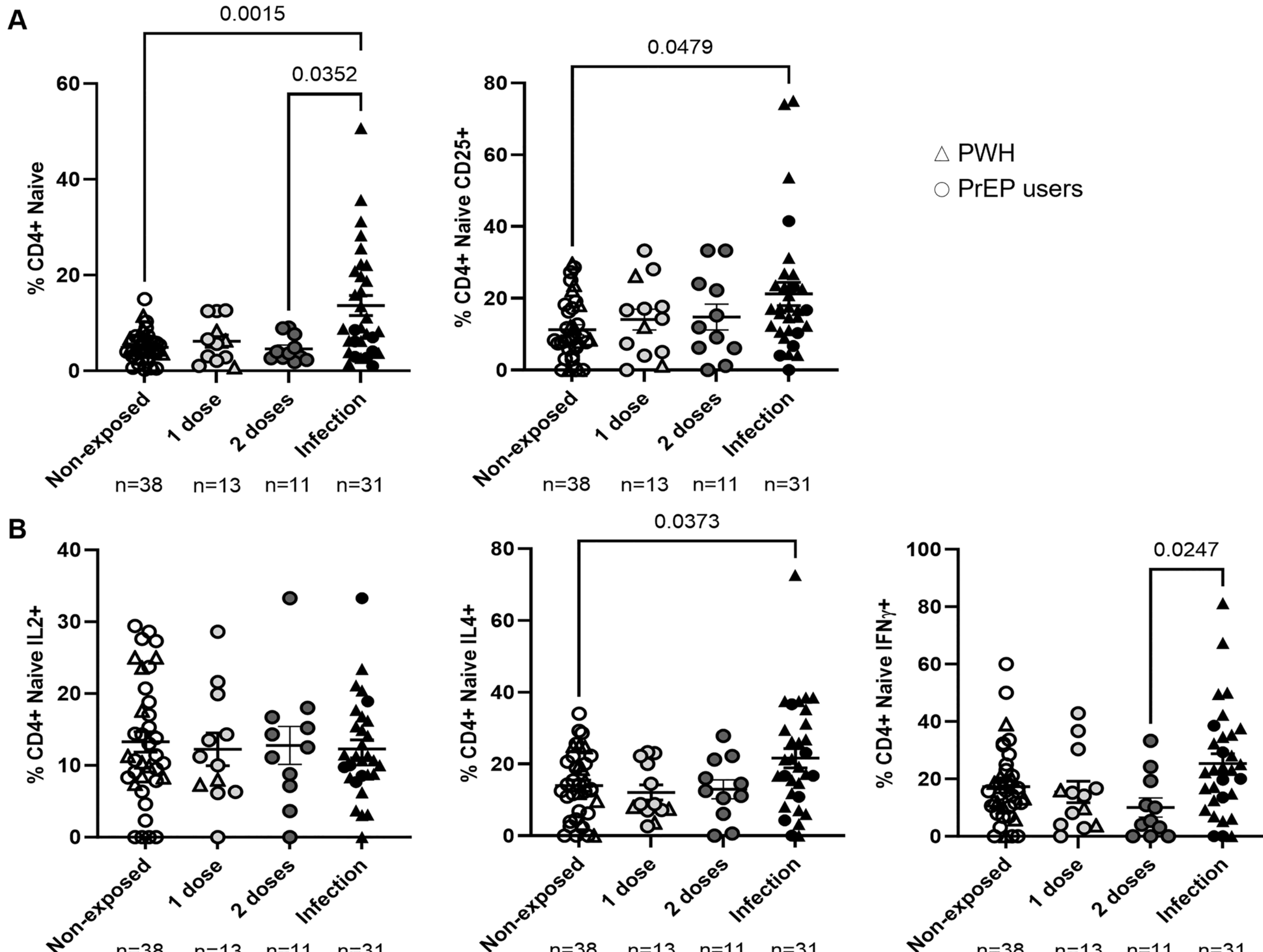

FIGURE 2 | Levels of CD4 + TN cells and production of specific cytokines in response to MHC II-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD4 + TN cells in participants from the different cohorts (left graph) and surface expression of activation marker CD25 (right graph). (B) Intracellular production of IL-2, IL-4, and IFNγ in CD4 + TN cells in response to MHC II-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

People from MPXV-infection cohort also showed higher levels of CD4 + TCM cells than the other cohorts (2-dose Vaccinated: 3.1-fold, $p = 0.0118$ and MPXV-nonexposed: 2.0-fold, $p = 0.0183$) (Figure 3A, left graph). In these cells, the expression levels of CD25 did not change between cohorts (Figure 3A, right graph). However, the production of IL-2 was decreased in the 2-doses Vaccinated cohort compared to MPXV-infection cohort (2.4-fold, $p = 0.0369$) (Figure 3B, right graph). People from MPXV-nonexposed cohort showed higher levels of IL-4 than CD4 + TCM cells from MPXV-infection cohort (1.3-fold, $p = 0.0408$) (Figure 3B, middle graph). There were not significant differences in the production of IFNγ between cohorts exposed to MPXV.

