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Original article

Kinetics of the humoral and cellular immune response up to 1 year from mpox virus infection

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ABSTRACT

Objectives: The immunological signature of mpox Clade IIb was described in the early stages of infection. We aimed to characterize the kinetics of both humoral and cellular immune responses against mpox from the onset of symptoms up to one year later.
Methods: Sixty-nine patients with mpox infected with Clade IIb during the 2022 outbreak were included in a longitudinal study. Blood samples were collected during the first 3 weeks and 3–4 (T3–4M), 6–8 (T6–8M), and 12 months (T12M) after infection. Mpox-specific IgM, IgA, IgG, and neutralizing antibodies (nAbs) titres were measured by immunofluorescence assay and 50% plaque reduction neutralization test. Interferon-γ producing specific T-cells to Modified Vaccinia Ankara (MVA) peptides was assessed by ELISpot assay. CD4+ and CD8+ T-cells phenotypic markers (CD38/CD57/PD-1) were performed by flow cytometry.
Results: All the humoral markers were detected as early as 4 days and peaked at week 2 (IgG) or 3 (IgM, IgA, nAbs) from symptoms onset. At T3–4M from onset, the antibody levels decreased, and IgM was detected in only one patient; IgA in 50% (13/26), IgG and nAbs in 92% (24/26) of participants. Further decreases in IgG and nAb mean titres were observed at 6–8M. At T12M, IgM, IgA, IgG, and nAbs were detected in 4 (2/47), 48 (23/47), 93 (44/47) and 78% (37/47) of patients, respectively. MVA-specific T-cell response was detected early in the acute phase of infection, peaked at T3M and are maintained until T12M.
Discussion: These data provide evidence of persistence of humoral and cellular immune response 1 year after natural infection, suggesting the maintenance of adequate immune memory. Further study is needed to assess longer persistence of immunity and the cross-protection against different mpox clades.
Valentina Mazzotta, Clin Microbiol Infect 2025;▪:1

Introduction

Alert on mpox (formerly monkeypox) resurged after the African outbreak linked to Clade Ib [1] and the first confirmed case in Sweden [2]. Concurrently, Clade IIb is circulating [3], and the global case count has reached over 100 000 cases [4], leading the WHO again to declare mpox a public health emergency of international concern [5].

The European Centre for Disease Prevention and Control mpox risk assessment considered the likelihood of infection with Clade Ib, as well as its severity, for contacts with an imported case in the European Regions much lower if they have been vaccinated or have

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a history of previous infection with mpox virus (MPXV) Clade IIb [6]. Although reinfections have been described, their clinical course seemed shorter and had less mucosal involvement than the first infection with Clade IIb, suggesting the role of natural immunity in mitigating the clinical picture [7].

The quality and persistence of the immune response after Clade IIb infection still need to be better defined, and the data available refer to small cohorts analysed by different laboratory assays. Our group previously reported the specific humoral and cellular T-cell response in the early phase of infection with Clade IIb in a cohort of 18 patients [8,9]. Briefly, we previously described the detection and the persistence of high titres of all the MPXV-specific antibodies, including the neutralizing antibodies (nAbs), during the first 3 weeks after infection and the early activation of T-cell response persisting until 20 days from infection and the expansion of MPXV-specific CD4+ and CD8+ T-cells.

More recently, Cohn et al. [10] conducted in-depth immunological analyses showing strong B-cell and T-cell activity in six patients with mild mpox between 1 and 3 months after infection. Two studies conducted across 6 months of follow-up from acute infection assessed the durability of MPXV immune response markers, showing specific IgG in 48% of the tested individuals, a sustained MPXV-T-cell response [11], and the persistence of MPXV-specific nAbs [12].

Furthermore, no correlates of immune protection are established, standardized protocols for immune marker assessment are unavailable [13,14], and the duration of immune response beyond 6 months from infection remains unknown.

The present study aimed to deeply characterize the kinetics of both humoral and cellular immune responses against mpox in a larger cohort of patients from the onset of symptoms up to 1 year later.