No significant differences were observed between cohorts regarding the levels of CD4 + TEM cells or in the expression of CD25 in response to MHC II-restricted MPXV-related peptides (Figure 4A). However, effector cells from MPXV-infection produced higher levels of IL-2 upon stimulation than cells from the 2-doses Vaccinated cohort (2.4-fold, $p = 0.0479$) (Figure 4B). No differences between cohorts were observed in the production of IL-4 or IFNγ.

Levels of CD4 + TEMRA cells from people in MPXV-infection cohort were lower than in other cohorts and this difference was significant with the 1-dose Vaccinated cohort (−1.4-fold, $p = 0.0176$) (Figure 5A, left graph), although they showed higher expression of CD25 upon stimulation compared to MPXV-nonexposed cohort (1.7-fold, $p = 0.0218$) (Figure 5A, right graph). There were no significant differences in the production of IL-2, IL-4, and IFNγ between cohorts in this CD4 subset (Figure 5B).

CD4 + T cell subpopulations from PWH who were included in the different cohorts did not show significantly different responses to MPXV-related peptides in comparison with those from PrEP users.

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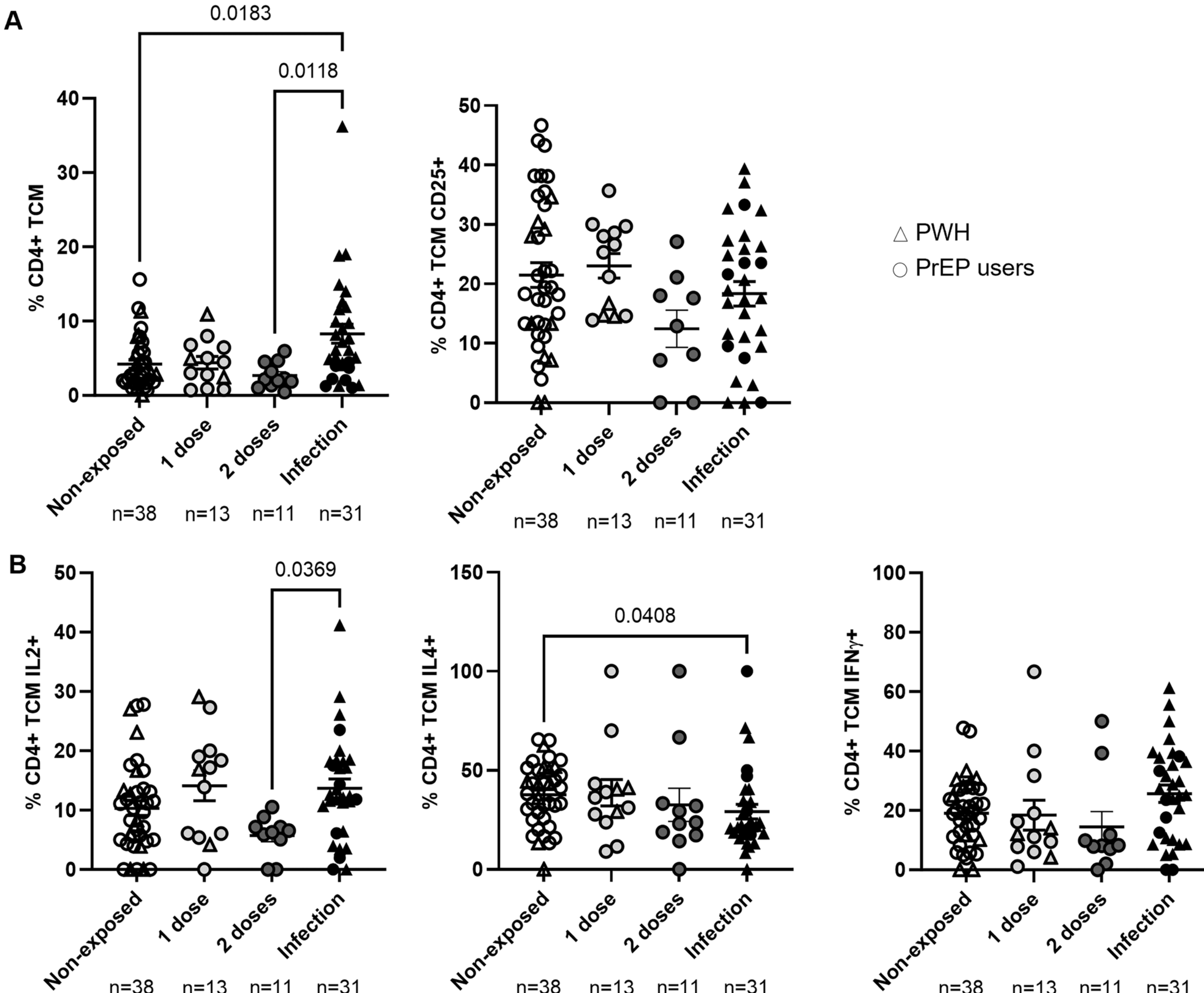

FIGURE 3 | Levels of CD4 + TCM cells and production of specific cytokines in response to MHC II-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD4 + TCM cells in participants from the different cohorts (left graph) and surface expression of activation marker CD25 (right graph). (B) Analysis by flow cytometry of intracellular production of IL-2, IL-4, and IFN γ in CD4 + TCM cells in response to MHC II-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

3.3 | Stronger CD8 + T Cell Response to MHC I-Restricted MPXV-Related Peptides in Individuals Who Had Mpox

The levels of CD8 + T cells did not change between cohorts (Figure 6A). However, CD8 + T cells from MPXV-infection cohort produced higher levels of IFNγ (1.6-fold, $p = 0.0321$, compared to 1-dose Vaccinated cohort; 1.7-fold, $p = 0.0296$, compared to 2-doses Vaccinated cohort; and 1.6-fold, $p = 0.0056$, compared to MPXV-nonexposed cohort) and TNF-α (2.1-fold, $p = 0.0084$, compared to 1-dose Vaccinated cohort), in response to MHC I-restricted MPXV-related peptides (Figure 6B). The capacity of CD8 + T cells to produce GZB did not change between groups.

Although there were not significant differences in the levels of CD8 + T cells with expression of CD107a on the surface between cohorts (Figure 7A), CD107a + CD8 + T cells from MPXV-infection cohort produced higher levels of TNF-α in response to MHC I-restricted MPXV-related peptides than 1-dose Vaccinated (3.2-fold; $p = 0.0004$) and MPXV-nonexposed (2.5-fold; $p = 0.0016$) cohorts (Figure 7B).

4 | Discussion

The emergence of mpox outbreaks outside Central and West Africa has heightened the urgency to vaccinate at-risk populations worldwide [9]. Because MPXV is primarily transmitted