Methods

Study design, participants, and sample collection

Patients with confirmed mpox diagnosis, admitted at the Lazzaro Spallanzani National Institute for Infectious Diseases IRCCS (Rome, Italy) during the outbreak in 2022, were enrolled in the 'Mpox-Cohort' protocol and followed up to 1 year from diagnosis. All patients provided written informed consent to participate in the study. The study was approved by the Ethical Committee of the Lazzaro Spallanzani Institute (*MpoxCohort protocol: "Studio di coorte osservazionale monocentrica su soggetti che afferiscono per sospetto clinico o epidemiologico di malattia del vaiolo delle scimmie (mpox)*"; approval number 40z, Register of Non-Covid Trials 2022).

Patients were evaluated at the time of diagnosis, every week until the clinical recovery, and then after 3–4 months (T3–4M), 6–8 months (T6–8M), and 12 months (T12M) from the onset of the symptoms.

Demographic, epidemiological, and clinical characteristics were recorded for all patients. Immunological sub-studies of the protocol involved collecting serum and blood samples at different time points to assess the immune response at each time point.

Samples from ten healthy donors matched for age and sex were used as controls for T-cell profile and functionality (for Methods section, see Supplementary Methods page 1–4, including Fig. S1).

We included in this study 69 people with samples available for analysis out of the 131 enrolled from May 19 to September 29, 2022.

Results

All 69 patients included were men, of those 92.8% self-reporting to be men who had sex with men. The median age was 39 years (interquartile range [IQR], 33–46). All were infected with Clade IIb MPXV during the 2022 outbreak.

Thirty-eight (55%) were people living with HIV (PLWH), all receiving antiretroviral therapy with undetectable viraemia and a median CD4 T-cell count of 626/mmc (IQR, 500–887). Six (8.7%) received smallpox vaccine during childhood. Among people without HIV (PLWoH), 9 (13%) were on HIV pre-exposure prophylaxis. The median duration of the disease was 19 days (IQR, 15–25); all patients successfully recovered, and 12 (17%) received treatment with cidofovir or tecovirimat. The main characteristics of the study population are detailed in Table 1.

Kinetics of the humoral response

To investigate the long-term dynamics of the humoral response to MPXV infection, we analysed the serum titres of anti-MPXV IgM, IgA, IgG, and nAbs in 178 serum samples collected from 69 patients. During the first week, we observed the highest titres and reactivity for IgG, followed by IgA, IgM and nAbs (Fig. 1 and Table S1).

Anti-MPXV IgM and IgA reached the highest titres and proportion of reactive patients at week 3 from symptom onset, showing mean titres of 40.0 (21.8–73.5) and 59.4 (36.4–96.9), with 85.7% and 92.9% positive patients, respectively. After 3–4 months from infection, a marked decrease in both the titres ($p < 0.0001$, and p 0.0003 vs. week 3) and proportion of reactive patients (4% and 50%)

Table 1
Main characteristics of the study population.

Participants	$n = 69$ (100%)
Sex at birth	
Male	69 (100.0%)
Median age, y (IQR)	39.0 (33.0–46.0)
Country of birth	
Italy	55 (84.6%)
Other European Countries	5 (7.2%)
Latin America Countries	4 (5.8%)
Northern Africa	1 (1.5%)
Sexual orientation	
Bisexual	3 (4.3%)
Heterosexual	2 (2.9%)
MSM	64 (92.8%)
PLWH, and among those:	38 (55.1%)
CD4 cell count (median, IQR)	626.0 (500.0–884.0)
HIV-RNA undetectable	38 (100.0%)
Current PrEP user	9 (13.0%)
Smallpox vaccine received during childhood	6 (8.7%)
Days from symptoms onset to mpox diagnosis	4.0 (2.0–7.0)
Symptomatic patients	69 (100.0%)
Fever	49 (71.0%)
Lymphadenopathy	48 (69.6%)
Myalgia	16 (23.2%)
Fatigue	11 (15.9%)
Headache	21 (30.4%)
Asthenia	2 (2.9%)
Diarrhoea	7 (10.1%)
Sore throat	8 (11.6%)
Skin rash	68 (98.6%)
Mucosal involvement	47 (68.1%)
Rectal/anal	12 (17.4%)
Ocular	3 (4.3%)
Genital	30 (43.5%)
Nasopharyngeal	14 (20.3%)
Median time of recovery	19.0 (15.0–25.0)
Concurrent STIs	20 (28.9%)
Treatment	
None	57 (82.6%)
Cidofovir	2 (2.9%)
Tecovirimat	10 (14.5%)

IQR, interquartile range; MSM, men who have sex with men; PLWH, people living with HIV; PrEP, pre-exposure prophylaxis for HIV infection; STI, sexually transmitted infection.