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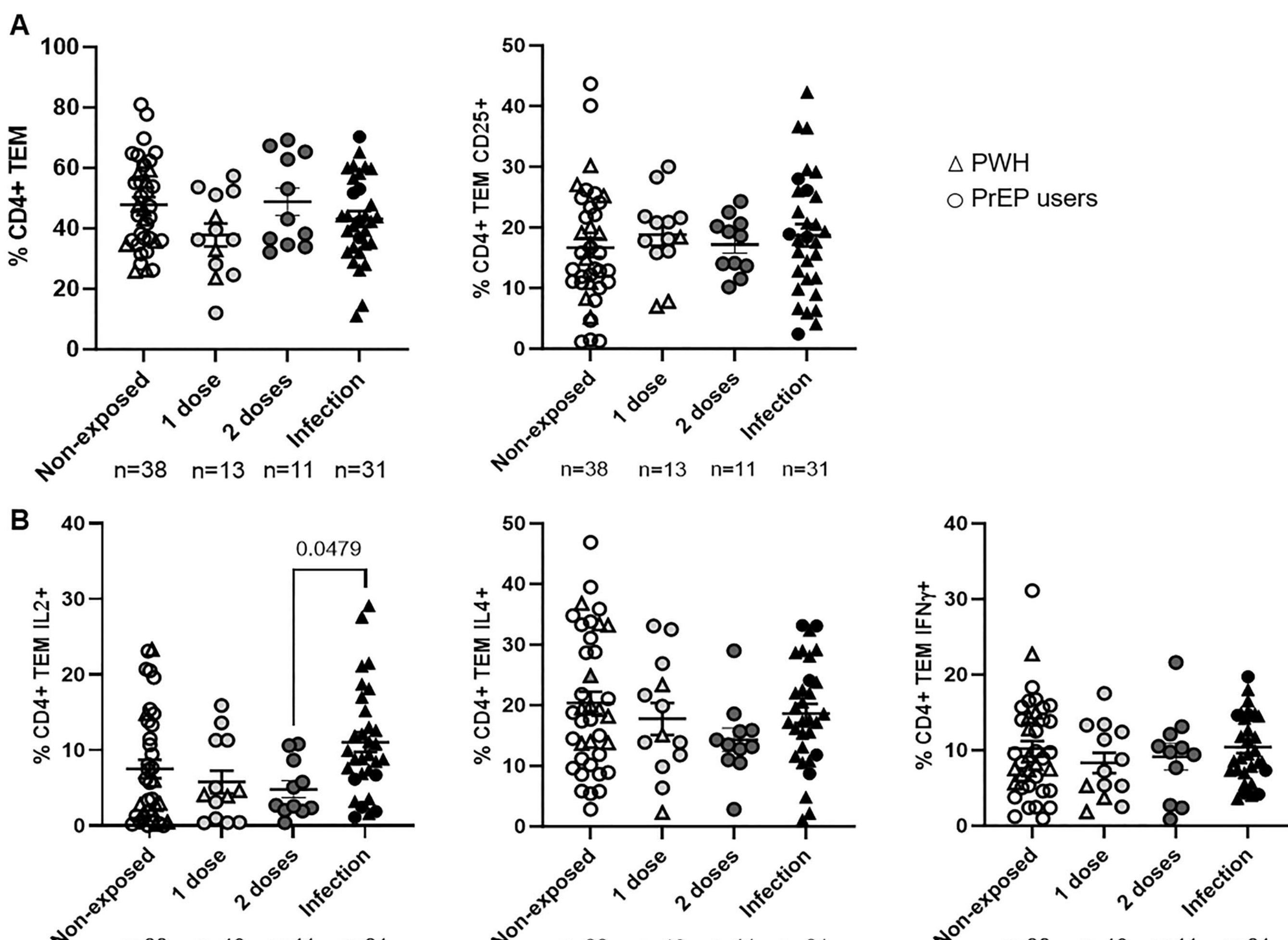

FIGURE 4 | Levels of CD4 + TEM cells and production of specific cytokines in response to MHC II-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD4 + TEM cells in participants from the different cohorts (left graph) and surface expression of activation marker CD25 (right graph). (B) Analysis by flow cytometry of intracellular production of IL-2, IL-4, and IFN γ in CD4 + TEM cells in response to MHC II-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

through direct contact [30] and can cause severe infection in immunocompromised individuals [13], PWH and MSM PrEP users are considered the most susceptible populations [31]. Since the start of the 2022 outbreak, Spain has been the most affected European country and the third worldwide in case numbers [32]. The peak incidence coincided with festive gatherings and overcrowding. In Spain, vaccination with Imvanex® and Jynneos® began in June 2022 [33], and rapid control of MPXV dissemination was achieved; by February 2023, cases had dramatically declined [34]. This decrease was likely due to immunity acquired via natural infection or vaccination, as well as changes in sexual behavior following widespread reporting of mpox cases [35]. However, WHO's second declaration of mpox as a PHEIC in August 2024, driven by a new clade I lineage distinct from previous strains [7], underscores the need to determine whether vaccine-induced immunity in at-risk groups matches that developed after infection.

The protective efficacy of Jynneos® against mpox is estimated between 66% and 86% [21, 22, 36], which is lower than that of other live attenuated vaccines such as smallpox (VE ~ 95%) [37], and the MMR vaccine (VE of 99%, 95%, and 90% against measles, mumps, and rubella, respectively) [38]. Several studies have analyzed the humoral response after vaccination with Jynneos® or Imvanex®; while the results vary [21, 22, 24, 25], vaccination consistently triggers a potent B-cell expansion [39]. In addition, Jynneos® induces specific antiviral Th1 responses in PWH, regardless of whether CD4 counts are normal or low, as early as 28 days after one dose [40]. Robust T-cell responses have been documented 2 months after a full two-dose regimen [14], yet the longevity of T-cell immunity remains less defined compared to the responses following natural infection, which elicits both B- and T-cell responses [14].

For this study, participants from key at-risk populations, PWH and PrEP users, who had received one or two doses of subcutaneous Jynneos® or Imvanex® were recruited at a median of 7.7 months postvaccination. There were no significant differences in CD4/CD8 ratios or T-cell responses between PWH and PrEP users. With only two participants having been vaccinated

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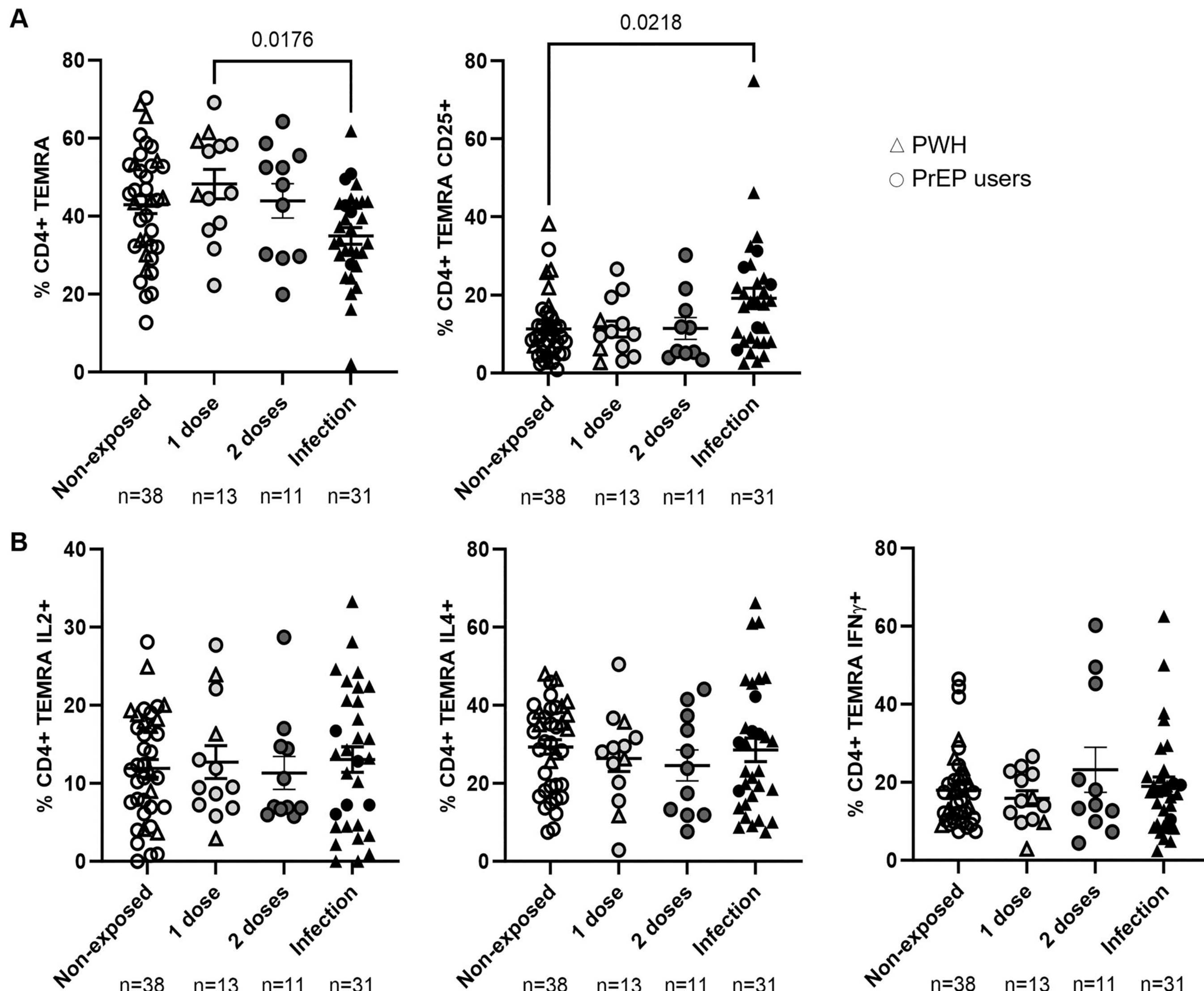

FIGURE 5 | Levels of CD4 + TEMRA cells and production of specific cytokines in response to MHC II-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD4 + TEMRA cells in participants from the different cohorts (left graph) and surface expression of activation marker CD25 (right graph). (B) Analysis by flow cytometry of intracellular production of IL-2, IL-4, and IFN γ in CD4 + TEMRA cells in response to MHC II-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

against smallpox in the 1970s, high levels of cross-immunity were not expected, even though some T-cell-specific responses have been observed in previously vaccinated individuals [41].