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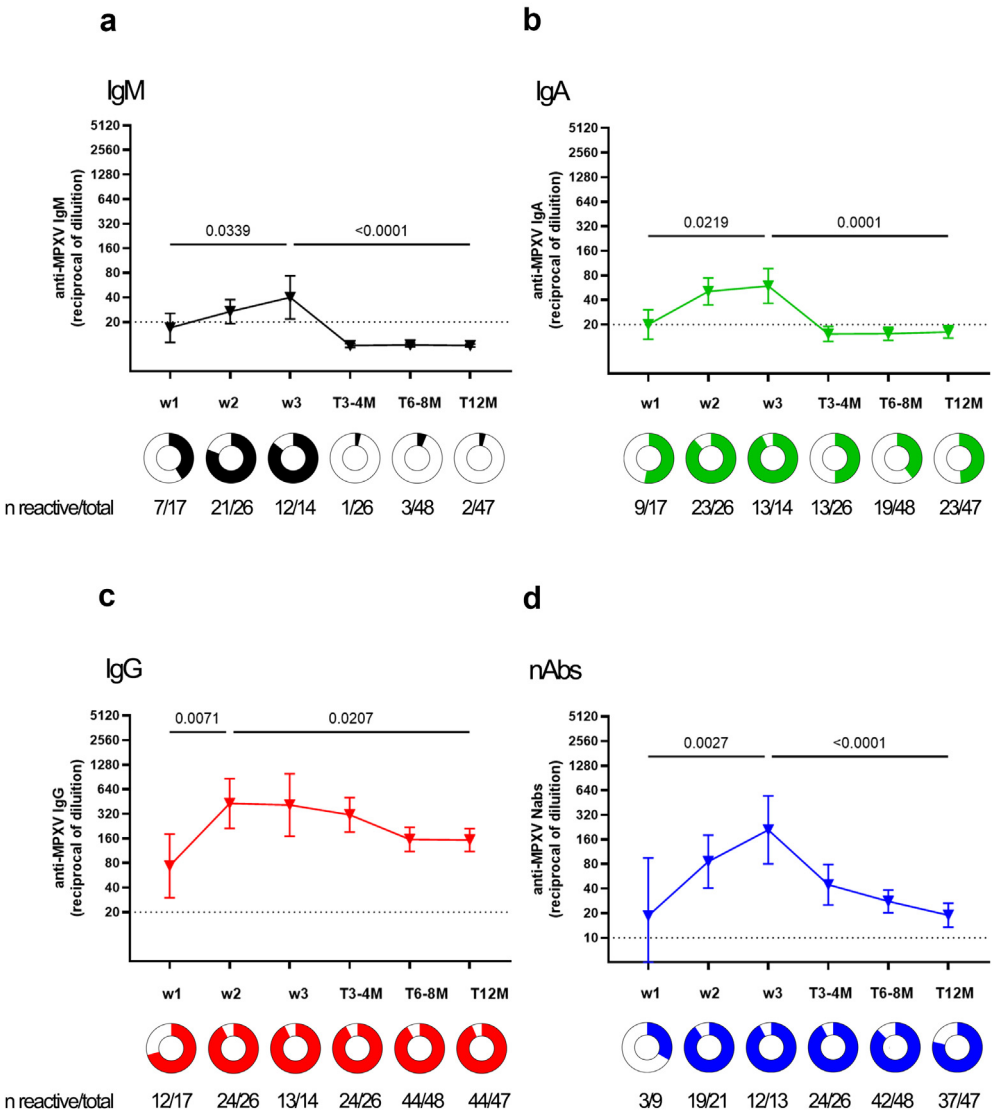

Fig. 1. One-year kinetics of the humoral response in mpox convalescents. Geometric mean titres are shown for IgM (a), IgA (b), IgG (c), and neutralizing antibodies (d) over the course of the follow-up. Error bars represent 95% CI, and dot lines indicate the limit of the assay detection. The percentage of reactive samples is depicted in donut graphs. Kruskall–Wallis, followed by Dunn's test, was used to compare antibody titres. p values are shown to compare peak antibody titres versus levels observed at baseline (w1) or at the last follow-up (T12M). M, month; MPXV, mpox virus; nAb, neutralizing antibody; w, week.

was observed for IgM and IgA (Figs. 1(a)–(b) and Table S1). At 6–8 months, IgM and IgA mean titres remained below the level of the detection, and over 39% of the individuals showed anti-MPXV IgA whereas only 6% had IgM. Similar results were obtained by testing serum samples collected 1 year after symptom onset (Figs. 1(a) and (b) and Table S1).

For anti-MPXV IgG, the peak level was observed at week 2 and remained stable during the third week when this humoral marker was observed in over 92% of the tested patients. Thereafter, the percentage of reactive individuals did not show significant variations (3–4 months: 92.31%, 6–8 months: 91.67%, 12 months: 93.6%). At 3–4 months, the mean level of anti-MPXV IgG was 311.6 (191.1–506.0), whereas lower levels were observed at 6–8 months (p 0.0422 vs. week 2) and 12 months (p 0.0207 vs. week 2) (Fig. 1(c) and Table S1).

The neutralizing activity was the highest at week 3 (208.9, 79.95–545.7), with over 92% of patients who developed anti-MPXV nAbs. Thereafter, the mean level of nAbs remained over the limit of the detection but progressively decreased to 44.5 (25.2–78.6) at 3–4 months, 27.9 (20.2–38.5) at 6–8 months, and 18.9 (13.4–26.5) at 12 months. Anti-MPXV nAbs were measured in 92.31%, 87.50%, and 78.72% of individuals at 3–4, 6–8 and 12 months, respectively (Fig. 1(d) and Table S1).

When comparing the humoral marker titres between PLWH and PLWoH, we observed differences in IgA levels at 3 weeks and nAbs at 3–4 months. At 3 weeks, PLWoH showed higher anti-MPXV IgA levels than PLWH. Anti-MPXV-nAbs at T3–4M were higher in PLWoH than PLWH. This difference was no longer evident at 6–8 and 12 months when nAbs mean titres were very similar in serum samples derived from PLWH and PLWoH (Fig. 2 and Table S2).

Kinetics of CD4 and CD8 T-cells profile

The analysis of the T-cell homeostasis and of MPXV-specific T-cell response was performed on 23 subjects of the 69 enrolled (12 PLWH and 11 PLWoH).

MVA-specific T-cell response was detected early after infection and peaked at 3–4 months (p 0.01 vs. 0–4 days and p 0.0001 vs.

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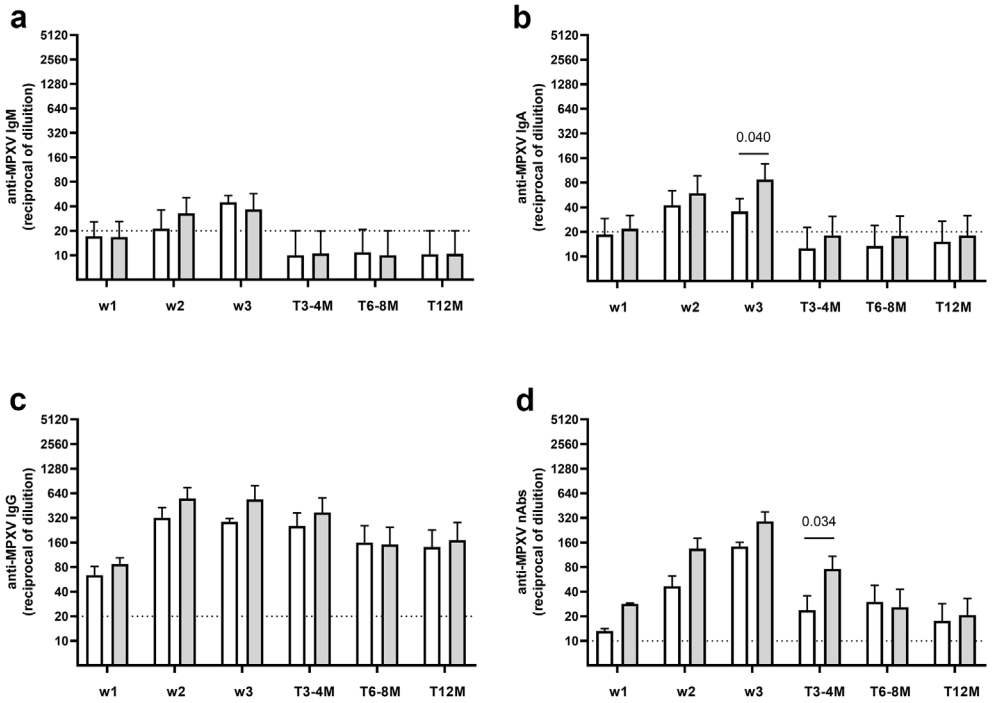