Although the complete 2-dose schedule is recommended for optimal protection [42], a single dose of Jynneos® may confer some immunity [43]. Notably, 1 month after vaccination, no differences in T-cell immunity were found between recipients of one versus two doses [41], a finding confirmed in our study when comparing responses at a median of 11.3 months after one dose to those at 4.2 months after two doses.

We also found that CD4 + T-cell levels were significantly reduced in individuals who had mpox a median of 8.6 months earlier. Although 83.9% of the MPXV-infection cohort were PWH, they maintained normal CD4/CD8 ratios, suggesting that the observed CD4 cytopenia was a long-term consequence of MPXV infection, possibly due to thymic and lymph node damage [44]. This CD4 cytopenia may persist for several months, similar to other acute viral infections [45]. The most reduced CD4 memory subset was the effector TEMRA population, while increases in TN and TCM subsets were observed compared to vaccinated individuals, likely reflecting CD4 self-renewal and thymic recovery [46]. Despite lower numbers, CD4 + T cells from individuals who had mpox were functionally competent, showing elevated expression of CD25 and IL-2 in response to MHC II-restricted MPXV peptides. Among CD4 subsets, TEM cells produced the most IL-2, whereas TN and TCM cells were the main producers of IFNγ and TNF-α, cytokines critical for Th1 polarization and antiviral defense, especially since MPXV can inhibit IFN-mediated responses [47, 48]. Similarly, CD8 + T cells from participants

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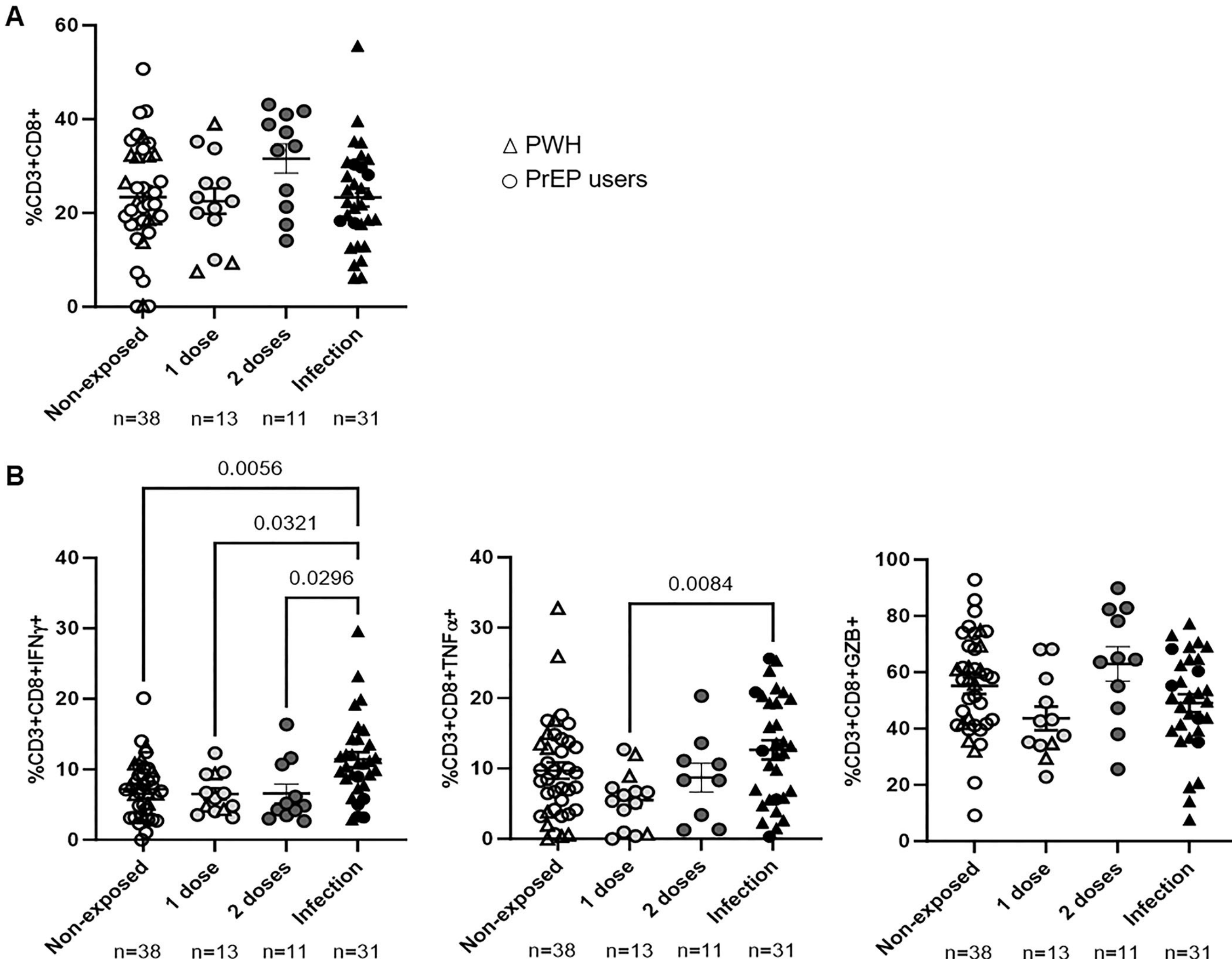