Fig. 2. Comparison of humoral response in people living with (PLWH) and without (PLWoH) HIV over 1 year from symptom onset. Geometric mean titres are shown for IgM (a), IgA (b), IgG (c), and neutralizing antibodies (d) over the course of the follow-up in PLWH (white bars) and PLWoH (grey bars). Error bars represent 95% CI, and dot lines indicate the limit of the assay detection. Mann–Whitney test was used to compare antibody titres in serum samples from PLWH and PLWoH. IFNγ, interferon-γ; M, month; MPXV, mpox virus; nAb, neutralizing antibody; w, week.

Healthly Donors). At 6–8 months, it dropped and remained stable up to 1-year from onset (T3–4M vs. T6–8M p 0.0002 T3–4M vs. T12M p 0.01) (Fig. 3(a) and Fig. S2). No evidence for a difference in MVA-specific T-cell response was found between PLWH and PLWoH (Fig. 3(b)).

Regarding the T-cells differentiating profile, both CD4 and CD8 T-cells showed a higher proportion of effector phenotype compared with healthy donors in the early phase of infection (CD4 p 0.03 and CD8 p 0.001). No differences were detected for this profile for CD4 T-cell phenotypes between patients and HD in the subsequent time points (T3–4M p 0.07; T6–8M; p 0.13; T12M p 0.08), whereas CD8 T effector memory cells were higher in patients than in HD across all the time points (T3–4M and T6–8M p 0.001; T12M p 0.01) (Figs. 4(a) and (b)) and also according to HIV status (Fig. S3(a) and (b)). In addition, CD38 and PD-1 expression on CD4 and CD8 T-cells was higher than HD early after infection, then plunged across T3–4M and T6–8M and finally decreased at the level of the healthy donors at T12M (Fig. S4). Expression of CD38 and PD-1 according to HIV status are shown in supplements (Fig. S5(a)–(f)). Conversely, a higher expression of CD57 senescence marker on CD8 T-cells was observed in patients when compared with healthy controls across the whole observation period (p 0.01, Fig. S5(f)). There was no evidence for a difference in CD57 expression on CD4 T-cells between patients and HD (Fig. S5(e)).

Interestingly, the frequency of CD8 T effector cells correlated with the number of Spot Forming Cells of interferon-γ-producing T-cells after MVA peptides stimulation (Figs. 4(c) and (d), and Table S3).

Discussion

The early rise of all antibody titres and the persistence of MPXV-specific IgA, IgG, and nAbs up to 1 year from infection suggest that natural infection elicits a robust, long-lasting humoral response.

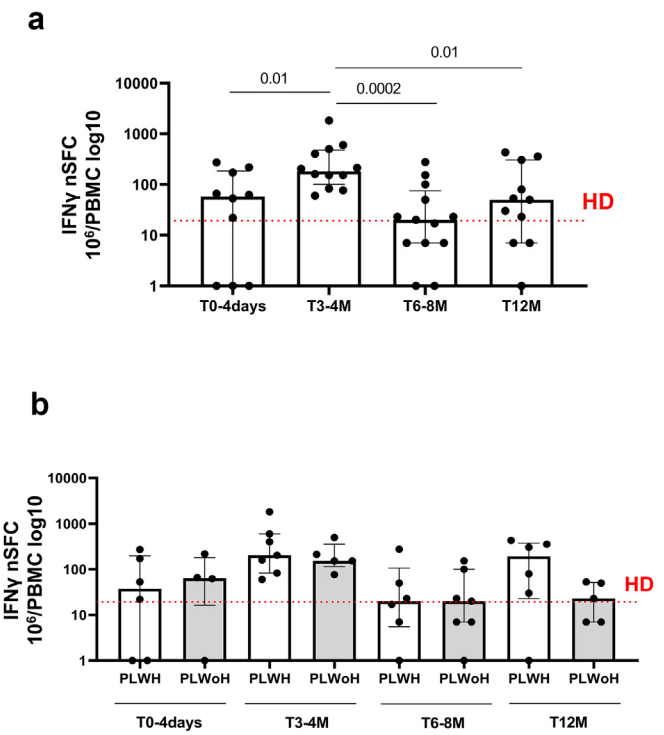

Fig. 3. Specific T-cells response to MVA peptides until 1-year post-infection. (a) The kinetic of poxvirus-specific T-cell response was tested by Elispot assay after MVA peptides stimulation in 23 mpox convalescents (a) and according to HIV stratification: PLWH (white) and PLWoH (grey) (b) at each time point. Results were showed as median value and interquartile range. The red dashed line identified the median of specific MVA T-cells in healthy controls ($n = 10$). Significant differences with HD were reported for (a): T3–4M vs. HD: p 0.0001; (b): T3–4M PLWH vs. HD: p 0.0001; T3–4M PLWoH vs. HD: p 0.0007; T12M PLWH vs. HD: p 0.03. IFNγ, interferon-γ; PLWH, people living with HIV; PLWoH, people living without HIV. MVA: Modified Vaccinia Ankara; HD: Healthly Donors; nSFC: number of Spot Forming cells; PBMC: peripheral Blood Mononuclear Cells.

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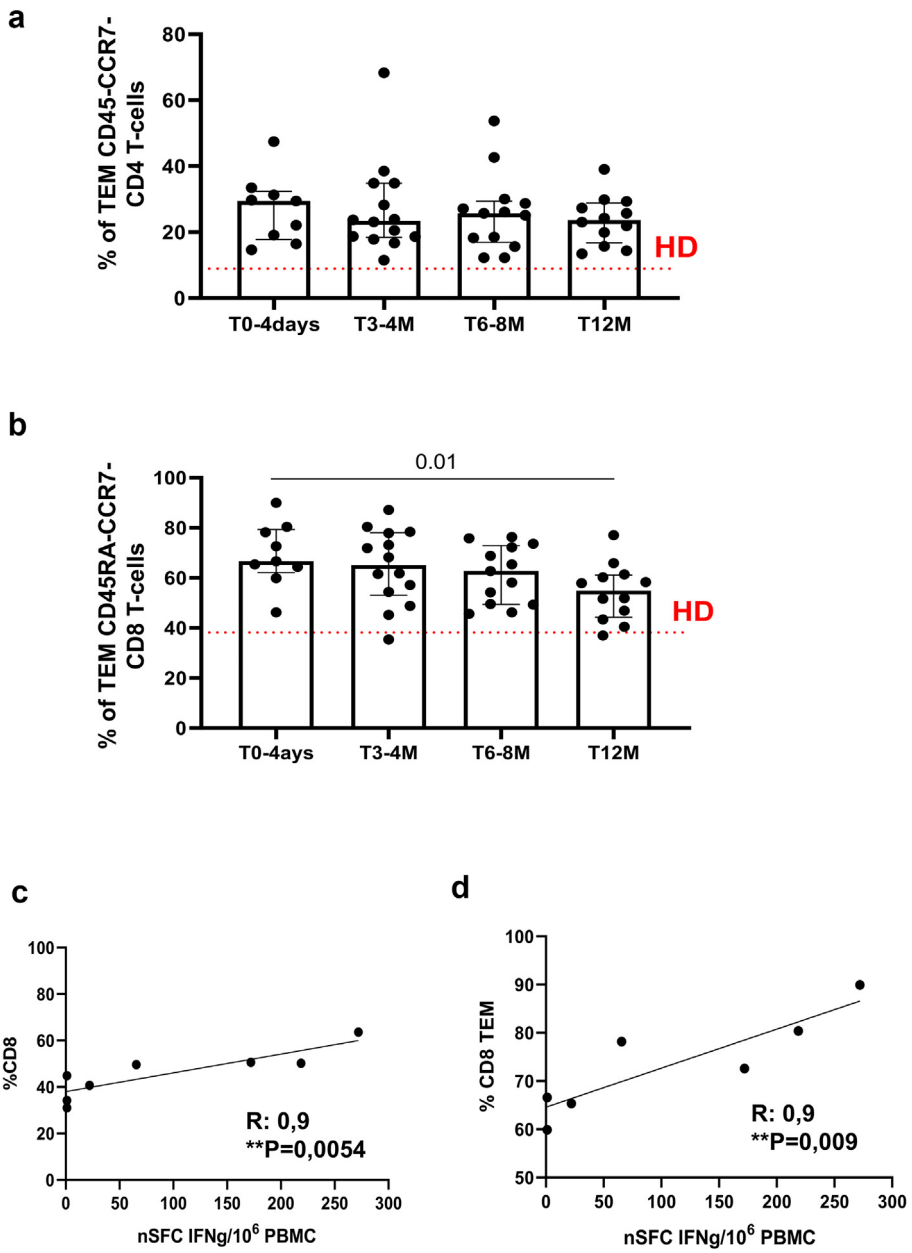