FIGURE 6 | Levels of CD8 + T cells and production of specific cytokines in response to MHC I-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD8 + T cells in participants from the different cohorts. (B) Analysis by flow cytometry of intracellular production of IFN γ, TNF-α, and GZB in CD8 + T cells in response to MHC I-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

who had mpox exhibited higher production of IFNγ and TNF-α compared to those from vaccinated individuals.

Additionally, IL-4 production was enhanced in CD4 + TN cells from individuals who had mpox following stimulation with MHC II-restricted MPXV peptides compared to vaccinated participants. Although IL-4 promotes Th2 polarization and humoral responses, it can also moderate Th1 responses [49]. However, not all CD4 subsets in the group who had mpox produced high levels of IL-4, with CD4 + TCM cells showing lower levels relative to the MPXV-nonexposed cohort.

One study limitation is that the humoral response was not evaluated due to the unavailability of validated assays at the time. Another potential limitation was the reliance on synthetic peptides, which, despite allowing precise epitope targeting and reducing biosafety concerns, may not fully capture the complexity of responses elicited by whole viral antigens.

5 | Conclusion

The most potent and durable CD4 and CD8 responses were observed following MPXV natural infection, persisting for at least 8 months, while responses in vaccinated individuals (after one or two doses) were minimal. PWH showed immune responses comparable to PrEP users; however, further studies with larger cohorts and more PWH with lower CD4/CD8 ratios are needed to fully assess the long-term protection provided by MVA-based vaccines against future MPXV outbreaks. Based on our findings, we recommend reinforcing vaccine strategies for at-risk populations by considering booster doses or improved vaccine formulations to enhance T-cell immunity, especially in the context of emerging mpox clades with uncertain cross-protection.