Fig. 4. Proportion of effector CD4 and CD8 T-cells and markers correlation at T0–4 days. (a) TEM phenotypic profile of CD4 (a) and CD8 (b) T-cells was analysed by multiparametric flow cytometry. Results were showed as median value and interquartile range. Red dashed line identified the median frequency of TEM in HD ($n = 10$). (c–d) Correlation of CD8 T-cell frequency (c) and CD8 TEM (d) with the interferon-γ (IFNγ) production after MVA-specific stimulation were shown (C: R: 0.9 **p 0.0054; D: R: 0.9 **p 0.009). Significant differences with HD were reported for (a): T0–4 days vs. HD: p 0.03; (b): T0–4 days vs. HD: p 0.001; T3–4M vs. HD: p 0.001; T6–8M vs. HD: p 0.001; T12M vs. HD: p 0.01. TEM, T effector memory.
MVA: Modified Vaccinia Ankara; HD: Healthly Donors; nSFC: number of Spot Forming Cells; PBMC: Peripheral Blood Mononuclear Cells.

As expected, IgM were detected predominantly in the early phase of infection, whereas from 3 months to 1 year, only 4% of sera were found reactive and with low titres. Studies carried out during the 2003 U.S. outbreak or in the Democratic Republic of Congo from 2007 to 2011 suggested an increased disease severity in IgM responders [15,16]. Nevertheless, we cannot confirm this finding because our study was not designed to test this interaction.

Specific IgA showed an early rise in titres that peaked at 3 weeks and dropped after 3 months. Of note, around 50% of patients display low levels of MPXV-specific IgA up to 1-year post-infection. A recent report [11] described the reactivity for anti-MPXV H3L IgA in only 5 of 29 (17%) patients with mpox 6 months after infection; this discordance could stem from the different assays used for antibody detection. Given the suggested role of IgA in accelerating viral clearance and decreasing disease severity [11], the persistence of reactivity for IgA could have a role in maintaining protection at the mucosal sites, representing a route of entry for MPXV. Like IgA, the concentrations of IgG directed against the MPXV A35R, A29L, and H3L proteins have been proposed to correlate with more rapid viral clearance and/or milder clinical course [11]. In our cohort, IgG against MPXV persists in over 93% of infected patients, showing robust median levels until the end of the follow-up.

Neutralizing antibodies have been historically considered the primary correlate of protection for Orthopoxvirus infections, and two

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different studies proposed nAb titres of 1:32 or 1:20 were protective against the smallpox disease [17,18]. Currently, because correlates of protection for mpox are not known and standardized protocols for assessing nAbs titres are missing, we cannot speculate on the minimum protective level of this immune marker. We detected nAbs in the vast majority (78%) of infected individuals 1 year after mpox, ranging from 1:10 to 1:320. Our data suggest a faster decay in specific IgA and nAbs levels in PLWH, an observation which needs to be confirmed in larger cohorts. Moreover, an enhanced complement-mediated neutralization in mpox has been described [19] but not tested in our cohort. This supports the hypothesis of a strong and persistent humoral response up to 1 year post-infection.