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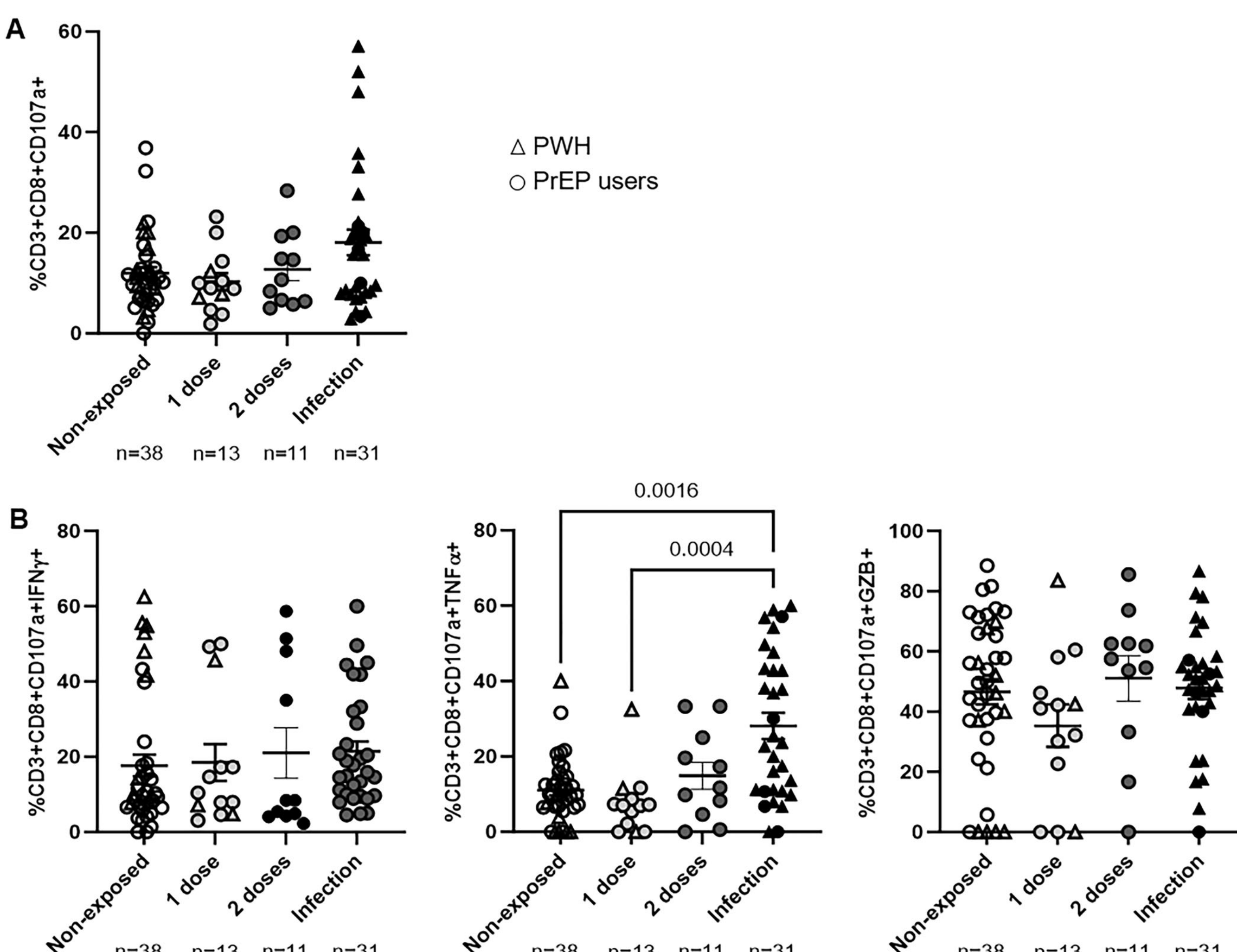

FIGURE 7 | Levels of CD8 + CD107a+ T cells and production of specific cytokines in response to MHC I-restricted MPXV-related peptides. (A) Analysis by flow cytometry of CD8 + CD107a+ T cells in participants from the different cohorts. (B) Analysis by flow cytometry of intracellular production of IFNγ, TNF-α, and GZB in CD8 + CD107a+ T cells in response to MHC I-restricted MPXV-related peptides. Each symbol corresponds to one sample and vertical lines represent SEM. Open symbols correspond to MPXV-nonexposed participants, light grey symbols correspond to 1-dose Vaccinated participants, dark grey symbols correspond to 2-doses Vaccinated participants, and closed symbols correspond to MPXV-infection participants. Triangles correspond to samples from PWH, and circles correspond to samples from PrEP users. Statistical analysis was performed with ordinary one-way ANOVA and Tukey's multiple comparison.

Author Contributions

Vicente Estrada, Montserrat Torres, and Mayte Coiras conceptualized the project. Olivia de la Calle-Jimenez, Guiomar Casado-Fernandez, Vicente Estrada, Montserrat Torres, and Mayte Coiras wrote the manuscript. Inés Armenteros-Yeguas, Javier Rodríguez-Añover, Reynaldo Homen, Eva Orviz-García, Jorge del Romero, Noemí Cabello, and Vicente Estrada selected and recruited the participants and collected the blood samples. Luis Lemus-Aguilar, Elena Mateos, Begoña Baza, Jorge Alfredo Perez-Garcia processed and stored blood samples. Olivia de la Calle-Jimenez and Guiomar Casado-Fernandez performed the analyses by flow cytometry. Olivia de la Calle-Jimenez, Guiomar Casado-Fernandez, Inés Armenteros-Yeguas, Montserrat Torres, and Mayte Coiras performed the functional analyses. Olivia de la Calle-Jimenez, Inés Armenteros-Yeguas, Vicente Estrada, Noemí Cabello, Montserrat Torres, and Mayte Coiras collected and analyzed the clinical data and laboratory results. Olivia de la Calle-Jimenez, Guiomar Casado-Fernandez, Inés Armenteros-Yeguas, Montserrat Torres, and Mayte Coiras performed the statistical analyses. María Paz Sánchez Seco, Anabel Negredo, Vicente Estrada, and Mayte Coiras provided funds for the study. All co-authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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