However, producing fully competent antibodies with different functions (neutralization, complement-mediated lysis, antibody-dependent cytotoxicity) needs a compelling interplay with T-cells in the secondary lymphoid organs. CD4 T-cells play a primary role in modulating the differentiation of naïve and/or memory B cells into antibodies-productive plasma cells. Furthermore, T-cells play direct antiviral roles, such as eradicating virus-infected monocytes (CD8), upregulating genes related to cytolytic activities (CD4), and more comprehensively controlling the orthopoxviral infection [20]. These mechanisms are consistent with our findings of the early rise of the amount of T-effector memory cells (CD4 and CD8) and the concomitant expression of activation and exhaustion markers: this subset gradually decreased across the time points, except for the effector/senescent CD8 T-cells, which persisted higher than HD up to 1 year from the symptom's onset. The CD8 effector profile directly correlated with the MVA-specific response after stimulation. Moreover, T-cell response to MVA-peptides stimulation peaked around 3 months after infection and was detected up to 1-year post-infection despite a slight decrease. These observations showed a prompt role of CD4 and CD8 T-cells in mpox acute infection and how already, after 3 months, they were able to restore their immune profile to values observed in healthy individuals in concomitance with viral clearance.

To our knowledge, the other reports published described T-cell response sustained only up to 6 months and detected it by diverse methods, only sometimes including characterization of both CD4 and CD8 T-cells [11,21].

Finally, the wide range of responses provided by the immune system, which recruits antibodies against more than 24 membrane and structural proteins [22,23] and T-cells capable of recognizing epitopes within a wide diversity of viral proteins, both structural and involved in the lifecycle [24–26], has been described. Concomitantly, Orthopoxviruses share a considerable similarity among immunologically relevant proteins and, subsequently, immune epitopes [27,28]. This immunological cross-reactivity related to the highly conserved nature of Orthopoxviruses [29] has been the milestone for using Vaccinia Virus (VACV)-based vaccines against smallpox and, later, mpox [20]. Accordingly, given the broad immune response detected in our cohort, the natural immunity developed after the infection with Clade IIb would likely be effective against Clade Ia and Ib.

Some limitations need to be stated. The study's observational nature may have introduced a potential selection bias that reduces the generalizability of our results. Although we described one of the largest cohorts of persons with mpox comprehensively characterized for humoral and cellular immune response, the stratified analyses could have lost power due to the sample size. We provided results up to 1 year from mpox infection, but further data are needed to assess the long-term persistence of immunity. The evaluation of the T-cell response was performed using MVA peptides, which were commercially available at the beginning of the outbreak, and this limits the comparison with other studies using MPXV mega pools. However, these have been derived from Orthopox mega pools. Finally, we could not provide information on specific Clade Ib immune responses, even though it has been supposed to overlap with those against Clade IIb.

In conclusion, in our cohort of convalescents from the Clade IIb MPXV infection prospectively evaluated for 1 year from the symptom's onset, we observed an early rise of all the MPXV-specific antibodies and the persistence of specific IgG nAbs and, to a lesser extent, IgA, although at decreased levels. Concurrently, T-cells with an effector phenotype were detected early, with the CD8+ persisting over time. After stimulation with MVA peptides, T-cell response persisted for up to 1 year, suggesting the maintenance of adequate immune memory. Taken together, these observations may provide helpful information for defining the possible need for vaccination in people who have had a natural infection with MPXV and long-term protection from different clades reinfection.

Author contributions

V.M. and A.A. conceived the study. V.M., G.M., and E.C. wrote the protocol. V.M. wrote the first draft of the manuscript. A.A., E.C., G.M., and F.M. revised the manuscript. V.M., G.M., E.C., and A.A. accessed and verified the data. G.M., F.C., and E.C. performed the statistical analysis. V.M., R.E., G.M., A.G., A.O., S.V., and J.P. enrolled and followed the patients during the study time points. E.C., S.N., R.C., G.G., E.T., and G.P. performed Peripheral Blood Mononuclear Cells storage. G.M., F.C., A.B., D.M., and F.M. provided serological assays on serum samples. E.C., S.N., R.C., E.T., and G.G. provided tests for T-cell response. A.A., E.G., E.N., and F.M. reviewed the final version of the manuscript. V.M. and G.M. contributed equally to this work. All authors approved the final version of the manuscript.

Transparency declaration

Potential conflict of interest

The authors declare that no conflicting financial interests or other competing relationships exist.

Financial report

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.04.026.

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